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ALPHA TOCOPHEROL, OXIDATIVE STRESS AND DIABETIC VASCULAR COMPLICATIONS

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ABSTRACT

Vascular complication with long time duration represents the main cause of morbidity and mortality in diabetic patients. Total 125 subjects were enrolled for the study. In study group of Diabetic patients were randomly given 400 mg of vitamin E once a day in a blind manner out of 95 patients. The present study was to investigate the role of alpha tocopherol and oxidative stress in diabetic vascular complications patients. Free radical determination was done by estimation of SOD, Catalase and MDA. In present study significant high units of catalase and MDA and lower units of SOD were found in patient .Alpha tocopherol administration results in faster reduction of oxidative stress. Antioxidant vitamin such as vitamin E might help in prevention of vascular complications in diabetes.

KEY WORDS:- Vitamin E, MDA, Catalase, SOD and Diabetes

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INTRODUCTION

Long term vascular complication still represents the main cause of morbidity and mortality in diabetic patients. Cardiovascular disease is the leading cause of mortality in the patients with diabetes[1]. Myocardial infarction and stroke constitute the cause of death in as many as 80% of subjects with NIDDM[2]. Diabetic microangiopathy still represents one of the main causes of blindness, terminal renal failure and amputation. Although prospective randomized long-term clinical studies comparing the effects of conventional and intensive therapy have demonstrated a clear link between diabetic hyperglycemia and the development of secondary complication of diabetes, they have not defined the mechanism through which excess glucose results in tissue damage . Evidence has accumulated indicating that the generation of reactive oxygen species (oxdative stress) may play an important role in the etiology of diabetic complications. This hypothesis is supported by evidence that many biochemical pathways strictly associated with hyperglycemia (glucose autoxidation,polyol pathway, prostaniod synthesis, protein glycation) can increase the productin of free radicals. Furthermore, exposure of endothelial cells to high glucose leads to augmented production of superoxide anion. Which may Quench Nitricoxide, a potent endothelium-derived vasodilator that participates in the general haemostasis of the vasculature[3].

Free radicals are atoms or molecules that have one or more unpaired electrons in their atomic structures and are highly reactive. the susceptibility of a given organ or organ system to oxidative stress is a function of the balance between pro-oxidant factors and those that scavenge them. Oxidative damage can therefore be the consequence of raised free radical production, insufficient antioxidant potential or both[4]. The non enzymatic free radical- mediated oxidation of biological molecules membranes, and tissues are associated with a variety of pathological events: Cancer[5]. Agenig[6], atheroscleriosis[7], inflammation[8], injuries due to oxygen toxicity and, as recently suggested, diabetes[9].

In further support of the consequential injurious role of oxidative stress, many of the advance effects of high glucose on endothelial functions, such as reduced endothelial-dependent relaxation and delayed cell replication, are reversed by antioxidants. A rational extension of this proposed role for oxidative stress is the suggestion that the different susceptibility of diabetic patients to microvascular and macrovascular complication may be a function of the endogenous antioxidant status. A number of cellular antioxidative substances protect The cell from potentially harmful effects of oxy-free radicals[10]. These substances (superoxide dismutase, Catalase, and glutathione peroxidise) are free radical scavenging enzymes and serve as the first line of cellular defence against oxidative injury .The other line of defence consist of nonenzymetic endogenous or exogenous scavengers such as Vitamin E (alpha-tocopherol), Ascorbate, Vitamin A, and sufhydryl compound[11,12].

Over the past decade, there has been increasing scientific and publish intrest in the so- called antioxidant hypothesis of atherogenesis and in the theoretical possibility that antioxidant vitamins might help in prevention of cardiovascular disease observational epidemiological data have suggested that individuals with a high intake of antioxidant vitamins (such as beta-carotene and vitamin E) have some what lower than average risk of cardiovascular disease[13,14,15]. Although the theoretical basis exist to reduce oxidative stress in diadetic patient, there are no reported studies examining the effects of antioxidants on cardiovascular events and mortality in diabetes.

