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Oxidative stress in diabetic retinopathy and diabetic nephropathy

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ABSTRACT

Background: Oxidative stress plays an important role in the pathogenesis of diabetic complications including diabetic retinopathy (DR) and diabetic nephropathy (DN) as suggested by experimental and clinical studies. The study aimed to measure as well as compare total antioxidant capacity (TAC) and malondialdehyde (MDA) levels in type 2 diabetic patients (T2DM) with diabetic retinopathy and diabetic nephropathy.

Materials and Methods: In this cross-sectional study we measured serum TAC, MDA, fasting plasma glucose (FPG), and HbA1c levels in 3 groups of T2DM patients with 110 cases in each group: 1st group without DR and/or DN, 2nd group with DN, and 3rd group with DR. This study was done in a tertiary care hospital from Dec 2019 to Dec 2022. Data was analyzed using version 20 of SPSS software.

Results: In DR patients, TAC levels were significantly lower (P=0.000), and FPG and HbA1c levels were significantly higher (P=0.003 & P=0.001 respectively) than in DM and DN patients. However, MDA did not show any significant difference in all groups. In the proliferative DR group, duration of diabetes, MDA levels, FPG levels, and HbA1c levels were significantly high (P=0.027, P=0.033, P=0.014 & P=0.000 respectively) and TAC levels were significantly low (P=0.033) as compared to non-proliferative DR group.

Conclusion: Weakening of the antioxidant defence system with increased oxidative stress in DM is associated with complications like DR and DN and also with the progression of DR to its vision-threatening proliferative stage.

1. Introduction

Being a progressive and long-standing disorder type 2 diabetes mellitus(DM) increases the risk of developing vascular complications including macro-vascular (cardiovascular, cerebrovascular, and peripheral artery diseases) and micro-vascular (diabetic retinopathy, nephropathy, and neuropathy) complications.[1]

Diabetic retinopathy (DR) is caused by injury to blood vessels in the retina of the eye and diabetic nephropathy (DN) is kidney damage caused by diabetes. These are some of the most specific complications of DM. DR is one of the important causes of preventable blindness in India with prevalence ranging from 4.8%[2] to 21.7%.[3]

In India, an increase in the prevalence of diabetic nephropathy has been noted[4] and is also the most common cause of end-stage kidney disease. The Chennai urban rural epidemiology study found that the prevalence of overt diabetic nephropathy and microalbuminuria in urban diabetic patients was 2.2% and 26.9% respectively.[5] The number of individuals with DN and DR is expected to increase very soon as a result of the substantial rise in the number of people with diabetes mellitus in India.

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Generation of free radicals due to glycosylation, auto-oxidation of the glycated products, and alteration in the activity and /or the tissue content of the antioxidant defense molecules are a few of the oxidative stress-producing mechanisms in diabetic patients.[6]

To improve knowledge of the pathogenesis of diabetic complications and to understand the role of oxidative stress in it, several clinical and experimental studies have been undertaken. In such a context, lipid peroxidation occurring in the body as a consequence of oxidative stress is measured by estimation of malondialdehyde (MDA), and the body's ability to counteract oxidative stress by antioxidant defense mechanism is measured by estimation of total antioxidant capacity (TAC).[7,8] Different end products of lipid peroxidation were found at a higher level and antioxidants were altered in the serum of diabetic patients. They are associated with the development of diabetic complications such as DR and DN.[9–11]

Up till now, there were few studies done to explore malondialdehyde and total antioxidant capacity in both DR and DN patients collectively. In this regard, we quantified MDA, TAC, fasting plasma glucose (FPG), and glycosylated hemoglobin (HbA1c) levels to know whether there was difference in the levels of these parameters in the patients with type 2 diabetes mellitus without DN and DR, with DR and with DN.

