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Evaluation of Oxidative Stress in Type 2 Diabetes Mellitus Patients

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Abstract:
Introduction: *Poorly controlled blood glucose levels accelerate hyperglycaemia-induced excess free-radical generation, dyslipidemia and thereby causing OS induced cellular inflammation, damaging blood vessels in type2diabetics.* ***Aim & Objectives:*** *To evaluate OS in T2DM, by estimating malondialdehyde (index of lipid peroxidation), uricacid & ceruloplasmin (physiological, endogenous free-radical scavengers), and correlating them with HbA_{1C} and lipid profile.* ***Methodology:*** *The study included 50 Type2diabetics and 50 age and sex matched controls (36-60yrs). Fasting & post-prandial glucose, HbA_{1C}, lipid profile, malondialdehyde, uricacid and ceruloplasmin were estimated using standard methods.* ***Results:*** *In Type2diabetics increased Triglycerides, Total-cholesterol, VLDL, LDL, MDA, UA & CP and decreased HDL values were observed. MDA,CP,UA,LDL showed significant positive correlation with HbA_{1c} while HDL showed significant negative correlation with HbA_{1C}. MDA, CP, UA showed significant positive correlation with LDL and significant negative correlation with HDL. MDA,CP,UA showed significant positive correlation with one another.* ***Conclusions:*** *It is of utmost importance to achieve tight control of blood glucose(HbA_{1C} < 6.5%), LDL(<100 mg/dL) and HDL(>40 mg/dL), earlier in the disease to reduce OS in T2DM.*

Keywords: *Oxidative stress(OS), Malondialdehyde (MDA), Uric acid (UA), Ceruloplasmin (CP), Type 2 diabetes mellitus(T2DM).*

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], [2]. 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 as ceruloplasmin [3] and uric acid [4].

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 [5]. 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 [6]. Alternatively, an increase in serum CP in type 2 diabetes could generate excess oxidized LDL, which causes atherosclerosis [7]. It could also cause vascular injury by generating free radicals, such as hydrogen peroxide, in the course of oxidization of serum homocysteine [8].

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 [9]. 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[10]

II. Materials & Methods

This study was conducted in the department of biochemistry, Siddhartha medical college, Vijayawada and diabetic clinic in the department of medicine, Government general hospital, Vijayawada.

2.1 Consent: This study was approved by the Institutional Ethics Committee. Informed oral consent was obtained from each participant, after explaining the purpose of this study in their own language, before obtaining the blood sample.

2.2 Study group: This study included 50 Type 2 diabetic patients without complications as cases and 50 age and sex matched apparently healthy individuals as controls.Complete history was obtained from all the participants and a thorough physical examination was done.

2.3 Inclusion criteria: Patient diagnosed by history, clinical examination and laboratory investigations to have Type 2 diabetes mellitus without any complications falling in the age group of 36 - 60 years were included in the study. Apparently healthy people who are age and sex matched with the sample group are used as controls for the study.

2.4 Exclusion criteria: Patients with macro-vascular complications such as cardiovascular, cerebro-vascular and peripheral vascular diseases and micro-vascular complications such as neuropathy, nephropathy and retinopathy were excluded. Patients with hemoglobinopathies, anemia,chronic alcoholism, renal, hepatic and thyroid disorders, febrile illness, diabetic ketoacidosis, renal failure, and those who were suffering from chronic diseases were also excluded from the study. Patients with Type I diabetes mellitus and Type II diabetes mellitus on Insulin therapy were excluded. Patients on lipid modifying drugs like statins or fibrates, steroids, beta blockers, thiazides, phenytoin, etc and pregnant females were excluded.

2.5 Sample collection: Fasting blood samples were taken after an overnight fasting of 12 hrs from all subjects. Under aseptic precautions, 8ml of blood was drawn from the median cubital vein of the study subjects in two vials, one plain and the other containing anticoagulant mixture of Potassium oxalate and sodium fluoride in the ratio of 2:1. Parameters were analysed from whole blood, plasma and serum.Whole blood is used for estimation of Malondialdehyde by Thiobarbituric acid method [11] and HbA1C by Ion exchange resin method [12]. Plasma is used for fasting glucose estimation by GOD-POD method [13]. Serum for estimation of Cholesterol by CHOD-POD method [14], Triglycerides by GPO method [15], HDL-C by Phosphotungstic acid method [16], Ceruloplasmin by Ravin's method [17], Uric acid by TBHB-POD method [18]. VLDL-C & LDL-C were calculated by Friedwald's formula [19]. Then 2ml of post prandial blood sample is collected for estimation of post prandial plasma glucose concentration.

