Research Article

Evaluation of oxidative stress and protein bound sialic acid in diabetes with and without retinopathy

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Abstract

Background: The prevalence of diabetic retinopathy (DR) is 18% in urban Indian population, which is the leading cause of blindness. Hyperglycemia alters retinal blood flow by increasing oxidative stress leading to pericytes loss and basement membrane thickening of retinal vessels. The main objective was to evaluate oxidative stress, and protein bound sialic acid in DR and to explore possible interrelationship between these factors among DR patients. **Materials and Methods**: Fasting serum glucose (FBG), postprandial serum glucose (PPBG), total cholesterol (TC), triglycerides (TG), high density lipoproteins (HDL), low density lipoproteins (LDL), very low density lipoproteins (VLDL), malondialdehyde (MDA), protein bound sialic acid (PBSA) were measured in diabetic patients without retinopathy (DM) (n=40), with retinopathy (DR) (n=40) and normal healthy controls (n=40). **Results:** Serum MDA and PBSA levels were significantly higher in diabetic and diabetic retinopathy subjects as compared to controls. Correlation analysis study showed a significant positive association of MDA and PBSA with FBS, PPBS and also with themselves. **Conclusion**: Increase in oxidative stress due to hyperglycemia and dyslipidemia seems to be related to genesis of acute phase response marker, protein bound sialic acid in diabetic retinopathy.

Keywords: Oxidative stress, sialic acid, diabetic retinopathy.

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Diabetes mellitus (DM), a chronic disorder is now recognized as a global epidemic, with the prevalence expected to rise to 439 million worldwide[1] and 87 million in India by 2030[2]. Oxidative stress generated by long term uncontrolled and sustained hyperglycemia may damage the microvasculature of the retina, leading to diabetic retinopathy[3]. The prevalence of diabetic retinopathy (DMR) is estimated to be 17.6% in anurbanstudy in India[4] and 33.2% in a large population study in USA[5]. Since the retina is rich in polyunsaturated fatty acids and related to high oxygen uptake, lipid peroxidation is a likely consequence in diabetic retinopathy[6]. Chronic hyperglycemia builds up oxidative stress by various mechanisms such as advanced end glycation end products, protein kinase C activation, polyol and hexosamine pathway, that induce overproduction of superoxide in endothelium of retinal blood vessels[7]. Malondialdehyde (MDA) is a marker of oxidative stress induced lipid damage. Several studies have shown that the malondialdehyde levels were elevated in diabetic retinopathy[8,9]. Sialic acid (SA) is an N-acetylated derivative of neuraminic acid attached to the terminal position of oligosaccharide chain of glycoprotein and glycolipid. The biological functions of sialic acid include membrane stability, cell- cell recognition, membrane transport and provision of binding sites for ligands. Changes in total serum sialic acid levels (TSSA) have been observed in various diseases such as atherosclerosis, diabetes, renal disease, neurodegenerative disorders, alcoholism, cancer, and oral pathology[10]. Glycoprotein portion of SA was reported to protect proteins from reactive oxygen species (ROS)[11]. Oxidative stress mediated sialylation and desialylation of proteins

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have not been fully elucidated in diabetic retinopathy. Conflicting results of TSSA contents of different tissues in diabetes patients have been consistently observed; most of the investigators observed an increase[12,13] while others have observed no difference at all [14,15]. We are not aware of any studies in the literature showing the association of oxidative stress markers and protein bound sialic acid (PBSA) in diabetic retinopathy patients. Hence the present study was carried out to evaluate the oxidative stress and protein bound sialic acid in diabetes with and without retinopathy, and to study the correlation of these factors with the presence of retinopathy.

MATERIALS AND METHODS

The present case-control study was conducted in the Department of Biochemistry and Ophthalmology of our college, after obtaining clearance from Institutional Ethics committee (IEC) and confirmed to the tenets of the declaration of Helsinki. Forty known diabetics without retinopathy (DM), 40 diabetics with retinopathy (DMR) and 40 healthy controls were included in our study. Patients were selected from among those attending the ophthalmology outpatient department. Diabetes mellitus was diagnosed based on a history of being diagnosed as diabetes or being on anti-diabetic medications. Diabetic retinopathy was diagnosed based on fundus examination done by ophthalmologist.