2. Materials and Methods

2.1. Study population and design

Our study was conducted from Dec 2019 to Dec 2022 in a tertiary care hospital. In this cross-sectional observational study, the sample size was calculated with the help of a study by Pieme et al.,[12] and from the formula: n= $(SD_1^2 + SD_2^2)(Z_{1-\alpha/2} + Z_{1-\beta})^2 / d^2$, which was a minimum of 50 individuals in each group. For the study, a total of 330 patients were included in 3 groups. The first group (DM) included 110 type 2 DM patients without DN and/or DR, the second group (DN) contained 110 type 2 DM patients with DN, and the third group (DR) included 110 type 2 DM patients with DR. Ethical clearance for the study was obtained from Institutional Ethics Committee.

2.2. Inclusion criteria

Age >40years, for the first group - known case of type 2 DM based on the criteria suggested by the American Diabetes Association,[13] DM for >10 years on treatment without DN and/or DR. For the second group - patients with DN diagnosed by department of nephrology with albuminuria >300mg/gm creatinine, no evidence of moderate, severe non-proliferative DR (NPDR) or proliferative DR (PDR). For the third group - patients with DR, no evidence of DN.

2.3. Exclusion criteria

Age< 40 years, Type 1 diabetic patient or any other type of diabetes, patients with liver disease, oncologic disease, thyroid disorders or other endocrine diseases, pregnant and lactating females, persons using antioxidant medications, tobacco users, and smokers. Patients who had mild NPDR, retinal vascular occlusions, glaucoma, uveitis, and mature cataracts.

After an explanation of the study, patients who accepted to take part in the study were selected consecutively according to selection criteria from the outpatient department. Written consent was taken from enrolled patients and blood was collected.

General characteristics like age, sex, etc., history, and clinical examination findings from their file were filled in a form for every individual. A full ophthalmic examination of patients was done. Diabetic retinopathy grading was performed according to the ETDRS scoring system.[14] DR patients were divided into non-proliferative DR (NPDR) and proliferative DR (PDR) depending upon scoring for this study. In the NPDR group, mild NPDR was excluded.

2.4. Sample collection and preparation

A fasting venous blood sample (5ml) was collected from each patient, 1ml in a fluoride oxalate vacutainer tube, 3ml in a plain vacutainer tube, and 1ml in EDTA vacutainer tube. Plasma and serum were separated and used for investigations. Plasma was used for the estimation of fasting plasma glucose (FPG) and serum was used for the measurement of total antioxidant capacity (TAC) and malondialdehyde (MDA).

2.5. Biochemical assay

Auto analyzer was used for measurement of FPG in mg/dl by enzymatic colorimetric method using glucose oxidase peroxidase test. Glycosylated hemoglobin (HbA1c) in % was estimated by immunoturbidimetric test. Spectrophotometer was used for the measurement of serum total antioxidant capacity (TAC) in mmol/L by the ferric-reducing ability of plasma (FRAP) assay[15] and serum malondialdehyde in μmoles/L was estimated by Thiobarbituric acid reactive substances (TBARS) assay using Kei Satoh method.[16]

2.6. Statistical analysis

SPSS software (SPSS Statistics, Version 20, IBM Corp., Chicago, Illinois, USA) was used for analyzing the data. The Chi-square test, Kruskal Wallis test, and Mann-Whitney U test were used for comparison of the groups. Spearman's rank correlation coefficients were estimated for correlation analysis. Statistical significance was considered with a P value ≤ 0.05.

3. Results

In the present study, a total of 330 individuals were studied who were divided into three groups as defined above. General characteristics and biochemical parameters in the three groups are displayed in Table 1. Gender wise all groups were similar (P=0.132). The mean age of patients in the DM group was significantly higher than the DN and DR groups (P=0.000). In all groups, serum MDA levels were statistically similar (P=0.464). Serum TAC levels were significantly lower (P =0.000) in the DR group than in other groups. Also, Serum TAC levels were significantly lower in the DN group than in the DM group. Fasting plasma glucose and HbA1c levels were significantly higher (P=0.016 & P =0.000 respectively) in the DR group than DM group. (Table 1)

In all groups, TAC correlated negatively with MDA (P=0.001) but no significant correlation was found between MDA, TAS, and FPG (Table 2).