2.6 Statistical analysis: Results were analyzed by descriptive statistical analysis using Excel and Medcal statistical software. Results were expressed in terms of Mean ± standard deviation for each variable. Comparisons of the parameters among groups were done using Analysis of variance (ANOVA) and $P < 0.001$ is considered is significant. Pearson's correlation is used to correlate between the variables and with the disease.

III. Results

In this study, 100 subjects were studied in which 50 were type 2 diabetic cases and 50 were apparently healthy controls. Both the groups were age and sex matched with 25 males and 25 females in each group. The mean age of the subjects involved in this study was 48.000±7.2843. The mean fasting plasma sugar level was higher in cases (152.860±23.8019) than controls (87.080±15.6868). The mean postprandial plasma sugar level was higher in cases (219.14±46.9055) than in controls (129.860±5.1824). The mean HbA_{1C} value of controls was 5.842±0.2853 and that of cases was 8.183±0.9681. The mean total cholesterol level of controls was 162.940±37.4321 and that of cases was 240.940±31.9128. The mean triglyceride level in controls was 127.080±44.2483 and that in cases was 208.120±40.0778. The mean HDL-C level in controls was 38.500±4.0520 and that of cases was 32.500±3.2655 which is statistically significant. The mean VLDL-C level in controls was 25.380±8.9257 and that of cases was 41.660±8.0600. The mean LDL-C level in controls was 99.360±34.4992 and that in cases was 167.420±29.2212. The mean MDA level in controls was 2.308±0.6076 and that in cases was 4.5388±0.7892. The mean serum ceruloplasmin level in controls was 45.500±8.1146 and that in cases was 70.200±8.4708. The mean serum uric acid level in controls was 3.536±0.3827 and that in cases was 4.426±0.5348**.** The mean value of all the above parameters except HDL-C was higher in cases than in controls and the difference is statistically significant ($P < 0.001$) as shown in Table: 1. The correlation of other parameters with Malondialdehyde was summarised in Table: 2, with Ceruloplasmin in Table: 3, and with Uric acid in Table: 4.

Table 1: Comparison of parameters

S.No	Parameter (Mean ± S.D)	Controls (n=50)	Cases (n=50)	Statistical significance
1.	Fasting plasma sugar	87.080±15.6868	152.860±23.8019	F-ratio:266.243(P<0.001)
2.	Postprandial plasma sugar	129.860±5.1824	219.140±46.9055	F-ratio:178.962(P<0.001)
3.	HbA_{1c}	5.842±0.2853	8.183±0.9681	F-ratio:269.034(P<0.001)
4.	Serum total cholesterol	162.940±37.4321	240.940±31.9128	F-ratio:125.724(P<0.001)
5.	Serum triglycerides	127.080±44.2483	208.120±40.0778	F-ratio:92.133(P<0.001)

6.	High density lipoprotein	38.500±4.0520	32.500±3.2655	F-ratio:66.466(P<0.001)
7.	Very low density lipoprotein	25.380±8.9257	41.660±8.0600	F-ratio:91.625(P<0.001)
8.	Low density lipoprotein	99.360±34.4992	167.420±29.2212	F-ratio:113.307(P<0.001)
9.	Malondialdehyde	2.308±0.6076	4.5388±0.7892	F-ratio:334.111(P<0.001)
10.	Ceruloplasmin	45.500±8.1146	70.200±8.4708	F-ratio:302.590(P<0.001)
11.	Uric acid	3.536±0.3827	4.426±0.5348	F-ratio:107.060(P<0.001)

Table 2: Correlation with Malondialdehyde

Parameter	Number of study subjects	Pearson correlation coefficient (r value)	Statistical significance
Fasting glucose	100	0.847	P < 0.0001
Postprandial glucose	100	0.829	P < 0.0001
HbA_{1c}	100	0.866	P < 0.0001
Total cholesterol	100	0.858	P < 0.0001
Triglycerides	100	0.742	P < 0.0001
HDL	100	- 0.652	P < 0.0001
VLDL	100	0.740	P < 0.0001
LDL	100	0.847	P < 0.0001
Ceruloplasmin	100	0.913	P < 0.0001
Uric acid	100	0.782	P < 0.0001

Table 3: Correlation with Ceruloplasmin

Parameter	Number of study subjects	Pearson correlation coefficient (r value)	Statistical significance
Fasting glucose	100	0.766	P < 0.0001
Postprandial glucose	100	0.800	P < 0.0001
HbA_{1c}	100	0.814	P < 0.0001
Total cholesterol	100	0.848	P < 0.0001
Triglycerides	100	0.708	P < 0.0001
HDL	100	-0.637	P < 0.0001
VLDL	100	0.708	P < 0.0001
LDL	100	0.840	P < 0.0001
Malondialdehyde	100	0.913	P < 0.0001
Uric acid	100	0.787	P < 0.0001