Exclusion Criteria

Diabetic patients with other complication such as ischemic heart disease, nephropathy, neuropathy and infections were excluded from the study.

Fasting and postprandial blood samples were collected after obtaining informed consent. Serum was separated after centrifugation and aliquoted for further biochemical analysis.

Biochemical Analysis

Serum glucose was measured by glucose oxidase - peroxidase method. Serum total cholesterol was measured by cholesterol oxidase – peroxidase methodand triglycerides (TG) levels was measured by glycerol kinase– peroxidase method. High density lipoprotein (HDL) was estimated by divalent cation precipitation method. All these parameters were estimated by colorimetric techniques using reagent kits adapted to automated chemistry analyzer, Chemwell autoanalyzer, CPC Diagnostics. Very low density lipoproteins (VLDL) cholesterol level was calculated by dividing the triglycerides concentration by 5 and low density lipoproteins (LDL) was calculated using Friedwald's formulae [LDL=TC-(VLDL+HDL)[16].

Estimation of Malondialdehyde

Malondialdehyde (MDA) was measured using thiobarbituric acid (TBA) reactivity assay method[17].

Estimation of protein bound sialic acid

The protein bound sialic acid (PBSA) was determined by modified Aminoff's method using thiobarbituric acid[18].

STATISTICAL ANALYSIS

All the data were presented as mean ± standard deviation. ANOVA was used for comparing the parameters among the study groups. Correlation analysis was performed with Pearson's method. A 'p' value of < 0.05 was considered statistically significant.

RESULTS

Baseline characteristics of the study population are shown in Table 1. Serum levels of glucose in both fasting and postprandial samples were significantly higher in DM and DMR patients, compared to controls. Serum triglycerides levels were significantly increased in DM, whereas very low density levels showed a significant increase in DMR compared to controls. Serum total cholesterol, high density lipoproteins and low density lipoproteins were increased in DM and DMR subjects compared to controls but not statistically significant. Table 2 displays the stress variables in the study group. Serum malondialdehyde and protein bound sialic acid were found to be significantly increased in DM and DMR compared to controls. Correlation analysis of stress parameters with biochemical parameters in table 3 revealed a significant positive association of MDA and PBSA with serum glucose. Malondialdehyde was found to be positively correlated with protein bound sialic acid as shown in table 4.

Table 1: Demographic and Biochemicalcharacteristicsof the study groups

Variables	Controls (n=40)	DM (n=40)	DMR (n=40)
Age (years)	46±9	51±9	55±10
Duration of diabetes (years)	-	7±4	8±5
FBG (mg/dl)	84±7	173±80*	201±92*
PPBG (mg/dl)	110±12	271±114*	306±103*
TC (mg/dl)	170±24	180±41	172±47
TG (mg/dl)	85±16	203±133*	142±54
HDL (mg/dl)	37±4	38±5	37±6
LDL (mg/dl)	116±22	102±38	107±41
VLDL (mg/dl)	17±3	40±26	29±11*†

*p<0.05 by ANOVA in comparison with control, † p<0.05 by ANOVA in comparison with DM.

FBG –fasting blood glucose, PPBG –postprandial blood glucose, TC- total cholesterol, TG- triglycerides, HDL-high density lipoproteins, LDL- low density lipoproteins, VLDL- very low density lipoproteins

Table 2: Oxidative stress parameters study groups

Variables	Controls (n=40)	DM (n=40)	DMR (n=40)
MDA (µmol/L)	3.92±1.25	6.19±2.94*	6.06±2.78*
PBSA (µg/mg of protein)	10.30±3.20	24±18.14*	19.90±8.83*

*p<0.05 by ANOVA in comparison with control. MDA-malondialdehyde, PBSA-protein bound sialic acid

Table 3: Correlation analysis of lipid and stress parameters with serum glucose among the study groups

Variables	FBG		PPBG	
	r	p	r	p
TG	0.219	0.006	0.307	0.001
VLDL	0.281	0.012	0.314	0.026
MDA	0.343	0.005	0.357	0.001
PBSA	0.216	0.001	0.289	0.042