Table 3 demonstrates general characteristics and biochemical parameters in DR patients. DR group was sub-divided into two groups: non-proliferative DR(NPDR) and proliferative DR(PDR) for comparison. Age and sex-wise no significant difference was found in the two groups (P=0.88 & P=0.468 respectively). In the PDR group, duration of diabetes, MDA levels, FPG levels, and HbA1c levels were significantly high (P=0.027)(P=0.033)(P=0.014)(P=0.000) and TAC levels were significantly low(P=0.033) as compared to NPDR group.

4. Discussion

In diabetes, environmental and genetic factors hasten chemical alterations in proteins, carbohydrates, and lipids along with their functions which could contribute to the progression of complications. Oxidative stress is one of the associated factors on which research is still going on.[17] Measurements of Malondialdehyde (MDA) as a pro-oxidant molecule and total antioxidant capacity (TAC) as an antioxidant molecule for oxidative stress in patients with diabetic complications mainly diabetic retinopathy and nephropathy were done in the present study.

There is a discrepancy in the timing between the diagnosis of diabetes and the development of DN.[18] Also, the prevalence of DR is low within the first 10 years of diagnosis of DM and it progresses occasionally.[19] So, we have selected patients in the DM group with more than 10 years of duration as controls. This explains the older age of the DM group than the DR and DN groups.

The present study showed that MDA levels were similar in all groups. However, TAC levels were significantly lower in the DN and DR groups. Our previous study had shown that MDA was significantly higher in diabetic patients compared to non-diabetic patients.[20] This suggests that oxidative stress was higher in diabetic patients irrespective of status i.e. with or without complications. However, the defence mechanisms to counter this oxidative stress were weaker in diabetic complications mainly in DR followed by DN than in the DM group. Enhanced MDA levels lead to detrimental physiological reactions such as alteration in cell membrane structural integrity, and inactivation of membrane-bound enzymes and cell surface receptors.[21] Also, in DM long-standing hyper-glycemia deteriorates the antioxidant defence mechanism which is shown by a decrease in TAC levels.[22] The vicious cycle ensues with the body's decreased efficiency in removing ROS, the persistence of oxidative stress with exacerbation of lipid peroxidation, and an increment in MDA.

Pieme et al. showed similar findings with higher MDA levels in diabetic patients with and without complications.[12] However, they did not find a significant difference between TAC levels in these two groups. Merzouk S et al. discovered that the plasma oxygen radical absorbance capacity (ORAC) assay which is an excellent indicator of the total antioxidant capacity was diminished in diabetic patients in general and had lower values in patients with complications such as renal failure and coronary artery disease than those without complications.[23] In contrast, Khalili F et al. found higher TAC levels in DR patients than in DM patients without complications.[24]

Higher fasting plasma glucose and HbA1c levels in the DR group than in other groups as noticed in this study were consistence with results in previous studies.[12,24] This suggests tissue damage with long-standing hyperglycemia.[25] A significant negative correlation between MDA and TAC without any correlation between MDA, TAS, and FPG was detected in this study. This suggests increased oxidative stress with weakened antioxidant defence mechanisms go hand in hand irrespective of FPG which may be regulated at that particular point in time. Conflicting results were found by Pieme et al.[12] with a significant positive correlation between catalase and fasting blood glucose levels.

PDR patients showed an increase in oxidative stress as shown by a significant increase in MDA levels and a decrease in TAC levels. Chronic hyperglycemia in PDR was suggested by the significant increase in FPG and HbA1c levels. Enhanced risk of PDR with extended duration of diabetes indicates a progressive deterioration in illness.

Carrizales et al. found significantly low serum levels of TAC but higher levels of serum erythrocyte catalase and glutathione peroxidase in severe DR cases.[26] Even though this study has found a significant rise in the serum antioxidant enzyme levels in severe DR cases, perhaps due to an adaptable mechanism dealing with pro-oxidants in diabetes, it is well recognized that estimation of TAC levels is more meaningful than individual antioxidant measurement as it provides collective information about