Vitamin E (alpha tocopherol) is a fat soluble naturally occurring antioxidant present in the cell membrane , it acts ad free radical scavenger by breaking peroxylradical chain reaction of membrane lipids[16]. With the above background knowledge we studies the status of oxidative stress by measuring the level of *S.O.D , Catalase, malonyldialdehde* which is one of many products of lipid peroxidation, in plasma and platelets of Diabetic patients, Furthermore, we analysed the effect of oral vitamin E administration on altered oxy-free radicals in diabetic patients.

PATIENTS AND METHODS

Two groups of patients were studied: (1) a study group which include 40 IDDM patients, 25 NIDDM patients, and 30 IDDM with vascular complication (14 patients with proteinurea, 8 with retinopathy, 4 having nonhealing ulcer. (2) a control group which included 30 age and sex matched healthy non diabetic volunteers. The younger age group was used as controls for IDDM cases and older age group for NIDDM cases .Informal consent and ethical committee approval were obtained. All IDDM patients had diabetes for more than 10 years, NIDDM patients had diabetes for 1.5 to 13 years. Diabetes was diagnosed by fasting plasma glucose and post prandial plasma glucose level. IDDM and NIDDM cases were differentiated of the basis of therapeutic history. All cases, after routine investigation (Hematocrit, blood sugar, blood urea nitrogen, serum cholesterol, electrocardiogram) were subjected to specific investigations (platelets and plasma levels of SOD, CATALASE AND MDA).

COLLECTION OF SAMPLES AND PREPRATION OF PLAIELET RICH PLASMA

Blood samples were taken in the study group for specific investigations on the Ist, 4th and 10th day of admission and in the control group only on day one. 9 ml venous blood was withdrawn and mixed with 1 ml of 3.8% sodium citrate solution, was transferred into plastic tubes and centrifuge at 400 g for 20 min . Platelet poor plasma was aspirated out and platelet was collected and suitable diluted with sodium citrate to obtain platelet rich plasma (PREP) . The platelet poor plasma (PPP) and platelate rich plasma (PREP) were analysed for SOD, catalase and MDA. All reagents used were from sigma Co. *Inc; St Louis, MO ,USA.*

VITAMIN 'E' ADMINISTRATION

In the study group of Diabetic patients were randomly given 400 mg of vitamin E once a day in a blind manner out of 95 patients (40 IDDM, 25 NIDDM and 30 patients of IDDM with vascular complication) 43 received vitamin E (vitamin E group) and 52 did not (without vitamin E group) . Care was taken that vitamin E was given at least 4 hours prior to taking blood for the estimation of free radicals on the first day.

BIOCHEMICAL ANALYSIS

Free radical determination was done by estimation of SOD, Catalase and MDA . Which are indirect indicators of oxy-free radicals. Superoxide dismutase was estimated according to *Green ang colleagues*[17] , at 30^0 C 3.0 ml of the control for the assay system contained 1.8 ml of 0.05 M sodium carbonate buffer Ph 10.2 and 0.6 ml of 0.15 M Kcl , 0.6 ml of 10 Mm epinephrine added to start the reaction . The jncrease is absorbance at 480 mm was recorde for one minute immediately after the addition of epinephrine. The experimental assay system contained 1.6 ml, 0.05 Sodium carbonate buffer , Ph 10.2, Enzyme preparation, and 0.15 M KCl to make up the volume 2.4 ml, 0.6ml of 10Mm epinephrine was then added to start reaction . The increase in optical density was recorded for minute. The amount of SOD was expressed as units of SOD formed per minute per milligram of protein. The unit of enzyme activity is defined here as the amount of enzyme required to inhibit the rate of autooxidation of 10 Mm of epinephrine by 50% under the experimental condition . Extinction coefficient of 4020/m cm was used.