Table 4: Correlation with Uric acid

Parameter	Number of study subjects	Pearson correlation coefficient (r value)	Statistical significance
Fasting glucose	100	0.700	P < 0.0001
Postprandial glucose	100	0.714	P < 0.0001
HbA_{1c}	100	0.730	P < 0.0001
Total cholesterol	100	0.712	P < 0.0001
Triglycerides	100	0.697	P < 0.0001
HDL	100	-0.582	P < 0.0001
VLDL	100	0.698	P < 0.0001
LDL	100	0.689	P < 0.0001
Malondialdehyde	100	0.782	P < 0.0001
Ceruloplasmin	100	0.787	P < 0.0001

IV. Discussion

Diabetes mellitus is a chronic disease characterized chronic elevations of glucose, nonesterified fatty acids,and oxidative stress. High values of Malondialdehyde, Ceruloplasmin and Uric acid indicate elevated OS in type 2 diabetics. In this study the lipid peroxidation product, MDA measured as TBARS is significantly

increased in type 2 diabetics than in controls. This finding is in accordance with findings of other studies as illustrated in Table: 5 [20], [21], [22], [23], [24], [25], [26], [27], [28].

TABLE 5: Comparison of present study with other studies in relation to MDA

Studies	Controls Mean ± S.D	Cases Mean ± S.D	Statistical significance*
Present study	2.308±0.6076	4.5388±0.7892	P < 0.001
Jamunarani a. et al., 2012	2.62	4.36	P < 0.001
Padalkar et al., 2012	6.13 ± 2.3	13.29 ± 0.72	P < 0.001
Vivian samuel T, 2011	3.62 ±0.24	5.14± 0.68	P < 0.001
Kedari G S R , 2011	2.41±0.12	6.98±0.13	P < 0.001
Sarita N. Shinde et al., 2011	3.59 ± 0.97	7.19 ± 0.64	P < 0.001
NatheerH al-rawi, 2011	1.1 ± 0.35	2.38 ± 0.97	P < 0.001
M. Salem et al., 2011	5.81 ± 2.39	11.13 ± 3.13	P < 0.001
Nakhjavani M et al., 2010	2.91 ± 0.59	3.82 ± 0.93	P < 0.001
S. A.moussa, 2008	1.3±0.3	3.0±0.7	P < 0.001

*P < 0.001 is significant

The present study showed significantly increased ceruloplasmin levels in cases when compared to controls. These findings are in accordance with other studies conducted by A. Sarkar et al., 2010 [29], and B.Vîrgolici et al,2008 [30]. The present study showed significantly increased levels of serum Uric acid in type 2 diabetic patients when compared to controls. These findings are in accordance with other studies conducted by Natheer H Al-Rawi, 2011; A. Sarkar et al., 2010 and B.Vîrgolici et al., 2008. The present study showed significant positive correlation of MDA, Ceruloplasmin and Uric acid with HbA_{1C} (P < 0.0001). The present study showed significant positive correlation of MDA with cholesterol, triglycerides, VLDL and LDL (P < 0.0001), and significant negative correlation with HDL (P < 0.0001). The present study showed significant positive correlation of Ceruloplasmin with dyslipidemia (P < 0.0001.). Similar findings are seen in the studies conducted by B.Vîrgolici et al., 2008 and Sarkar et al., 2010. The present study showed significant positive correlation of Uric acid with cholesterol, triglycerides, VLDL and LDL(P < 0.0001). HDL showed highly significant negative correlation with Uric Acid (P < 0.0001). Similar findings are seen in the studies conducted by B.Vîrgolici et al., 2008 and Natheer H Al-Rawi, 2011.

V. Conclusion

Type 2 diabetes is a chronic progressive disease, characterised by hyperglycaemia and dyslipidemia, causing an increased susceptibility of cells to lipid peroxidation and inflammation due to oxidative stress which plays a major role in the pathogenesis of diabetes and its complications. The results of this study and previous works provide ample evidence that poor metabolic control and dyslipidemia in patients with type 2 diabetes mellitus were associated with increased MDA, CP and UA. These observations suggest that supportive therapy aimed at oxidative stress may help prevent the development of complications in type 2 diabetes mellitus. Approaches such as regular physical exercise and/or antioxidant therapy, concurrent with standard treatment, may improve the quality of life and reduce disease progression in type 2 diabetic patients.

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