Table 1: General characteristics and biochemical parameters

Variables	Group 1 (N=110)	Group 2 (N=110)	Group 3 (N=110)	P value
Gender				
Male	61(55.5%)	75(68.2%)	71(64.5%)	0.132
Female	49(44.5%)	35(31.8%)	39(35.5%)	
Age (years)	63.94±8.56 ^{b,c}	60.68±7.76^{a}	58.87±7.02 ^{a}	0.000**
MDA (μmoles/L)	3.88±1.81	3.78±2.17	3.56±1.82	0.283
TAC (mmoles/L)	1.74±0.37 ^{b,c}	1.12±0.21^{a,c}	0.88±0.28 ^{a,b}	0.000**
FPG (mg/dl)	195.48±79.18^{b,c}	224.57±78.07^{a}	230.23±82.12^{a}	0.003**
HbA1c (%)	7.72±1.69 ^{c}	7.78±1.92^{c}	8.55±1.96^{a,b}	0.001**

Group 1- DM, Group 2- DN, Group 3- DR

Data were stated in terms of percentage and mean± SD. MDA- malondialdehyde, TAC- Total antioxidant capacity, FPG- Fasting plasma glucose & HbA1c- Glycosylated hemoglobin

^{a}-Significantly different from group 1(P<0.05), ^{b} - Significantly different from group 2 (P<0.05) and ^{c} - sSignificantly different from group 3 (P<0.05)

*P<0.05.

Table 2: Correlation between biochemical parameters

Biochemical parameters		Group 1	Group 2	Group 3
MDA and TAC	Spearman's ρ (rho)	-0.319	-0.366	-0.317
	P- value	0.001**	0.000**	0.001**
MDA and FPG	Spearman's ρ (rho)	0.096	0.012	0.105
	P- value	0.320	0.897	0.273
TAC and FPG	Spearman's ρ (rho)	0.001	0.071	0.053
	P- value	0.995	0.464	0.581

MDA- Malondialdehyde, TAC- Total antioxidant capacity, FPG- Fasting plasma glucose.*P<0.05.

Table 3: General characteristics and biochemical parameters in DR patients

Variables	NPDR (N=64)	PDR (N=46)	P value
Gender			
Male	42(65.6%)	29(63%)	0.468
Female	22(34.4%)	17(37%)	
Age(years)	58.95±7.50	58.76±6.37	0.93
Duration of diabetes	8.20±7.10	11.30±7.98	0.027*
MDA(μmoles/L)	3.24±1.58	4.01±2.04	0.033*
TAC(mmoles/L)	0.93±0.26	0.81±0.31	0.033*
FPG (mg/dl)	213.82±75.06	253.06±86.77	0.014*
HbA1c	7.86±1.55	9.52±2.08	0.000**

NPDR- Non-proliferative diabetic retinopathy, PDR- Proliferative diabetic retinopathy; MDA- Malondialdehyde, TAC- Total antioxidant capacity, FPG- Fasting plasma glucose.*P<0.05

antioxidant status. The lowest levels of TAC and highest levels of TOS were detected in the aqueous humor of PDR patients compared to NPDR patients in a study by Erdinç Bozkurt et al.[27]

Some studies found higher MDA levels in the blood and vitreous samples of PDR patients compared to NPDR patients.[9,10] They concluded that high levels of MDA in diabetic patients reflect a similar rise at the level of the vitreous body and it indicates the possible involvement of oxidative stress and lipid peroxidation in the advancement of DR to the proliferative form.[10]

Increased oxidative stress detected in the DN group compared to the DM group was consistent with the results found in previous studies.[11,28] In this study we have not taken into consideration the detailed classification of DR including diabetic macular edema. Also in the DN group, different stages of DN were not included. The sample size was less for this type of detailed study. Further study with a large sample size will be needed.

5. Conclusion

Weakening of the antioxidant defence system with increased oxidative stress in DM is associated with complications like DR and DN and also with the progression of DR to its vision-threatening proliferative stage. Monitoring TAC and MDA levels can provide insights into the oxidative stress status and the degree of antioxidant defence in individuals with diabetic retinopathy and diabetic nephropathy. These markers may help in understanding the underlying mechanisms of these complications and may

assist in the development of potential therapeutic strategies aimed at reducing oxidative stress and its detrimental effects. This may help delay complications which will improve the quality of life of DM patients.

6. Source of Funding & Conflict of Interest

None.

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