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Vitamin E and oxidative stress in early diabetic retinopathy

KEYWORDS: Vitamin E, Oxidative stress, Diabetic Retinopathy

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ABSTRACT

Introduction: Diabetic Retinopathy is one of the major causes of blindness. It is a chronic micro vascular complication of long standing Diabetes Mellitus. Other than duration of diabetes and glycemic status, oxidative stress is also being considered as a causative factor of diabetic retinopathy nowadays. This study aims to find out whether supplementation of vitamin E reduces the oxidative stress in paients having early diabetic etinoathy.

Methods: 49 patients having early diabetic retinopathy were selected for study. HbA1c and catalase activity were estimated in them using washed red blood cells and estimation of superoxide dismutase activity, malondialdehyde and tocopherol levels were done in serum in all these patients. 27 of them were randomly selected and given vitamin E 200mg orally for 6 months in addition to their oral hypoglycemic agents. 22 patients were studied as controls. All the parameters were repeated after 6 months.

Results: HbA1c and MDA levels were found to decrease in cases which was statistically significant. SOD and catalase activities showed statistically insignificant decrease in cases than controls. Vitamin E level was done to ensure patient compliance.

Conclusion: Vitamin E supplementation to patients with early diabetic retinopathy brought about a reduction in HbA1c level. There was a reduction in MDA level also in those patients. There was no change in the activities of superoxide dismutase or catalase activities.

INTRODUCTION

Diabetic Retinopathy is one of the major causes of blindness in our population. It is a chronic micro vascular complication of long standing Diabetes Mellitus. Development of Diabetic Retinopathy in a diabetic patient depends mainly on two factors.

Duration of Diabetes: -

95% of patients having Type I Diabetes Mellitus for 20 – 30 years have diabetic retinopathy. Similarly in patients having Type II Diabetes Mellitus for more than 16 years, 64% have Non-Proliferative Diabetic Retinopathy & 10% have Proliferative Diabetic Retinopathy[1]. Even though long standing hyperglycemia is found to result in retinopathy, euglycemic control of blood sugar is not found to prevent development of retinopathy[2]. In addition to duration of diabetes and glycemic state, oxidative stress is also implicated in the pathogenesis of diabetic retinopathy[3,4,5]. Oxidative stress could be considered as the causative factor in cases where proper glycemic control does not prevent the the development of diabetic retinopathy.

Diabetic retinopathy is asymptomatic in early stages. Symptoms like diminished vision present mainly when macula is involved. In most cases, mild diminution in vision is attributed by the patients to their advancing age and medical advice is sought only in late stages. In the retina, diabetes produces a series of slow changes leading to features like micro aneurysms, hard exudates, retinal edema, dot and blot hemorrhages, cotton wool spots, neo vascularization, vitreous hemorrhages, retinal detachment etc. which can be detected by careful examination of the fundus. According to the presence of these features, Diabetic retinopathy is classified into different stages namely,

- Back ground retinopathy
- Non-Proliferative retinopathy – Mild --Moderate --Smevere
- Proliferative retinopathy

Once fully developed, the changes of diabetic retinopathy are irreversible and even maintenance of total euglycemic state is found to be ineffective in treatment of diabetic retinopathy [1,6].

In a pilot study conducted earlier by us, it was found that oxidative stress was directly related to the severity of diabetic retinopathy, irrespective of the duration of diabetes mellitus or glycemic state[7]. The results of this study suggested that, in patients having diabetes mellitus antioxidants might have a prophylactic role in preventing the development of retinopathy. Vitamin E is the major natural antioxidant in the body that can also be supplemented orally. Moreover there are other studies that prove the effectiveness of Vitamin E in controlling other vascular complications of Diabetes mellitus[3].

These facts prompted us to conduct a study to assess the effectiveness of Vitamin E in alleviating the oxidative stress in Diabetic Retinopathy.

OBJECTIVES

1. To assess the anti oxidant status in patients with early diabetic retinopathy.
2. To assess whether vitamin E has beneficial .effect in reducing the oxidative stress in these patients.

METHODS

Patients attending the out – patient department of Ophthalmology and Diabetic clinic of Calicut Medical College were selected for study. Individuals of age 18 – 60 diagnosed to have early diabetic retinopathy were selected. Patients clinically diagnosed to have coronary artery disease; renal disease, hypertension, hyperthyroidis m, malignancies, acute infections, and rheumatoid arthritis were excluded from study, as these conditions are well known to accentuate oxidative stress. Pregnant and lactating women, chronic smokers and alcoholics also were excluded from study.

