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COMPARATIVE STUDY ON MDA, SOD AND HbA1c LEVELS IN PATIENTS OF TYPE 2 DIABETES MELLITUS WITH RETINOPATHY AND WITHOUT RETINOPATHY

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Keywords:

Diabetic Retinopathy; Diabetes Mellitus; Risk Factors; Oxidative Stress; Biomarker; MDA; SOD; HbA1c

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ABSTRACT: Background: Diabetes mellitus comprises a group of metabolic disorders that share the common phenotype of hyperglycemia, association with the biochemical alteration of glucose and lipid peroxidation. Abnormalities in metabolism, including elevated polyol pathway, increased non-enzymatic glycation, accumulation of AGEs, uncontrolled oxidative stress, protein kinase C activity (PKC) and the expression of vascular endothelial growth factor (VEGF) result from glucose dysmetabolism and evidently also contribute to the development of retinopathy. The aim of this study was to analyze and correlate determine the levels of Malondialdehyde and Superoxide dismutase in patients of Type 2 diabetes mellitus with and without retinopathy. **Materials and Methods**: The study population comprised of 54 type 2 diabetics with retinopathy and 54 type 2 diabetics without retinopathy in the age group of 35-74 years. HbA1c, MDA, and SOD were assayed for both subjects. Results were analyzed carried out by using SPSS 16.0 version (Chicago, Inc., USA). **Results:** Serum MDA levels were highly significant increased in Type 2 Diabetes Mellitus with Retinopathy in comparison to without Retinopathy (4.25±1.03 µmol/l vs 2.12±1.55 µmol/l and $p<0.0001$*). SOD; which acts as an antioxidant was highly significant decrease in diabetic retinopathy, in comparison to without Retinopathy (0.53±0.07 U/mg protein/min vs 0.91±0.10 U/mg protein/min and $p<0.0001$*). HbA1c, which acts as a biomarker of diabetes was significant higher diabetic retinopathy in comparison to without Retinopathy (9.28±2.31% vs 7.95±1.77 % and $p < 0.001$). **Conclusion:** The present study indicates that the MDA and HbA1c level increase while there is a decrease SOD level, leading to oxidative stress in diabetic complications. Oxidative stress in diabetes mellitus, increasing over time may play a role in the pathogenesis of diabetic retinopathy that should be considered in further research.

INTRODUCTION: Diabetes Mellitus is a chronic metabolic disorder characterized by hyperglycemia with disturbance of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both.[1]

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The effects of diabetes mellitus include long term damage, dysfunction and failure of various organs.[2] Diabetes-specific micro vascular disease is a leading cause of blindness, renal failure and nerve damage. [3] India faces a huge case load of type 2 diabetes mellitus (DM), which is projected to affect about 69.9 million Indians by the year 2025.[4] Though all traditional risk factors such as hyperglycemia, dyslipidemia, hypertension and duration of diabetes are associated with development and progression of Diabetic Retinopathy. [5]

Oxidative stress is increased in DM, owing to an increase in the production of oxygen free radicals and a deficiency in the antioxidant defense mechanisms. The lipid peroxidation of the cellular structures, a consequence of the increased oxygen free radicals, is thought to play an important role in the atherosclerosis and the micro-vascular complications. [6] Oxidative stress has been implicated in the pathogenesis of diabetic retinopathy. It has been hypothesized that hyperglycemia may damage the vascular endothelium and the retina by inducing the synthesis of oxidant reactive species and thereby causing oxidative stress. [7]

Diabetic Retinopathy is a progressive disorder. It is the most common cause of blindness in people aged 30-60 years. The retina has high content of polyunsaturated fatty acid and glucose oxidation relative to any other tissue. This phenomenon renders the retina more susceptible to oxidative stress. [8] Retinopathy is characterized by increased vascular permeability, by vascular closure mediated by the formation of new blood vessels neo-vascularization, on the retina and posterior surface of the vitreous. [9]