Catalase was assayed at 25^0 C by spectrophotometric method of Beers and Sizer (1952). Control curette contained 50 Mm of Phosphate Buffer, PH 7.0 and 0.05 ml to 0.10 ml of enzyme preparation, the total volume is 3.0 ml. The experimental cuvette contained 2.0ml of 50 mM phosphate buffer , Ph 7.0 and 40 Mm of H_2O_2. 0.05 ml to 0.10 ml of catalase solution was pipette quickly into it and the contents were mixed. The decrease in absorbance was recorded at 240 mm for 60 seconds taking reading at every 10 sec interval. A molar extinction coefficients of 0.04 cm^2/micro mole at 240 mm was used for calculating the amount of H_2O_2 decomposed . One unit of catalase is defined here as the amount of enzyme required for the decomposition of one m Mole of H_2O_2 in 1 min . under experimental conditions. Specific activity was expressed as units per mg. Protein.

Malonyldialdhyde (MDA) was estimated according to placer etal; one ml. of sample

preparation was incubated at 37^0C for one hour with rapid shaking . Lipid per oxide formation was terminated by mixing 1.5 ml of 20% (w/v) cold TCA . In control tubes TCA was added prior to incubation . Contents were centrifuged at 600 rpm for 10 minutes, then cooled under tap were shaken well, heated in a boiling water bath for 10 minutes, then cooled under tap water and volume reconstituted to original of 2.0 ml. Optical density was measured at 535 mm . The amount pf MDA formed was expressed as micromoles of MDA formed per gram of tissue. An extinction coefficient of 1.56x 105/m.cm was used.

Protein determination of platelets and plasma done by method of Folin and Ciocalteu's (1927).

The result of oxidative stress in the study groups were analysed as fallows:

1) Comparison of SOD, Catalase and MDA between control group and without vitamin E group including IDDM , NIDDM with vascular complications.
2) Comparison of SOD, Catalase and MDA between control and vitamin E group.
3) Comparison of SOD, Catalase and MDA between with and without vitamin E groups.
4) Comparison of SOD, Catalase and MDA Ist and 4th day and between Ist and 10th day in both vitamin E group and without vitamin E group.

Data were analysed for statistical significance using student t test. P value less than 0.5 were considered significant.

RESULTS

In the present study significant high units of catalase and MDA and lower units of SOD were found in patient than healthy controls. Administration of vitamin E tends to normalize the levels of SOD, Catalase and MDA in these patients. Comparison of values SOD, Catalase and MDA between control subjects and study group of IDDM patients, NIDDM patients with complications who did not receive Vitamin E (Table-1) and those received vitamin E (table-2) shows that a level of platelets as well as plasma Catalase, SOD and MDA differed significantly on 1st day , just significantly on 4th day and not significantly on 10th day . table-1 and table -2 also depicts that mean value of Catalase and MDA is much higher in the without Vitamin E group IDDM patients with diabetic vascular complications (DVC) , then the IDDM and NIDDM patients without diabetic vascular complications (DNVC). Table-1 also shows that levels of plasma SOD is approximately same as in platelets, but Catalase and MDA of plasma are higher than platelets. To find out the exact significance of the effect of Vitamin E in lowering the oxidative stress (Catalase, SOD and MDA in the study groups we compared the values of SOD, catalase and MDA between the two study groups without and with Vitamin E on each day of estimation(1st, 4th and 10th day) as shown in table-3 . SOD does not show any significant difference in the two study groups on the 1st day, while catalase and MDA estimates show significant difference on the same day . On the 4th day the differences of SOD , Catalase and MDA in the without vitamin E and with Vitamin E groups are statistically significant .This is explained by the effect of vitamin E in causing a rapid fall in catalase and MDA level . On the 10th day SOD and Catalase differs significantly in the two study groups, while for MDA the difference was not found to be statistically significant. We also compared the levels of SOD, Catalase and MDA (table-4) of plasma and table-5 of platelets between the 1st day and the 4th day and between the 1st day and the 10th dai in both study groups , to again find out the significance of vitamin E in inducing or expediting the rate of fall of MDA , and Catalase in normalizing the SOD level . This table also shows that difference between 1st Day and 4th day were not statistically significant in the without vitamin E group, becomes highly significant in the vitamin E group ($P<0.001$) similarly the difference of levels of SOD , Catalase and MDA ,between 1st day and 10th day, which were just significant ($P<0.05$) in the without Vitamin E group , becomes highly significant in the vitamin E group ($P<0.001$).