After selection, each patient was randomly allocated to case or control group by drawing lots. Those patients coming under case group received Vitamin E 200 mg. tablets orally for 6 months in addition to the Oral Hypoglycemic agents (OHA) they were on. Those patients coming under control group received only OHA as treatment.

Heparinized and plain blood samples were collected from each patient by venepuncture under aseptic precautions after getting

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informed consent. Serum was used for estimating levels of MDA, vitamin E and superoxide dismutase activity. Hemolysate prepared was used for estimating HbA1c and catalase activity.

Chemicals of analar quality obtained from Sigma, BDH and E Merck were used for various estimations. CE certified kit manufactured by Monozyme India ltd. Was used for estimation of HbA1c.

1.Estimation of HbA_{1c}

Principle

Hemolysate prepared was subjected to ion exchange chromatography by passing through readymade columns containing cationic exchange resin. Hemoglobin A_{1c} is specifically eluted after washing away the HbA_{1a+b} fractions using suitable buffers supplied in the kit. Hb A_{1c} is quantified from the elute by reading the optical density at 415nm.

Calculation - $\%\ HbA1c = \frac{AHbA1c}{3 \times AHbTotal} \times 100$

Where AHbA1c = absorbance of the HbA1c fraction at 415 nm
AHbTotal = absorbance of hemolysate

2. Estimation of Catalase

The hemoglobin content of blood was determined and adjusted to 5 g/100 ml.

PRINCIPLE

The decomposition of hydrogen peroxide of specific concentration is followed colourimetrically by decrease in extinction at 240 nm. The decrease in extinction (ΔE_{240}) per unit time is a measure of catalase activity[8].

CALCULATION

Rate constant $K = \frac{2.3 \times \log (E1-E2)}{15}$

Where, 2.3 = factor
15 = time (in seconds for which decrease in extinction is read)
E_1 = extinction at 0 sec.
E_2 = extinction at 15 sec.

3.Estimation of Malondialdehyde

The thiobarbituric acid method based on Valipasha and Sadasivudu's procedure was used to estimate malondialdehyde level in serum[9]. Malondialdehyde can react with thiobarbituric acid to generate a colored product. In acidic solutions, the product absorbs light at 530 nm. The molar extinction coefficient of malondialdehyde – thiobarbituric acid product at 530 nm is used to calculate the amount of malondialdehyde formed.

CALCULATION

E = KCL
$C = \frac{E}{KxL}$ nmoles/dl.
K = Molar extinction coefficient = 1.5×10^5
E = Extinction / absorbance
C = concentration in moles/l
L = length of cuvette used = 1 cm.

4. Estimation of Superoxide dismutase

Serum Superoxide dismutase activity was measured by the method suggested by Marklund and Marklund and modified by Nandi et al[10]. This method utilizes the inhibition of autoxidation of pyrogallol by superoxide dismutase.

Calculation

Units of SOD/ 3 ml of assay mixture = $\frac{(A-B) \times 100}{A \times 50}$

Absorbance of control – A
Absorbance of sample – B

Definition of unit = One unit of superoxide dismutase is the amount of enzyme required to cause 50 % inhibition of pyrogallol autoxidation per 3 ml of assay mixture.

Units x 10 = units/ ml of sample solution

5. Estimation of serum Tocopherol:-[11]

Serum tocopherols can be measured by their reduction of ferric to ferrous ions, which then form a red complex with 2, 2' dipyridyl. Tocopherols and carotenes are first extracted into xylene and the absorbance is read at 460nm to measure the carotenes. A correction for carotenes is made after adding ferric chloride and reading at 520nm.

Calculation

S. Tocopherol mg/l = $\frac{A' \text{ of unknown}}{A' \text{ of standard}} \times 10$ where, $A' = A^{520} - 0.29 \times A^{460}$

In addition to the biochemical parameters mentioned above detailed Ophthalmologic examination was done for each patient and the findings were recorded.

Patients were given Vitamin E 200mg soft gels for 6 months along with their AHAs. After 6 months, patients were reviewed and the same biochemical parameters were again estimated.The results were analysed statistically using paired t test. p value less than .05 was considered significant.

