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ORAL *A- TOCOPHEROL* SUPPLEMENTATION AMELIORATES THE SERUM MALONDIALDEHYDE, SOD, GPX, CAT, GSSH, BLOOD LIPIDS AND GLYCEMIC CONTROL IN TYPE 2 DIABETIC SUBJECTS

[1]Dr. Suhail Ahmed Almani, [2]Dr. Ali Naseer, [3]Dr. Shahzore Gul, [4]Dr. Sanjay Kumar Maheshwari, [5]Dr. Raza Naseer and [6]*Dr. Arsalan Ahmed Uqaili,

[1]Professor, Department of Medicine Liaquat University of Medical and Health Sciences Jamshoro, Sindh, Pakistan.
[2]Postgraduate Student, Department of Medicine Liaquat University of Medical and Health Sciences Jamshoro, Sindh, Pakistan.
[3]House Officer, Department of Medicine Liaquat University of Medical and Health Sciences Jamshoro, Sindh, Pakistan.
[4,5]House Officer, Department of Medicine Abbasi Shaheed Hospital Karachi, Sindh, Pakistan.
[6]*Lecturer, Faculty of Medicine and Allied Medical Sciences Isra University, Hyderabad, Sindh, Pakistan.

*Corresponding Author: Dr. Arsalan Ahmed Uqaili
Lecturer, Faculty of Medicine and Allied Medical Sciences Isra University, Hyderabad, Sindh, Pakistan.
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ABSTRACT

Background: Vitamin E (α- tocopherol) is a natural anti oxidant vitamin found in large quantities in blood and cell membrane. The α-tocopherol exerts anti oxidant and anti lipid peroxidant activity and influences the insulin sensitivity. **Objective:** To analyze the effects of Oral *α- tocopherol* supplementation on the serum malondialdehyde, SOD, GPX, CAT, GSSH, blood lipids and glycemic control in type 2 Diabetic (T2DM) subjects. **Methods:** The present observational study was conducted at the Department of Medicine, LUMHS Jamshoro/Hyderabad from January 2014 to March 2015. 50 diagnosed cases of type 2 DM were labeled as controls and 50 type 2 DM+ oral α- tocopherol 400 mg/day as cases. At the end of three months, blood samples were centrifuged at 4000 rpm and sera were stored at -20^0C. HOMA-IR was calculate by formula; fasting insulin × fasting glucose/22.5. Blood glucose, HbA1c, fasting insulin, serum creatinine and serum bilirubin were estimated. Assay kits were used for the estimation of SOD, GPX, CAT, GSSH and MDA. Data was analyzed on *Statistix 8.1* (USA) at 95% confidence interval (P ≤ 0.05). **Results:** Malondialdehyde (MDA) was reduced in T2DM subjects taking Oral α- tocopherol; cases 4.78±2.85 vs. controls 6.08±2.16 µmol respectively (p=0.012). Systemic blood pressure, blood glucose, blood lipids, serum insulin, HOMA-IR, serum SOD, GPX, CAT, GSSH and serum bilirubin were improved T2DM supplemented with oral α- tocopherol compared to controls. **Conclusion:** Oral α-tocopherol supplementation for 3 months exerted anti-peroxidant and anti oxidant effects significantly with improved glycemic status and blood lipids.

KEYWORDS: Oral α- tocopherol Malondialdehyde Antioxidant enzymes Glycemic control Diabetes mellitus.