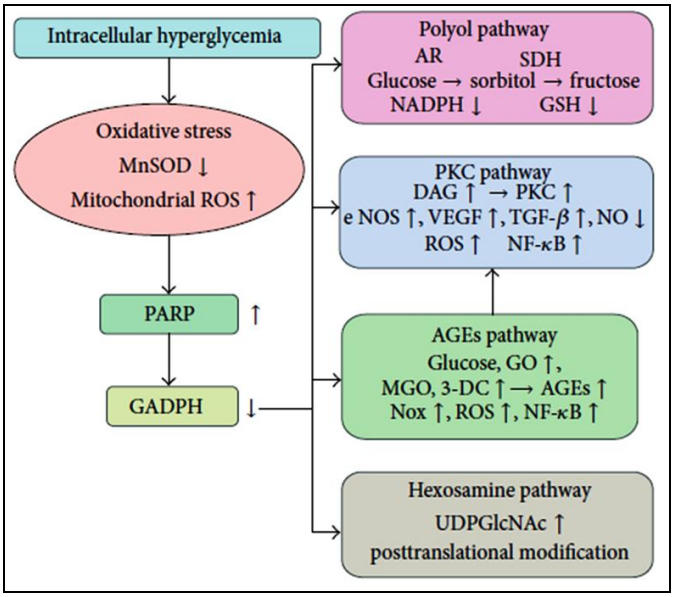

FIG. 1: RELATION BETWEEN HYPERGLYCEMIA, OXIDATIVE STRESS, AND PATHWAYS ASSOCIATION WITH PATHOGENESIS OF DIABETIC RETINOPATHY [10]

Oxidative stress has been implicated in the pathogenesis of diabetic retinopathy. [11] The epidemiology of diabetic retinopathy and has been previously described, largely in the Wisconsin Epidemiological Study of Diabetic Retinopathy (WESDR). [12] Glycosylated hemoglobin (HbA1c) level at baseline has been found to be strongly related to the incidence, Progression or both of Diabetic Retinopathy.[13]

Diabetes increases oxidative stress in the retina: the levels of lipid peroxide, thiobarbituric acid substances, and superoxide are increased in the retina. [14] This increase in oxidative stress can be the result of several diabetes-induced abnormalities, including auto-oxidation of glucose, the formation of advanced glycation end products, and impairments in the antioxidant defense system. The activity of superoxide dismutase (SOD), an enzyme known to scavenge superoxide, is decreased in the retina in diabetes, and its expression is down regulated. [15, 16]

Oxygen free radicals liberated by metabolic processes can cause tissue damage. Endogenous oxidative damage to proteins, lipids and DNA is thought to be important etiologic factor in the pathophysiology of the complications of diabetes mellitus. Normally the body has an abundant supply of antioxidants, such as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase, Vitamin A, Vitamin C etc. which are naturally occurring substances that delay or inhibit oxidation and neutralize the oxygen free radicals. Free radicals in diabetes mellitus and increasing over time may play a role in the development of diabetic retinopathy which is an important complication of the disease.[17] Little is known about the relationship in between oxidative stress and diabetic retinopathy in India.

Studies on patients with long term and poorly controlled diabetes suggest that free radicals in diabetes mellitus and increasing over time may play a role in the development of diabetic retinopathy. [18] The ophthalmic complications of diabetes include corneal abnormalities, glaucoma, iris neo-vascularization, cataracts and neuropathies. However, the most common and the potentially most blinding of these complications is diabetic retinopathy. [19]

Diabetes duration and sustained hyperglycemia are among the primary risk factors for the development of diabetic retinopathy. [20] HbA1c test analysis may be ordered. The use of HbA1c testing may help

predict those at-risk for diabetes, diabetic retinopathy or other complications of diabetes. [21]

The study was confirm whether these exists an involvement in between antioxidant nutrient intake and reduction in the improvement of diabetic complications particularly retinopathy. [32] Supplementary Vitamin C may be helpful in decreasing blood glucose type 2 diabetes and thus reducing the risk of complications. [33] The diabetes complications and control trial (DCCT) established HbA1c as the gold standard to assess glycemic control.[34]

MATERIALS AND METHODS: The present study was conducted in the Department of Biochemistry, Central Research Laboratory and Central Clinical Laboratory and P.G Research Laboratory in Integral Institute of Medical Sciences & Research, I.U, Lucknow, India. Clinically diagnosed & confirmed cases of diabetic retinopathy in age group 35 to 74 years. The study was approved by the Institute Ethics Committee, Integral Institute of Medical Sciences & Research Lucknow, India and informed consent was obtained from all the case and control subjects.