RESULTS:

Baseline parameters of cases and controls were comparable. Base line data of cases and controls are given below. Table (1)

Table - 1

	Cases	Controls
Age (years)	49.26	51.86
Males (No)	17	11
Females (No.)	10	11
Duration of diabetes (years)	14.2	11.27
Treatment		
insulin	12	9
Oral hypoglycemic agents	14	12
Both	1	0
Others	0	1

HbA_1c levels of cases were found to decrease after 6 months. For controls, it was found to increase as given in table 2.This was found to be statistically significant.

Table -2
HbA_1c levels of cases and controls in %

	Initial value	Value after 6 months	Mean difference	P value
Cases	11.53	9.51	-2.459	.005*
Controls	10.67	11.42	.4918	

Mean MDA level was also found to decrease in cases after 6 months. For controls it was not so. Here also, the difference was statistically significant as given in table - 3

Table –3
Mean MDA levels of cases & controls in nmol/100ml

	Mean initial MDA con.	Mean MDA con. after 6 mths	Mean difference	p value
Cases	97.53	73.01	-27.04	.016
Controls	76.03	85.31	6.01	

The reduction in the mean MDA level of cases after 6 months of Vitamin E was found to be very significant statistically when compared to the values of control group Table (3).

Volume - 7 | Issue - 4 | April-2017 | ISSN - 2249-555X | IF : 4.894 | IC Value : 79.96

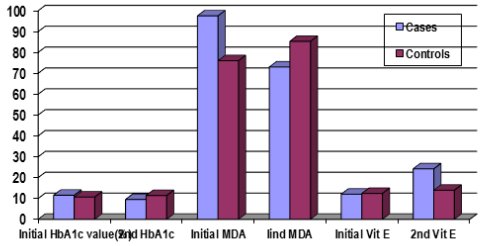
Mean vitamin E levels showed increase after 6 months in cases. The slight increase in value shown by controls was not statistically significant as give in table - 4.

Table – 4
Mean vitamin E levels of cases & controls in mg/Ltr

	Mean initial vitamin E con.	Mean Vit E con after 6 mths	Mean difference	p value
Cases	12.04	24.19	12.25	.000*
Controls	12.406	13.943	1.6	

This was done with the objective of confirming patient compliance in this study.

Mean HbA_1c, MDA and Vitamin E levels of cases & controls



Mean SOD level was found to decrease after 6 months for both cases and controls and the change in serum level was not found to be significant on statistical analysis Table - 5.

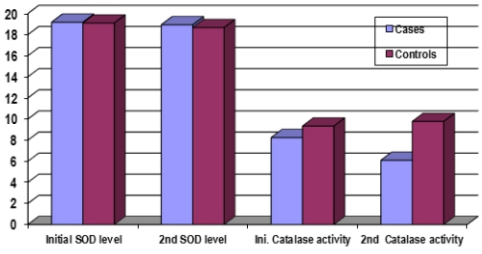
Table – 5
Mean SOD levels of cases & Controls in units/ml

	Mean initial SOD level	Mean SOD level after 6mths	Mean difference	p value
Cases	19.17	18.93	-045	.872*
Controls	19.09	18.66	.38	

Mean catalase activity was found to decrease after 6 months in cases but it was not statistically significant as given in table - 6.

Table – 6
Mean catalase activity of cases and controls in K/ml of blood

	Mean catalase activity initially	Mean catalase activity after 6 mths	Mean difference	p value
Cases	8.24	6.09	-2.64	.378
Controls	9.33	9.77	-.47	



Discussion:
Many studies have been done to analyze the role of oxidative stress in diabetic retinopathy.

Greismacher A et al[12] studied the relationship among HbA_{1c}, lipid peroxidation measured as TBARS and free vitamin E in 158 patients. They found that compared with controls, serum levels of TBARS were found to be significantly elevated in diabetic patients. Diabetic patients with good metabolic control had (HbA_1C < 6.5%) significantly lower TBARS than those with poor metabolic control. They have suggested that enhanced lipid peroxidation is contributed to an increased formation of free radicals in diabetes mellitus.