INTRODUCTION

Lipid peroxidation plays major role in the pathophysiology of type 2 Diabetes mellitus (T2DM). Malondialdehyde (MDA) is a surrogate marker of lipid peroxidation. Chronic hyperglycemia of DM has been linked with exaggerated lipid peroxidation and oxidative stress which deplete the anti-oxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), reduced glutathione (GSSH) and bilirubin. Lipid peroxidation and oxidative stress interfere with endothelial faunctions which culminate eventually into the cardiovascular disease.[1,2] Concomitant depletion of anti- oxidation SOD, GPX, CAT and GSSH multiplies the risk of complications. Non-enzyme antioxidants such as vitamin E, vitamin A, vitamin C, glutathione, bilirubin and uric acid show depletion.[2] Antioxidants systems- both enzymes and non-enzymes protect against oxidative and peroxidative load and prevent cell destruction. Oxidative injury occurs through formation of oxygen derived free radicals which injure the cell parts resulting complications of DM.[3,4] Elevated oxidant and lipid peroxidant disrupts cell membrane phospholipids and damage cell organelles resulting in a vicious cycle which casues more cellular damage.[4] T2DM is an increasing health problem of World. Prevalence and incidence is increasing in populations of developing countries notoriously.[5] Diabetes mellitus is increased much in Pakistan, which now accounts as one of the major health problems.[6] Diabetic complications may be delayed by optimal

glycemic control[7] as this reduces the oxidative burden. Since the last decade, much interest has developed on the oxidative stress and its role in the pathophysiology of diabetic complications.[8]

Lipid peroxidation and oxidative stress correlates with the poor glycemic control,[9] so that the diabetics may be supplemented with antioxidant like vitamin E (α-tocopherol). Low concentrations of α- tocopherol (α- TP) in diabetics have been reported in previous clinical studies.[11,12] A previous study reported the diabetic complications may be delayed by α- tocopherol supplementations.[11] The α- tocopherol has modifying effect on the glycemic control because of its anti oxidant activity. Its anti oxidant effect influences the insulin sensitivity, non-enzymatic glycation of proteins, and lipid peroxidation.[12] Effects of α- tocopherol supplementations on glycemic control are contradictory. Some studies reported improvement in glycemic control,[13,14] while others could not prove its efficacy in diabetics.[15,16] As DM is increasing in Pakistan and in future it will be major health problem. Chronic complications of DM increase morbidity and mortality, hence there is need to analyze the effects of α-tocopherol on the lipid peroxidation (MDA) and anti oxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), reduced glutathione (GSSH) and bilirubin in our population. The present study hypothesized there is no effect of oral α-tocopherol supplementation on the MDA, SOD, GPX, CAT, GSSH and bilirubin in type 2 diabetic patients.

SUBJECTS AND METHODS
A case control study was conducted at the Diabetic clinic, Department of Medicine, Faculty of Medicine and Allied Medical Sciences, Isra University Hyderabad, Sindh, Pakistan. The study was conducted from January 2014 to March 2015. Diabetic subjects presenting at the diabetic clinic of institute were approached as per ethical criteria of clinical research. The subjects were interviewed if they are willing to participate voluntarily. Subjects were told about the merits and demerits or any losses. They were informed that if they don't participate, it will not affect their medical care. Volunteers who showed willingness were told about purpose of study. Fifty diagnosed cases of type 2 DM were labeled as controls and fifty diagnosed cases of type 2 DM (oral α-tocopherol 400 mg/day) were labeled as cases. Sampling technique was non-probability purposive sampling. Inclusion criteria were diagnosed T2DM of ≥5 years. Age range was 30-60 year. Diabetics with diabetic kidney disease, ischemic heart disease, smokers, chronic lung affections, viral hepatitis and cardiac failure were exclusion criteria. Diabetics taking multi vitamin supplements, mineral supplements, and cholesterol lowering agents were also excluded. Volunteers were asked of their right to withdraw from study protocol without telling any reason and this behavior will also not affect their medical therapy. Volunteers were told about to sign the consent form. Ethical approval was taken from the institutional authority. A Performa was designed for data collection. Controls and cases were examined by a medical officer followed by a consultant physician. Age, gender, body weight and systemic blood pressure were noted in the Performa. A subject who met inclusion and exclusion criteria was selected finally. Diabetics were given oral α- tocopherol 400 mg daily (Merck Pharmaceutical) for three months and blood samples were collected again. Ante cubital fossa was watched for the prominent vein. Area was cleaned with alcohol swab. 10 ml blood was taken into disposable syringe (BD, USA). Blood samples were centrifuged at 4000 rpm (for ten minutes). Sera were stored at -20^0C for analysis. Random blood glucose, HbA1c, fasting blood glucose, fasting insulin and serum creatinine and serum bilirubin were estimated by standard methods. Formula used for HOMA was; HOMA = fasting insulin × fasting glucose/22.5.[17] Assay kits were used for the estimation of SOD, GPX, CAT, GSSH and MDA (Fortress Diagnostics and Cayman Chemical, USA). Clinical laboratory uses Cobas e 411 analyzer (Roche Diagnosis GmbH, Mannheim, Germany) for biochemical testing. Results were typed on Microsoft Excel sheet. Complete data was copied to *Statistix 8.1* (USA) for analysis. Student's t test and Chi square test were used for the data calculation of continuous and categorical variables. Data was analyzed at 95% confidence interval (P ≤ 0.05).

