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OXIDATIVE STRESS AND HIGH SENSITIVITY C-REACTIVE PROTEIN IN DIABETIC RETINOPATHY

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ABSTRACT

Indians as an ethnic group have a higher risk of type 2 diabetes mellitus, most likely due to genetic susceptibility. Diabetic retinopathy is one of the most devastating microvascular complications of diabetes mellitus[1] and it is the most common cause of blindness. Oxidative stress plays an important role in the pathogenesis of microvascular and macrovascular complications of diabetes mellitus[2]. High sensitivity C-Reactive Protein (hsCRP) is a significant marker of inflammation and plays an important role in the pathogenesis of diabetic retinopathy. Fasting blood samples were collected from healthy controls, diabetics without retinopathy and with retinopathy. Serum malondialdehyde (MDA), vitamin C and vitamin E were estimated as a marker of oxidative stress. Serum hsCRP was estimated to validate its usefulness in gauging the severity of diabetic retinopathy. Our study showed a significant increase in serum MDA and decrease in serum antioxidant vitamins in diabetic retinopathy as compared to diabetics without retinopathy ($p<0.001$). Our study demonstrated increased serum hsCRP in diabetics with retinopathy as compared to those without retinopathy ($p<0.001$). Our study clearly indicates a close relationship between oxidative stress and diabetic retinopathy. Supplementation of anti-oxidants may prevent or delay onset of diabetic retinopathy. Increased hsCRP in diabetic retinopathy points to its usefulness in risk stratification.

KEY WORDS: **Diabetic retinopathy, Anti-oxidant vitamins, Malondialdehyde, hsCRP.**

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INTRODUCTION

Diabetic retinopathy is one of the most devastating microvascular complications of diabetes mellitus[1]. It is considered as the leading cause of adult onset blindness[3] and the prevalence of retinopathy is strongly related to the duration of diabetes mellitus. It is the most common cause of blindness in actively working population arising from a combination of microvascular leakage and occlusion. According to WHO, approximately 5 million individuals have diabetic retinopathy worldwide. By 2025, it is expected to increase to 150 million and 75% are expected to be from developing countries. Oxidative stress plays a key role in the pathogenesis of diabetic retinopathy. The study was conducted to assess the extent of oxidative stress in these patients, which gives information regarding the therapeutic use of anti-oxidants to prevent or delay the onset of diabetic retinopathy. Inflammation is a prominent feature seen in diabetic retinopathy. Serum hsCRP was used as a marker of inflammation to look for any significant relation with diabetic retinopathy.

MATERIALS AND METHODS

Present study comprised of 150 patients out of whom 50 were healthy controls, 50 clinically diagnosed diabetics without retinopathy and 50 diabetics with retinopathy. Study was conducted at Navodaya Medical College Hospital and Research Centre, Raichur from May 2009 to May 2010. Patients attending outpatient department and those admitted were included in the study. The patients and controls voluntarily participated in the study. Approval of ethical committee was taken. Inclusion criteria: 1) Clinically diagnosed diabetics without retinopathy (Group I) and another group consisting of diabetic retinopathy (Group II). Group II was further classified based on severity of diabetic retinopathy into Group IIA (background and non-proliferative diabetic retinopathy) and Group IIB (proliferative diabetic retinopathy and advanced eye disease). Diabetes was diagnosed based on fasting plasma glucose ≥ 126 mg/dL as per WHO diagnostic criteria. 2) Age and sex matched controls included in the study were those with FPG < 100 mg/dL with no past history of diabetes. Exclusion criteria: 1) Juvenile diabetics 2) Diabetics with renal and cardiovascular complications 3) Acute or chronic inflammatory conditions 4) Metabolic ketoacidosis 5) Cerebrovascular accidents 6) Hypertension 7) Alcoholics and smokers. 6ml of blood was drawn in fasting state under asceptic precautions from cubital vein and serum was separated by centrifugation. Oxidative stress was measured by estimating serum levels of MDA, vitamin C and vitamin E. Serum MDA was estimated using Thiobarbituric acid method[4], serum Vitamin E by Baker and Frank method[5] (reference range: 6-19 mg/L) and serum vitamin C by 2,4 Dinitrophenylhydrazine method[6] (reference range: 0.4-1.5 mg/dL). Serum hsCRP was estimated by latex high sensitivity method[7] (Biosystems).