Memisogullari P et al[13] have suggested that antioxidant deficiency and excessive peroxide mediated damage may appear in NIDDM. Matsumoto et al[14] studied the generation of superoxide in streptozocin induced diabetic mice and they also demonstrated a reduction of superoxide dismutase in these mice. Bae SC et al[15] found that rheumatoid arthritis patients showed significantly lower level of activity of plasma superoxide dismutase and glutathione peroxidase when compared with controls. Roussal AM et al[16] studied antioxidant effects of Zn supplementation in Tunisians with type 2 DMand found that after 6 months of Zn administration, there was a decrease in plasma TBARS level (15%) while there was no significant change in TBARS level in placebo group. Supplementation did not alter significantly HbA_{1C} levels.

In this study, the antioxidant effect of Vitamin E supplementation showed results similar to these studies. On administration of Vitamin E 200mg orally daily for 6 months, there was a significant reduction in MDA level in case group when compared to control group.

Similarly, Anwar MM et al[17] have demonstrated that garlic oil or melatonin may effectively normalize the impaired anti oxidant status in streptozocin induced diabetes.

In a study, Palanduz S et al[18] investigated the relationship between plasma oxidants and antioxidants in diabetes mellitus. They have suggested that replenishment by antioxidants when necessary may be useful in the prevention of diabetic complications.

Koya D et al[3] have summarized that oxidative stress by diabetes could play a crucial role in the development and progression of diabetic nephropathy and antioxidant treatment could be a potential therapeutic procedure for diabetic nephropathy. As diabetic retinopathy and nephropathy are both microvascular complications of diabetes, the same results can be applied to diabetic retinopathy also. Kuznetsov NS et al[19] showed a favourable effect of alpha tocopherol acetate on plasma lipid peroxidation and total lipid levels in diabetes. S.N.Chugh et al[4] evaluated oxidative stress in 50 patients (10 IDDM & 40 NIDDM) of recently diagnosed diabetes mellitus. And found that after 4 weeks of Vitamin E supplementation (400mg daily), further fall in MDA and rise in glutathione suggested beneficial effect of vitamin E over and above optimal blood sugar control. Normalisation or near normalization was not achieved with vitamin E therapy indicating persistence of oxidative stress.

The significant rise in Vitamin E level in cases in our study can be taken as an indication of patient compliance. Vitamin E levels were more or less same before and after 6 months in the case of control group.

Absence of significant charge in SOD and catalase activities in our study could be due to inadequate dosage (200mg daily) of vitamin E given in our study. Other similar studies were done using higher doses. Kuznetsov et al[19] gave 300mg alpha tocopherol daily to get a favourable result.Balabolkin et al[20] prescribed Vitamin E in daily dose of 600 and 200mg. S.N.Chugh et al gave Vitamin E 400mg daily for assessing its antioxidant effect. A study conducted by Daga MK et al[21] on the effects of exogenous vitamin E in COPD patients showed results similar to this study.

Detailed studies including more patients and longer duration is necessary to find out the real relationship between antioxidants, free radicals and cytoprotective enzymes.

Moreover from the interrelationship between cytoprotective enzymes, antioxidants and free radicals, it is clear that action of tocopherols is mainly in checking the effects of free radicals like lipid peroxidation which occurs late in the chain of free radical induced

Volume - 7 | Issue - 4 | April-2017 | ISSN - 2249-555X | IF : 4.894 | IC Value : 79.96

reactions. They do not affect the synthesis of ROS or hydrogen peroxide which are the earliest reactions in the chain of reactions.[16] Thus, because the levels of SOD and catalase were not changed after vitamin E supplementation, we can assume that free radical generation was unaffected and the effects of free radical induced damages was reduced as a result of vitamin E supplementation in the subjects studied.

CONCLUSION

1. Vitamin E supplementation to patients with early diabetic retinopathy brought about a reduction in HbA_1c level.
2. There was a reduction in MDA level also in those patients
3. There was no change in the activities of superoxide dismutase or catalase