RESULTS
The present observational case control study evaluated the lipid peroxidant and antioxidant status of type 2 DM. Mean± SD age in controls and cases was found 51.6±5.3 and 50.2±8.7 years respectively (P>0.05). Male and female in controls and cases were calculated as 28(56%) and 22(44%), 33(66%) and 17(34%) respectively (P>0.05). Body weight 78.3±12.6 and 76.5±11.8 kg was noted in controls and cases (P>0.05). The controls and cases showed statistically significant differences for the systolic BP, diastolic BP, Random blood glucose, fasting blood glucose, fasting insulin and HbA1c (p<0.05) as shown in table I. HOMA-IR% of controls and cases was noted as 5.47 and 5.11 respectively (P >0.05 Non-significant). Serum SOD, GPX, CAT, GSSH and bilirubin were found raised in cases (type 2 diabetics fed oral α- tocopherol) compared to controls as shown in table I (p≤0.014) and graphs 1-4. Malondialdehyde (MDA) was elevated in controls compared to cases 6.08±2.16 vs. 4.78±2.85 µmol respectively (p=0.012) as shown in table I and graph 5. MDA showed positive correlation with HbA1c (r=0.505, p=0.0001) and serum creatinine (r=0.296, p=0.001). Serum bilirubin showed negative correlation with HbA1c (r= -0.305, p=0.001) and serum MDA (r= -0.0312, p=0.001). Pearson's correlation was significant at the 0.01 level (2-tailed) as shown in table II.

TABLE. I. DEMOGRAPHIC CHARACTERISTICS AND LABORATORY FINDINGS OF STUDY SUBJECTS

|	Controls (Diabetes mellitus)	Cases (DM+ α-tocopherol)	p-value
Systolic BP (mmHg)	153.4±24.1	140.8±19.2	0.005
Diastolic BP (mmHg)	90.0±14.7	73.3±11.2	0.0001
Blood glucose (R) (mg/dl)	273.9±49.7	171.2±66.0	0.0001
HbA1c (%)	12.18±1.7	10.6±3.5	0.007
Blood glucose (F) (mg/dl)	143.9±41.7	139.9±29.7	NS
Fasting insulin (μIU/ml)	15.5±4.5	14.9±5.5	NS
HOMA-IR%	5.47	5.11	NS
Cholesterol	273.8±49.5	173.5±54.8	0.0001
Triglycerides	409.0±129.0	211.7±108.3	0.0001
LDL-c	197.3±27.9	129.9±29.7	0.0001
HDL-c	26.3±5.9	42.5±6.71	0.0001
Serum SOD (μM/ml)	105.0±21.9	182.8±14.4	0.0001
Serum GPX (μM/ml)	108.8±27.9	231.1±42.1	0.0001
Serum CAT (μM/ml)	466.8±90.9	847.5±104.1	0.0001
Serum GSSH (μM/ml)	3.6±0.43	4.96±0.73	0.0001
Serum creatinine (mg/dl)	1.05±0.24	0.93±0.18	0.011
Serum Bilirubin (mg/dl)	1.01±0.28	1.13±0.17	0.014
Malondialdehyde (μmol/ml)	6.08±2.16	4.78±2.85	0.012