TABLE-1
COMPARISON OF SOD, CATALASE AND MDA BETWEEN DIABETIC PATIENTS WITHOUT VITAMIN 'E'

Platelets			**Plasma**		
Control (n= 30)	W. Vitamin E (n = 52)	P value	Control (n= 30)	W. Vitamin E (n= 52)	P value
SOD					
9.10 ± 0.08	0.54 ± 0.02 (DNVC)	<.001	9.22 ± 0.06	0.63 ± 0.06 (DNVC)	<.001
	0.44 ± 0.06 (DVC)	<.001		0.43 ± 0.07 (DVC)	<.001
Catalase					
0.36 ± 0.09	0.54 ± 0.02 (DNVC)	<.001	0.41 ± 0.06	0.99 ± 0.07 (DNVC)	<.001
	0.44 ± 0.06 (DVC)	<.001		1.42 ± 0.28 (DVC)	<.001
MDA					
0.46 ± 0.16	0.54 ± 0.02 (DNVC)	<.001	0.53 ± 0.12	0.98 ± 0.09 (DNVC)	<.001
	0.44 ± 0.06 (DVC)	<.001		1.24 ± 0.26 (DVC)	<.001

SOD= Super oxide dismutase, MDA= malonyldialdehyde , W. Vitamin E= Without Vitamin E, DNVC= Diabetics with no vascular complications, DVC= Diabetics with vascular complications, NS= Not significant, DVC n= 22, DNVC n= 30

TABLE-2
COMPARISON OF SOD , CATALASE AND MDA BETWEEN CONTROL AND DIABETIC PATIENTS WITH VITAMIN 'E'

Platlets			**Plsama**		
Control n= 30	With Vitamin E (n = 43)	P value	Control n= 30	With Vitamin E (n = 43)	P value
SOD			SOD		
Day- 1 9.10 ± 0.08	0.54 ± 0.02 (DNVC)	<0.001	Day- 1 9.22 ± 0.06	0.63 ± 0.06 (DNVC)	<0.001
	0.44 ± 0.06 (DVC)	<0.001		0.43 ± 0.07 (DVC)	<0.001
Day- 4 9.12 ± 0.08	6.33 ± 9.80 (DNVC)	<0.05	Day- 4 9.22 ± 0.06	6.93 ± 0.42 (DNVC)	<0.05
	4.74 ± 0.08 (DVC)	<0.05		5.25 ± 0.06 (DVC)	<0.05
Day-10 9.08 ± 0.08	8.98 ± 0.09 (DNVC)	NS	Day-10 9.22 ± 0.06	9.12 ± 0.09 (DNVC)	NS
	8.90 ± 0.09 (DVC)	NS		8.94 ± 0.08 (DVC)	NS
Catalase			Catalase		
Day- 1 0.36 ± 0.09	6.63 ± 0.22 (DNVC)	<0.001	Day- 1, 0.41 ± 0.06	0.69 ± 0.02 (DNVC)	<0.001
	1.12 ± 0.32 (DVC)	<0.001		1.18 ± 0.26 (DVC)	<0.001
Day- 4 0.37 ± 0.08	0.43 ± 0.04 (DNVC)	<0.05	Day- 4, 0.41 ± 0.06	0.48 ± 0.02 (DNVC)	<0.05
	0.53 ± 0.60 (DVC)	<0.05		0.57 ± 0.01 (DVC)	<0.05
Day-10 0.38 ± 0.09	0.39 ± 0.02 (DNVC)	NS	Day-10, 0.41 ± 0.06	0.42 ± 0.04 (DNVC)	NS
	0.40 ± 0.08 (DVC)	NS		0.43 ± 0.06 (DVC)	NS
MDA			MDA		
Day- 1 0.46 ± 0.16	0.73 ± 0.09 (DNVC)	<0.001	Day- 1 0.53 ± 0.21	0.79 ± 0.08 (DNVC)	<0.001
	0.94 ± 0.27 (DVC)	<0.001		0.94 ± 0.04 (DVC)	<0.001
Day- 4 0.46 ± 0.15	0.62 ± 0.01 (DNVC)	<0.05	Day- 4 0.53 ± 0.21	0.69 ± 0.02 (DNVC)	<0.05
	0.83 ± 0.04 (DVC)	<0.05		0.84 ± 0.01 (DVC)	<0.05
Day-10 0.48 ± 0.17	0.43 ± 0.01 (DNVC)	NS	Day-10 0.53 ± 0.21	0.46 ± 0.03 (DNVC)	NS
	0.46 ± 0.02 (DVC)	NS		0.49 ± 0.06 (DVC)	NS