STATISTICAL ANALYSIS

One way ANOVA was used to test the difference between the three groups. Further analysis between the two groups was done using Schefee test. A 'p' value of 0.05 or less was considered as indicating statistical significance.

RESULTS

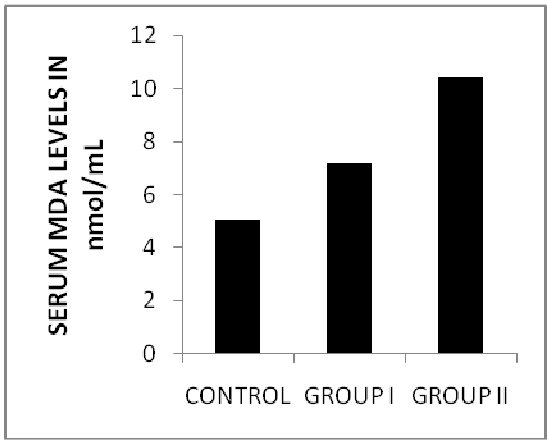
The results are shown in Table 1. Our study showed a significant increase in serum MDA in Group II in comparison to Group I ($p<0.001$) as shown in Graph 1. Significant decrease in anti-oxidant vitamins E, C were observed in Group II compared to Group I ($p<0.001$) as shown in Graph 2 and 3. An increased hsCRP was noted in Group II compared to Group I ($p<0.001$) as shown in Graph 4. Serum hsCRP was increased in Group IIB compared to Group IIA (table 2).

TABLE 1
Mean serum values of MDA, vitamin E, vitamin C and hsCRP.

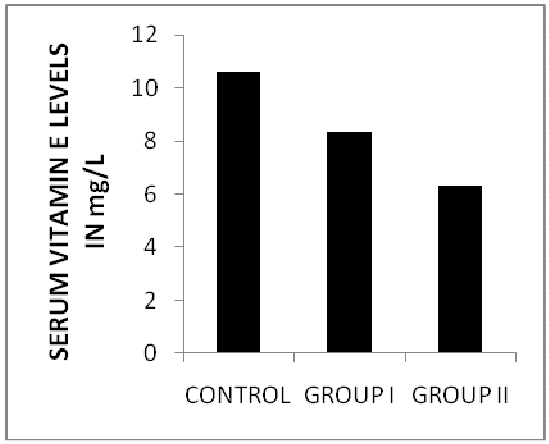
		n	Mean MDA (nmol/mL)	Mean vitamin E (mg/L)	Mean vitamin C (mg/dL)	Mean hsCRP (mg/L)
Control		50	5.08±0.64	10.63±1.38	1.2±0.09	5.89±1.51
Group I		50	7.18±1.25	8.32±0.89	1.03±0.11	10.17±2.09
Group II		50	10.39±0.9	6.30±0.59	0.46±0.03	11.24±2.02
ANOVA		F	383.8	229.3	975.5	111.3
		p	< 0.001	< 0.001	< 0.001	< 0.001
Comparison between different groups	Control vs Group I	p	< 0.001	< 0.001	< 0.001	< 0.001
	Control vs Group II	p	< 0.001	< 0.001	< 0.001	< 0.001
	Group I vs Group II	p	< 0.001	< 0.001	< 0.001	< 0.001