REFERENCES

1. Gyanam G.Murthy, Diabetic Retinopathy – its relation to blindness. Ophthalmology, Vol.IV, No.1 : January-February, 2003.
2. Jack K.Kauski. Retinal vascular diseases in clinical ophthalmology. Clinical Ophthalmology, 5th Ed., 2003; p.439.
3. Koya D, Hayashi K, Kitada M, Kashiwagi A, Kikkawa R, Haneda M. Effects of antioxidants in diabetes-induced oxidative stress in the glomeruli of diabetic rats. 120: J Am Soc Nephrol. 2003; Aug. 14 (8 Suppl.3) S 250-3.
4. Chugh SN, Kakkar R, Kalra S, Anjali Sharma – an evaluation of oxidative stress in DM during uncontrolled and controlled state and after Vit E supplementation. JAPI 1999; Vol.47: No.4, 380-383.
5. Ashok Kumar, Rajagopal G. Lipid peroxidation in erythrocytes of patients with type 2 diabetes mellitus. Indian Journal of Clinical Biochemistry 2003; 18(1): 71-74.
6. Robert N.Frank. Etiologic mechanisms in diabetic retinopathy. Retina. Edited by Andrew P, Schachat MD, Robert P Murphy MD. Vol.2, II Ed. Pg.1246.
7. Dr.Padma B.Prabhu MBBS, DO; Dr.Nimi Bharathan, MBBS, Dr.Geetha Damodaran MD Biochemistry, Dr.Parvathy K et al. Oxidative stress in diabetic retinopathy.
8. Nishchal HK, Sharma MP, Gojal RK, Kaushik GG. Serum superoxide dismutase levels in diabetes mellitus with or without microangiopathic complications. JAPI; 1998, Vol.46, No.10.
9. Screening for Diabetic Retinopathy. Diabetic Retinopathy, Vol.24. Supplement 1. American Diabetes Association. Clinical Practice Recommendations, 2001.
10. Mark lund S, Mark lund G (Modified by Nani et al, 1988). Assay of superoxide dismutase activity in animal tissue. J. Biochem 1988, 12(3): 305 – 15.
11. Harnett ME, Stratton RD, Browne RW, Rosner BA, Lanham RJ, Armstrong D. Serum markers of oxidative stress and severity of diabetic retinopathy. Diabetes Care, 2000 Feb. 23 (2): 234-40.
12. Greismacher A, KindHanser M, Andert SE, Schreiner W, Toma C, Knoebi P, Peitschman P, Prager R, Schnack C, Scheruthaner G et al. Enhanced serum level of TBARS in diabetes mellitus. American Journal of Medicine, 1995; May 98 (5): 469-75.
13. Menisogullari R, Jaysi S, Bakan E, Capoglu I. Antioxidant status status and lipid peroxidation in type II D.M. Cell Biochem Funct. 2003; Sep. 21(3): 291-6.
14. Matsumoto S, Koshiishi I, Inoguchi T, Naiwata H, Utsumi H. Confirmation of so generation in a xanthine oxidase in Streptozocin induced diabetic mice. Free radical research. 2003 July; 37 (7): 767-72.
15. Bae SC, Kim SJ, Sung MK. Inadequate antioxidant nutrient intake and altered plasma antioxidant status of Rheumatoid Arthritis Patients. J Am Coll Nutr. 2003; Aug 22(4): 311-5.
16. Rouseel AM, Kerkein A, Zonari N, Mahjoub s, Matheau JM, Anderson RA. Antioxidant effects of Zn supplementation in Tunisians with type 2 DM. J Am Coll Nutr. 2003 Aug. 22 (4): 316-21.
17. Anwar MM, Meki AR. Oxidative stress in streptozocin induced diabetic rats : effects of garlic oil and melatonin. Comp Biochem Physiol Amol Integr Physiol. 2003; Aug. 135 (4): 539-547.
18. Palanduz S, Ademoghi E, Gokkusu C, Tamer S. Plasma antioxidants and type II DM. Res. Comm. Mol. Pathol. Pharmacol. 2001; 109 (5-6): 309-18.
19. Kuznetsov NS, Abulela AM, Neskoromnyi VN. The use of antioxidants (alpha stoeopherol acetate) in the treatment of diabetic mellitus. Probl. Endocrinol – Mork 1993 Mar-Apri; 39 (2): 9-11.
20. Balabolkin MI, Mikhailoma EV, Kniazena AP, Pankova SS. Effect of high doses of tocopherol on the processes of lipid peroxidations and insulin secretion in patients with NIIDM- Probl-Endokrinol-Mark, 1994; May June; 40(3): 10-2.
21. Daga MK, Chhabra R, Sharma B, Mishra TK, Effects of exogeneous vitamin E supplementation on the levels of oxidants and antioxidants in chornic obstructive pulmonary disease.