SOD= Super oxide dismutase, MDA= malonyldialdehyde , DNVC= Diabetics with no vascular complications, DVC= Diabetics with vascular complications, NS= Not significant, DVC n= 18, DNVC n= 25

TABLE-3
COMPARISON OF SOD , CATALASE AND MDA BETWEEN DIABETIC PATIENTS WITH VITAMIN 'E' ON EACH DAY OF ESTIMATION

DAYS OF COMP		**PLATELETS**			**PLASMA**		
WITH VITAMIN E N= 43			**WITHOUT VITAMIN E N= 52**	**P VALUES**	**WITH VITAMIN E N= 43**	**WITHOUT VITAMIN E N= 52**	**P VALUES**
SOD					**SOD**		
DAY 1	(DNVC)	0.626±0.062	0.626±0.062	NS	0.538±0.022	0.538±0.022	NS
	(DVC)	0.430 ±0.074	0.430 ±0.074	NS	0.442±0.062	0.442±0.062	NS
DAY 4	(DNVC)	6.928±0.042	0.724±0.132	<0.001	6.328±0.980	0.486±0.032	<0.001
	(DVC)	5.248±0.062	0.526±0.182	<0.001	4.736±0.082	0.512±0.082	<0.001
DAY 10	(DNVC)	9.120±0.092	0.828±0.052	<0.001	8.980±0.090	0.882±0.032	<0.001
	(DVC)	8.940±0.082	6.428±0.032	<0.001	8.902±0.086	0.412±0.084	<0.001
CATALASE					**CATALASE**		
DAY 1	(DNVC)	0.686±0.024	0.988±0.068	<0.001	0.626±0.220	0.988±0.068	<0.001
	(DVC)	1.180±0.262	1.420±0.282	<0.001	1.120±0.320	1.420±0.282	<0.001
DAY 4	(DNVC)	0.482±0.022	0.846±0.032	<0.001	0.426±0.042	0.888±0.042	<0.001
	(DVC)	0.566±0.012	1.224±0.018	<0.001	0.532±0.062	0.986±0.032	<0.001
DAY 10	(DNVC)	0.422±0.042	0.812±0.028	<0.001	0.386±0.026	0.712±0.012	<0.001
	(DVC)	0.432±0.062	0.988±0.032	<0.001	0.400±0.082	1.288±0.032	<0.001
MDA					**MDA**		
DAY 1	(DNVC)	0.786±0.082	0.988±0.092	<0.001	0.782±0.086	0.942±0.086	<0.001
	(DVC)	0.942±0.042	1.242±0.262	<0.001	0.938±0.274	1.320±0.032	<0.001
DAY 4	(DNVC)	0.688±0.022	0.892±0.032	<0.001	0.622±0.012	0.836±0.012	<0.001
	(DVC)	0.842±0.012	1.032±0.012	<0.001	0.832±0.042	1.198±0.032	<0.001
DAY 10	(DNVC)	0.456±0.032	0.566±0.022	NS	0.432±0.012	0.612±0.012	NS
	(DVC)	0.486±0.060	0.522±0.012	NS	0.458±0.024	0.732±0.082	NS