Sample collection and storage: Under aseptic conditions 4ml of venous blood was collected. Out of this 1 ml was collected in EDTA estimation of HbA1c and 3 ml collected in without anticoagulant (Plain) estimate Malondialdehyde (MDA) & Superoxide dismutase (SOD) centrifuged (3,000 rpm, for 3-5min at 37°C) to obtain serum that was also stored at -80 °C for further biochemical measurements**.**

Numbers of Cases selected for the study were:

- 54 cases of Type 2 diabetes mellitus with retinopathy
- 54 controls of Type 2 diabetes mellitus without retinopathy

Biochemical measurement:

Estimation of HbA1c by Nephelometry method using analyzer: [22] The test procedure and the calibration data according to NGSP method is provided in the smart card along with the kit. Insert the smart card and follow the instructions. Insert card to card reader slot & display will prompt to add R1+sample. Pipette 180µl R1 & 5µl sample to cuvette & place the cuvette holder. After incubation display will prompt to add R2. Pipette 60 µl R2 using attached sensor pipette to the cuvette.

The result will show in the display and print out.

The HbA1c concentration was calculated according to the following formula:

ΔOD of samples = OD Sample – OD Blank HbA1c %

The sample is calculated by interpolation of OD of sample on the calibration curve. For calculation of results according to IFCC, use IFCC calibrator values (see calibrator insert), or use following equation.

NGSP= (0.915 × IFCC) + 2.15

Estimation of Malondialdehyde by Satoh k. (1978) method: [23] The TCA–TBA-HCl solution will be freshly prepared by mixing equal volume of 15% TCA, 0.375% TBA and 0.25N HCl. 0.8 ml of serum + 1.2 of TCA-TBA-HCl reagent. Mixed immediately + kept in a boiling water bath for 10 minutes. Cooled +2ml of 1N NaOH (freshly prepared) to eliminate centrifugation. O.D at 535nm against blank which contained normal saline in place of serum. The MDA concentration was calculated according to the following formula:

MDA (µmol/l) =OD 532 × 1.75/0.15

Estimation of Superoxide dismutase (SOD) using Nitroblue tetrazolium (NBT) method: [24] The assay mixture contained 1.2ml of sodium pyrophosphate buffer, 0.1ml of PMS, 0.3ml of NBT, 0.2ml of the enzyme preparation and water in a total volume of 2.8ml. The reaction will be initiated by the addition of 0.2ml of NADH. The mixture was incubated at 30^{o}C for 90 seconds and arrested by the addition of 1.0ml of glacial acetic acid. The reaction mixture will be then shaken with 4.0ml of n-butanol, allowed to stand for 10 minutes and centrifuged. The intensity of the chromogen in the n-butanol layer will be measured at 560 nm in a spectrophotometer. One unit of enzyme activity is defined as the amount of enzyme that gave 50% inhibition of NBT reduction in 1 minute. One unit of SOD activity is the amount of the enzyme that inhibits the rate of auto oxidation of NBT by 50% and was expressed as Units /mg protein/min.

The enzyme unit can be calculated by using the following equation :-

Rate ® = (final OD – initial OD) / 3 min

% of inhibition = {(blank OD – R) / blank OD} x 100

Enzyme unit (U) = (% of inhibition / 50) x common dilution factor.

[50% inhibition = 1 U] Specific activity = (U / mg) protein

Statistical Analysis: The results are presented in mean±SD and percentage. Chi-square test was used to compare the categorical variables between cases and controls. Unpaired t-test was used to compare the study parameters between cases and controls. The Pearson correlation coefficient was calculated among the study parameters. The p-value<0.05 was considered significant. All the analysis was carried out by using SPSS 16.0 version (Chicago, Inc., USA).

RESULT AND OBSERVATION: In this study, 54 cases and 54 controls of either sex were included.

Table 1 Shows the comparison of MDA, SOD & HbA1c level between cases and controls. MDA was highly significant (p=0.0001*) higher among cases (4.25±1.03) than controls (2.12±1.55). SOD was highly significant (p=0.0001*) lower among cases (0.53±0.07) as compared with controls (0.91±0.10). HbA1c level was significant (p=0.001) higher among cases (9.28±2.31) as compared with controls (7.95±1.77).