FBG –fasting blood glucose, PPBG –postprandial blood glucose, TG- triglycerides, VLDL- very low density lipoproteins, MDA-malondialdehyde, PBSA-protein bound sialic acid.
p value is calculated by Pearson's method. p<0.05 is considered statistically significant;

Table 4: Correlation analysis between Malondialdehyde and protein bound sialic acid in the study groups

Variables	MDA	
	r	p
PBSA	0.23	0.014

MDA-malondialdehyde, PBSA-protein bound sialic acid
'p' value is calculated by Pearson's method. A 'p'<0.05 is considered statistically significant.

DISCUSSION

Chronic hyperglycemia induced oxidative stress is associated with the development of diabetic complications. In our study, serum malondiladehyde and protein bound silaic acid levels were significantly increased in diabetes with and without retinopathy as compared to controls. In correlation analysis, serum MDA and PBSA levels revealed positive association with hyperglycemia. We also found a positive correlation between serum malondialdehyde and protein bound sialic acid. In the present study, serum triglycerides showed a significant increase in DM compared to controls, whereas very low density lipoprotein levels were significantly high in DMR compared to DM and controls. Though, the oxidative stress levels were raised in diabetes with and without retinopathy in our study, the two groups showed no significant difference of these levels. This may be either due to smaller sample size or due to lack of difference in the diabetic duration between the diabetes and diabetic retinopathy subjects. Studies which have shown a significance of serum MDA levels between DM and DMR, had a mean diabetic duration of more than 10 years[8,19]. Oxidative stress plays an important role in the development of microangiopathic complications of diabetes, although the mechanism of which is unclear. Kumari et al demonstrated a significant elevation of serum malondialdehyde levels in diabetes with and witout retinopathy as compared to controls. Hence our results suggest that increased lipid peroxidation may be a cause for the progression of microvascular complications of diabetes[8]. Serum sialic acid is a component of circulatory glycoproteins and a marker of the acute phase response. Sialic acid (SA) attached to glycoprotein portion was reported to protect proteins from ROS[20]. The association of total serum sialic acid (TSSA) in diabetes and its complications have been reported previously[21,22]. Nayak S et al showed significant increase in TSSA in diabetes with complications compared to diabetes and controls[23]. Crook et al,in his pilot project showed significant increase in total serum sialic acid in the diabetic patients with retinopathy[12]. In contrast, some studies have found no association between TSSA and diabetic retinopathy[14,15]. However to the best of our knowledge, there are no reports regarding the protein bound sialic acid in diabetic retinopathy. In the present study, we found a significant higher levels of protein bound sialic acid in DM and DMR on comparison with control group and additionally revealed positive correlation with FBG, PPBG and MDA. Although the mechanism by which the protein bound sialic acid levels are elevated in diabetic retinopathy is unclear, previous studies have demonstrated that the tissue injury due to diabetic vascular complications stimulates the production of acute phase proteins containing sialic acid.Other possible reason could be either due to increased sialylation or reduction in desialylation of plasma glycoproteins [12]. Dyslipidemiareduces the electron transfer through the electron transport chain (ETC), thereby increasing oxidative mitochondrial stress in diabetes mellitus and its complications. Our study revealed a significant elevation and positive correlation of TG and VLDL with both fasting and postprandial serum glucose. This result is consistent with previous reported studies[8,24]. Elevated TG and VLDL were related to the progression of natural antioxidant reserve reduction and increased reactive oxygen species (ROS)[25]. From the present study, it is suggested that the increase in oxidative stress due to chronic hyperglycemia and dyslipidemia in diabetes with and without retinopathy alters the microvasculature endothelium and may lead to the generation of protein bound sialic acid.

CONCLUSION

The present study demonstrates higher levels of Malondialdehyde and protein bound sialic acid in diabetes with and without retinopathy patients. The estimation of these parameters may serve as a tool in the early diagnosis and treatment for diabetic patients, prior to the development of retinopathy complication. However in order to reach a definitive conclusion, further studies are conducted with large sample size.

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