SOD= Super oxide dismutase, MDA= malonyldialdehyde , DNVC= Diabetics with no vascular complications, DVC= Diabetics with vascular complications, NS= Not significant

TABLE-4
COMPARISON OF SOD, CATALASE AND MDA OF PLASMA BETWEEN FIRST AND FOURTH DAY AND BETWEEN FIRST AND TENTH DAY IN WITHOUT VITAMIN 'E'AND WITH VITAMIN 'E' GROUP

		Ist Day	**4th Day**	**P Value**	**Ist Day**	**10th Day**	**P Value**
WITHOUT VITAMIN 'E' (N= 52)							
SOD	(DNVC)	0.626±0.062	0.724±0.132	NS	0.626±0.062	0.828±0.052	<0.05
	(DVC)	0.430±0.074	0.526±0.182	NS	0.430±0.074	0.428±0.032	<0.05
CATALASE	(DNVC)	0.988±0.068	0.846±0.032	NS	0.988±0.068	0.812±0.028	<0.05
	(DVC)	1.420±0.282	1.224±0.018	NS	1.420±0.282	0.988±0.032	<0.05
MDA	(DNVC)	0.988±0.092	0.892±0.032	NS	0.988±0.092	0.566±0.022	<0.05
	(DVC)	1.242±0.262	1.032±0.012	NS	1.242±0.262	0.522±0.012	<0.05
WITH VITAMIN 'E' (N= 43)							
SOD	(DNVC)	0.626±0.062	6.928±0.042	<0.001	0.626±0.062	9.120±0.092	<0.001
	(DVC)	0.430±0.074	5.248±0.062	<0.001	0.430±0.074	8.940±0.082	<0.001
CATALASE	(DNVC)	0.686±0.024	0.482±0.022	<0.001	0.686±0.022	0.422±0.042	<0.001
	(DVC)	1.180±0.262	0.566±0.012	<0.001	1.180±0.262	0.432±0.062	<0.001
MDA	(DNVC)	0.786±0.082	0.688±0.022	<0.05	0.786±0.012	0.456±0.032	<0.001
	(DVC)	0.942±0.042	0.842±0.012	<0.05	0.942±0.042	0.486±0.060	<0.001

SOD= Superoxide dismutase, MDA= malonyldialdehyde , DNVC= Diabetics with no vascular complications, DVC= Diabetics with vascular complications, NS= Not significant

TABLE-5

COMPARISON OF SOD, CATALASE AND MDA OF PLATLETS BETWEEN FIRST AND FOURTH DAY AND BETWEEN FIRST AND TENTH DAY IN WITHOUT VITAMIN 'E'AND WITH VITAMIN 'E' GROUP