TABLE 1: COMPARISON OF MDA, SOD& HBA1C LEVELS BETWEEN (CASES AND CONTROLS)

Groups	Case (N=54)	Controls (N=54)	t- value	p- value
MDA (µmol/L)	4.25±1.03	2.12±1.55	8.4106	<0.0001*
SOD(U/mg protein/min)	0.53±0.07	0.91±0.10	22.8764	<0.0001*
HbA1c (%)	9.28±2.31	7.95±1.77	3.3584	<0.001

Abbreviations: MDA, Malondialdehyde; SOD, Superoxide dismutase; HbA1c, Glycosylated hemoglobin

Correlation among the Parameters:

TABLE 2: PEARSON CORRELATION COEFFICIENT AMONG THE BIOCHEMICAL PARAMETERS IN CASES

		Age	HbA1c	MDA	SOD
Age	Pearson Correlation	1	-.262	-.415**	.175
	Sig. (2-tailed)		.056	.002	.204
	N	54	54	54	54
HbA1c	Pearson Correlation	- .262	1	.625**	-.753**
	Sig. (2-tailed)	.56		.000	. 000
	N	54	54	54	54
MDA	Pearson Correlation	-.415**	.625**	1	-.455**
	Sig. (2-tailed)	.002	.000		.001
	N	54	54	54	54
SOD	Pearson Correlation	.175	-.753**	-.455**	1
	Sig. (2-tailed)	.204	.000	.001	
	N	54	54	54	54

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

Abbreviations: Age, Age distribution; MDA, Malondialdehyde; SOD, Superoxide dismutase; HbA1c, Glycosylated hemoglobin

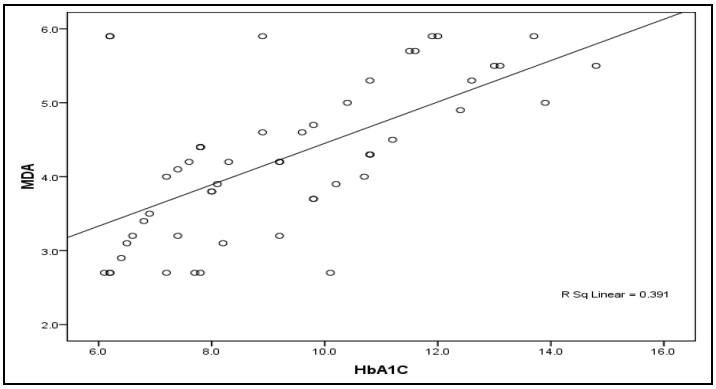

FIG. 2: SCATTER DIAGRAM SHOWING ASSOCIATION BETWEEN HbA1c AND MDA IN CASES

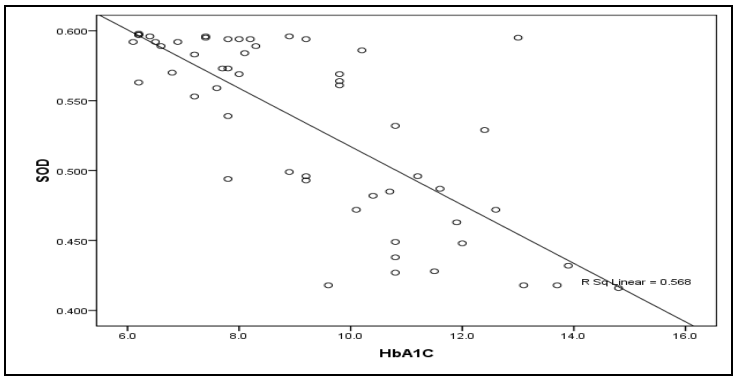

FIG. 3: SCATTER DIAGRAM SHOWING ASSOCIATION BETWEEN HBA1c AND SOD IN CASES

TABLE 3: PEARSON CORRELATION COEFFICIENT AMONG THE PARAMETERS IN CONTROLS

		Age	HbA1c	MDA	SOD
Age	Pearson Correlation	1	-.233	-.334*	.422**
	Sig. (2-tailed)		.090	.014	.001
	N	54	54	54	54
HbA1c	Pearson Correlation	-.233	1	.018	-.572**
	Sig. (2-tailed)	.090		.898	.000
	N	54	54	54	54
MDA	Pearson Correlation	-.334*	.018	1	-.053
	Sig. (2-tailed)	.014	.898		.704
	N	54	54	54	54
SOD	Pearson Correlation	.422**	-.572**	-.053	1
	Sig. (2-tailed)	.001	.000	.704	
	N	54	54	54	54

*. Correlation is significant at the 0.05 level (2-tailed).