p<0.05 indicates statistical significance

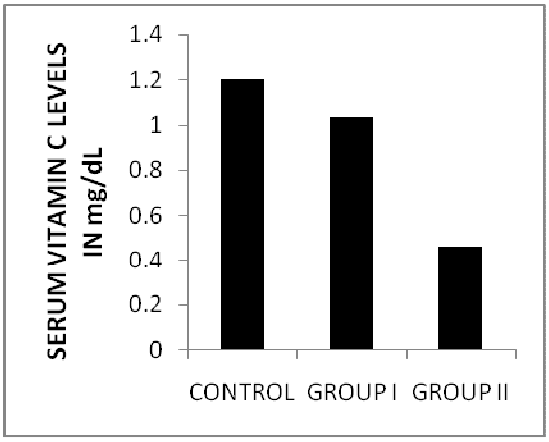
GRAPH 1
Serum MDA levels



GRAPH 2
Serum vitamin E levels



GRAPH 3: ***Serum vitamin C levels***



GRAPH 4: ***Serum hsCRP levels***

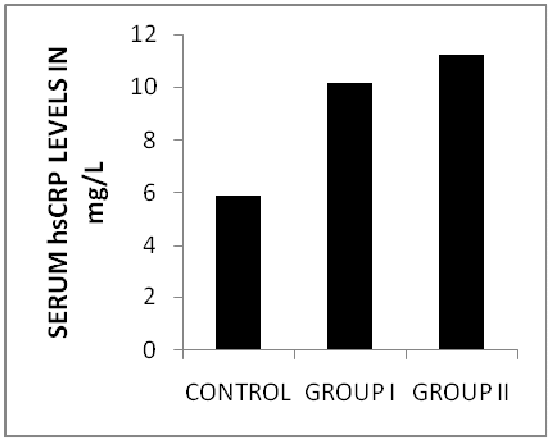


TABLE 2
Serum hsCRP in Group IIA and Group IIB

	hsCRP (mg/L)
Group IIA (n=40)	10.63±1.9
Group IIB (n=10)	11.39±2.4

DISCUSSION

In our study, a significant rise in serum MDA levels and a significant fall in vitamin E and vitamin C levels were seen in diabetics as compared to controls. A similar significant difference was seen in those with retinopathy as compared to those without retinopathy. The significant fall in vitamin E and vitamin C explains their utilization in the scavenging process. Chronic hyperglycemia and increased glycation of proteins correlate with development of retinopathy in diabetics. Our study also showed a significant negative correlation between MDA and vitamins E and C in Group I and Group II. Our findings are in agreement with various other studies done on oxidative stress. In 2004, Polak M and Zagorski showed a significant rise in serum MDA in diabetic retinopathy as compared to healthy controls as well as diabetics without retinopathy[8]. In 2005, Madhur M Gupta and Suresh Chari showed in their study a significant increase in MDA, while a significant decrease was noted in superoxide dismutase and vitamin C levels in diabetics as compared to controls. Similar findings were observed in diabetics with PDR as compared to controls and diabetics without complications[9]. In 2008, Hong Zhi Pan et al showed in their study a significant increase in MDA levels in diabetics as compared to controls, which was more pronounced in those with diabetic retinopathy[10]. In 2008, S Kumari et al showed that plasma MDA was markedly increased with significant decrease in vitamin E and vitamin C in diabetic groups compared to controls and was more distinct in those with diabetic retinopathy. Correlation study revealed a significant negative correlation between plasma MDA and vitamins E, C[11]. Our study showed a significant elevation in serum hsCRP levels in cases as compared to controls. Serum hsCRP rise in those with retinopathy was highly significant as compared to diabetics without retinopathy. In 2005, Kang ES et al showed no difference between serum hsCRP levels of those with and without retinopathy[12]. Muni RH et al demonstrated that increasing quintiles of baseline hsCRP level may be associated with higher risk of clinically significant macular oedema in the DCCT

cohort[13]. Another study showed that increased serum hsCRP was one of the independent risk factors for the development of retinopathy in patients with type 2 diabetes mellitus[14].

CONCLUSION

Our study clearly portrays the role of oxidative stress in the development of retinopathy in diabetic patients. Thus the use of antioxidants in diabetics may delay the onset or progression of retinopathy as evidenced by several studies[9,15,16]. Our study showed a significant increase in serum hsCRP levels in those with diabetic retinopathy compared to diabetics without retinopathy. This explains the possible role of hsCRP in the risk stratification of diabetic retinopathy as it does in the risk stratification of cardiovascular diseases. However, in order to reach a definitive conclusion, a study with a larger population size is required.

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