		Ist Day	4th Day	P Value	Ist Day	10th Day	P Value
WITHOUT VITAMIN 'E' (N= 52)							
SOD	(DNVC)	0.538±0.028	0.486±0.032	NS	0.538±0.028	0.882±0.032	<0.05
	(DVC)	0.422±0.062	0.512±0.082	NS	0.422±0.062	0.412±0.084	<0.05
CATALASE	(DNVC)	0.988±0.068	0.888±0.092	NS	0.988±0.068	0.712±0.012	<0.05
	(DVC)	1.420±0.282	0.968±0.032	NS	1.420±0.282	1.288±0.032	<0.05
MDA	(DNVC)	0.942±0.086	0.836±0.012	NS	0.942±0.086	0.612±0.012	<0.05
	(DVC)	1.320±0.032	1.198±0.032	NS	1.320±0.032	0.732±0.082	<0.05
WITH VITAMIN 'E' (N= 43)							
SOD	(DNVC)	0.538±0.022	6.328±0.980	<0.001	0.538±0.022	8.980±0.090	<0.001
	(DVC)	0.442±0.062	4.736±0.082	<0.001	0.442±0.062	8.902±0.086	<0.001
CATALASE	(DNVC)	0.626±0.220	0.426±0.042	<0.001	0.626±0.220	0.326±0.026	<0.001
	(DVC)	1.120±0.320	0.532±0.602	<0.001	1.120±0.320	0.400±0.082	<0.001
MDA	(DNVC)	0.782±0.086	0.622±0.012	<0.05	0.782±0.086	0.432±0.012	<0.001
	(DVC)	0.938±0.274	0.832±0.042	<0.05	0.938±0.274	0.458±0.024	<0.001

SOD= Superoxide dismutase, MDA= malonyldialdehyde , DNVC= Diabetics with no vascular complications, DVC= Diabetics with vascular complications, NS= Not significant

DISCUSSION

The balance between hyperglycemia and the different genetically determined antioxidant cellular defences , mainly enzymatic ones (superoxide dismutase, catalase , Glutathionr peroxidise), account for the different effects of hyperglycemia related free radical productin in the development of diabetic complications the increased susceptibility to oxidant injury . Which is a function of antioxidant endogenous potential, may be mechanism by which the " toxic" effect of glucose is amplified in some diabetic patients , the vulnerability to free radical induced injury might be expected to have marked consequences in tissues or cells in which antioxidant potential is low . Pancreatic Beta-cells are physiologically equipped with very low free radical scavenging enzyme activities[18] . There are many ways hyperglycemia may increase the generation of free radicals . Hyperglycemia, seen in diabetic cataract can stimulate oxidative stress by the auto oxidation of glucose in the presence of transition metals Hence malondialdehyde (MDA) [31] .The term autoxidation describes the capability of glucose to enolize, thereby reducing molecular oxygen and yielding oxidizing intermediates[19]. The reduced oxygen products formed in the auto oxidative reaction are superoxide anion (O_2), the hydroxyl radical (OH), and hydrogen peroxide (H_2O_2). All can damage lipids, as well as protein through cross-linking and fragmentation. Free radical also accelerates the formation of advanced glycosylation end products (AGE_S), which in turn supplies more free radicals; this process is termed autooxidative glucosylation, or glycooxidation[9] is believed to be important in the pathogenesis of diabetic micro and macrovascular complication[20] .

In tissues where glucose uptake is independent of insulin, including retina, lens, kidney, peripheral nerves, all tissue sites of diabetic complications, exposure to elevated glucose level causes an increase in intracellular sorbitol and fructose levels due to increased activity of Aldose reductase (AR) and Sorbitol dehydrogenase (SDH). These two enzymes constitute the polyol pathway. Increased substrate flux through the Polyol pathway not only increases cellular levels of sorbitol and fructose but also decreases the ratio NADPH to $NADP^+$ and increases a cytosolic NADH to NAD^+ ratio[21] the hyperglycemia- induced increase in the NADH to NAD^+ is referred to as hyperglycemiapseudohypoxia[21] and is thought to play a role in diabetic complications.

Hyperglycemia can also induced alterations in the coagulation system, affecting all stage coagulation, including thrombin