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

Abbreviations: Age, Age distribution; MDA, Malondialdehyde; SOD, Superoxide dismutase; HbA1c, Glycosylated hemoglobin

HbA1c was association with SOD in controls: (Table 3 and Fig. 4)

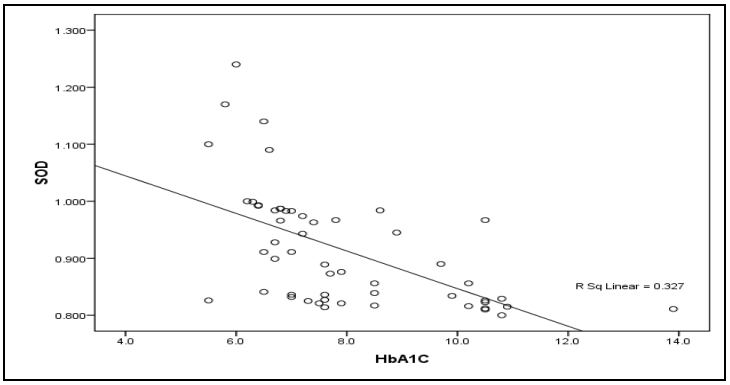

FIG. 4: SCATTER DIAGRAM SHOWING ASSOCIATION BETWEEN HbA1c AND SOD IN CONTROLS

DISCUSSION: Diabetic retinopathy (DR) is a vascular disorder affecting the microvasculature of the retina. It is estimated that diabetes mellitus affects 4 % of the world's population, almost half of whom have some degree of DR at any given time.[25] DR occurs both in type 1 and type 2 diabetes mellitus (DM) and has been shown that nearly all type 1 and 75 per cent of type 2 diabetes will develop DR after 15 yr duration of diabetes as shown in earlier epidemiological studies. [26]

Free radicals and oxidative stress are found to be responsible for the development of diabetic macroangiopathy and microangiopathy. Free radicals or reactive oxygen species (ROS) causes the oxidative stress which leads to development of diabetic retinopathy, so an imbalance due to increased production of reactive oxygen and as well as reduction in antioxidant defenses which alter cellular redox status. MDA is highly toxic compound formed by lipid peroxidation due to free radical damage. Many studies have shown increase in MDA levels in diabetic retinopathy correlating with poor glycemic control. In their study showed a significant increase in MDA levels in diabetic retinopathy cases when compared to diabetics without retinopathy controls. [27] A total number of 120 subjects out of which 40 were controls without type 2 DM and the rest 80 were type 2 DM patients were included in the study of those 80 diabetics, 44 patients did not have DR and 36 patients had DR. Serum MDA levels were found to be higher in diabetics as compared to controls (P = 0.00).

Superoxide dismutase (SOD) is the antioxidant enzyme that catalyses the dismutation of superoxide anion (O_2) into hydrogen peroxide and molecular oxygen.[28] SOD plays important protective roles against cellular and histological damages that are produced by ROS. It facilitates the conversion of superoxide radicals into hydrogen peroxide, and in the presence of other enzymes it converted into oxygen and water. [29] In the present study, SOD was significantly (p=0.0001*) lower among cases (0.53±0.07) as compared with controls (0.91±0.10). Scientists had also observed similar finding in which an inverse relationship was observed of HbA1c with superoxide dismutase (SOD) levels in type 2 diabetic retinopathy and diabetes without retinopathy groups. [30] Glycated hemoglobin (HbA1c) is the marker of both severity and long term control of the disease. It reflects the average level of blood glucose concentration over the preceding 6-8 weeks and is unaffected by diet, insulin therapy and other drugs. The values in our study are in accordance with several studies which have shown increase in HbA1c levels in diabetes retinopathy [31].

Their study found significant association with Diabetic retinopathy, hyperglycemia (as measured by HbA1c) is considered an important risk factor associated with diabetic retinopathy.[32] One of the most important risk factors for diabetic retinopathy, regardless of the type of diabetes, is how well the diabetes is controlled. This is often measured by levels of a glycated hemoglobin or HbA1c, which is representative of blood sugar levels over a 3 to 4 month period. In this study, HbA1c level was significantly (p=0.001) higher among cases (9.28±2.31) as compared with controls (7.95±1.77).

CONCLUSION: This study demonstrates significant abnormalities in diabetic retinopathy patients suffer from more oxidative stress. When compared, oxidative stress is still higher in diabetic patients with complications than patients without complications. Although other factors play an equally important role, if not more, in the pathogenesis of diabetic complications, oxidative stress plays a significant role in diabetes and its complications. This fact is to be kept in mind when planning strategies for prevention of complications of diabetes mellitus for better quality of life of diabetics. More extensive study is required the evaluation on oxidative damage risk factor for type 2 diabetes mellitus with retinopathy.

CONFLICT OF INTERESTS: Nil

SOURCE OF SUPPORT: Nil

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