formation and its inhibition, fibrinolysis and platelet and endothelial function[22]. Thrombosis is concerned one of the most important factors in the pathogenesis of myocardial infraction and also contributes to the acceleration of atherogenesisi[2]. In present clinical study we have evaluated the oxidative stress (SOD, Catalase and MDA of plasma and platelets were significantly raised in diabetics, while the level of SOD was significantly lowered in Diabetics. The differences of level of plasma Catalase and MDA and platelets SOD between control group and diabetic patients are highly significant, these difference were much more significant in diabetic with Vascular complications. This differences in level of Catalase, SOD and MDA had a normalizing trend with respect to time. The differences between 1st and 4th day was not statistically significant, while the differences between 1st and 10th day was just significant in patients without vitamin E. On the other hand, in patients with vitamin E, this differences, both between 1st and 4th day as well as between 1st and 10th day was highly significant. The normalizing trend of vitamin E was faster in case of diabeteic without vascular complications than that of diabetes with vascular complications. Thus it appears that vitamin E expedited the normalizing trend of platelets and plasma SOD, Catalase and MDA level in diabetics. Hence the reduction in oxidative stress with respect to time is significantly greater in patients talking vitaminE compared to those patients not talking Vitamin E . This fact was further supported by the observation that one each day of estimation the patients who were talking vitamin E demonstrated significant reduction in MDA and Catalase and significant increase in SOD level compared to those not taking Vitamin E.

There is also evidence that acute elevation in glucose level may depress natural antioxidant defence . For example, incubation of purified bovine Cu , Zn-SOD with different glucose concentration (10-100 m ml/l) reduced the enzyme activity by 60% [23] ; this may lead to reduced protection against the damaging effects of superoxide radicals. In general, antioxidant enzymes, such as SOD, Catalase and Glutathione peroxidise have been reported to be decreased, increased, or unaltered in non vascular beds of diabetic animals, with wide variations from one tissue to another[24] . These discrepancies may depend on variation in enzyme activity to face raised oxidative stress), as well as the type of tissue under examination. Thus depending on the particular moment of the analysis and the particular tissue examined, one could obtain divergent results. In vascular bed [25] and cardiac tissue of diabetic animals, there is a selective increase in Catalase, but not in SOD or glutathione peroxidise, which has been interpreted as evidence that diabetic blood vessels are chronically exposed to peroxidative stress due to level production of H_2O_2 in vivo. Similar findings were found in our study of levels of Catalase, SOD of platelets and plasma of Diabetic patients.

It appears that many reactions associated with hyper glycemia may acutely and chronically increase the production of free radicals, resulting in an oxidant/ antioxidant imbalance. The circulating levels of malonyldialdehyde (MDA) are higher in the plasma and platelets of diabetic as compared with those in nondiabetics. The interpretation of the result showing elevated lipid peroxide levels or conjugated dienes in diabetes is complicated by the high rate of vascular disease in clinical studies[26], although increased lipid peroxide levels have been found in diabetic patients regardless of the presence of vascular disease.[27] Despite numerous studies of the action of Vitamin E as an antioxidant, the precise mechanism remain undetermined. It is generally assumed to protect the unsaturated lipid bilayer of vital cellular and sub cellular membrane against endogenous or exogenous oxy- free radicals, which initiate or propagate nonenzymatic lipid peroxidation damage[28]. In arterial tissue an optimal Vitamin E deficiency results in enhanced tissue susceptibility toward free radicals and in an increased lipid peroxidation in vivo[29]. In fact vitamin E is the principal ;lipid soluble, chain breaking, antioxidant in mammalian tissue[24] and in particular is capable of quenching the propagation of free radical reaction within cell membranes[25] without altering the production of free radicals compounds through the donation of a hydrogen atom from the phenolic hydroxyl

group with subsequent formation of tocopherol dimmers or quinones[30].

To conclude, several experimental studies related to alpha tocopherol (Vitamin E) confirmed its antioxidative properties in diabetes . Clinical studies regarding its protective role in diabetic vascular complications are lacking. Recently, consumption of Vitamin E has been to be associated with reduced risk of vascular complication in diabetics. The present study suggest antioxidative properties of vitamin E in the Diabetes . Such changes might contribute to accelerated aging, atherogenesis and to microangiopathic complications. Alpha tocopherol administration results in faster reduction of oxidative stress. Antioxidant vitamin such as vitamin E might help in prevention of vascular complications in diabetes.

Conflicts of Interest and Source of Funding

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