Table II. Pearson`s correlation

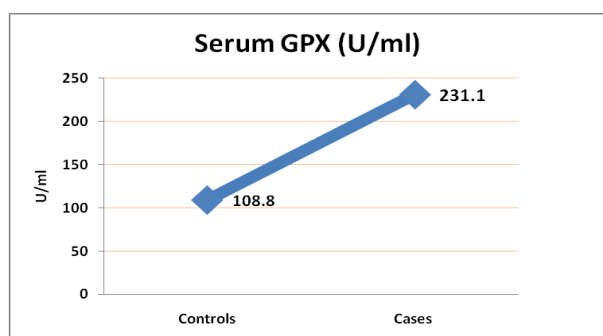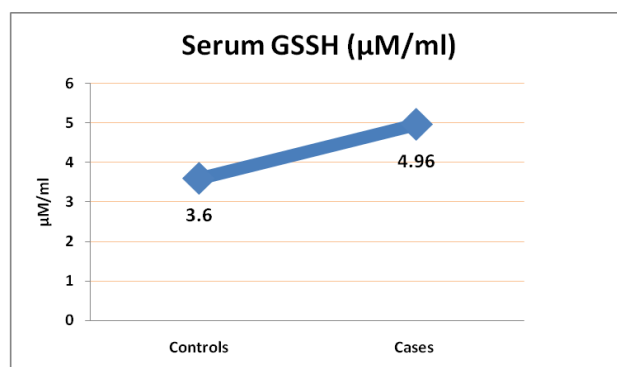|		HbA1c	MDA	S. Creatinine	S. Bilirubin
Glycated HbA1 (%)	r-value	-	0.505[**]	0.423[**]	-0.305[**]
	p-value		0.0001	0.001	0.001
Serum Malondialdehyde (μmol/dl)	r-value	0.505[**]	-	0.296[**]	-0.312[**]
	p-value	0.0001		0.001	0.001
**. Correlation is significant at the 0.01 level (2-tailed)					
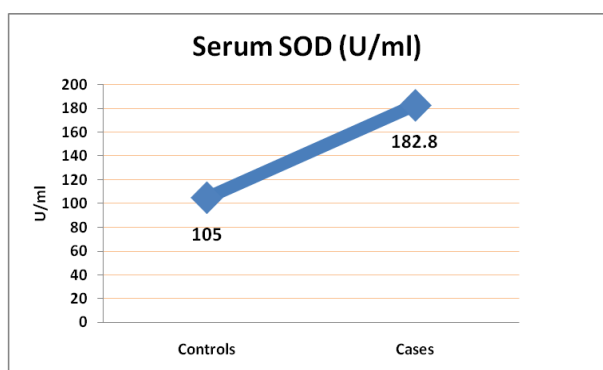
Graph 1. Mean serum SOD in controls and cases
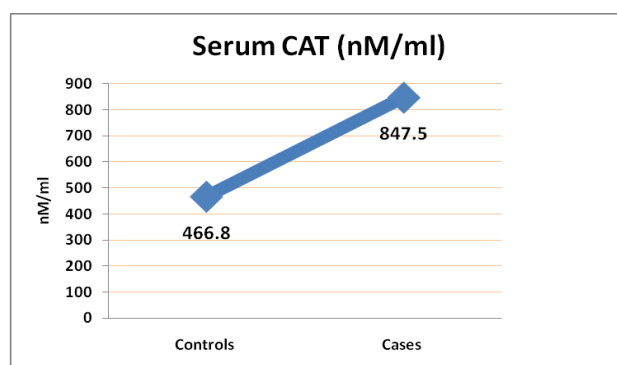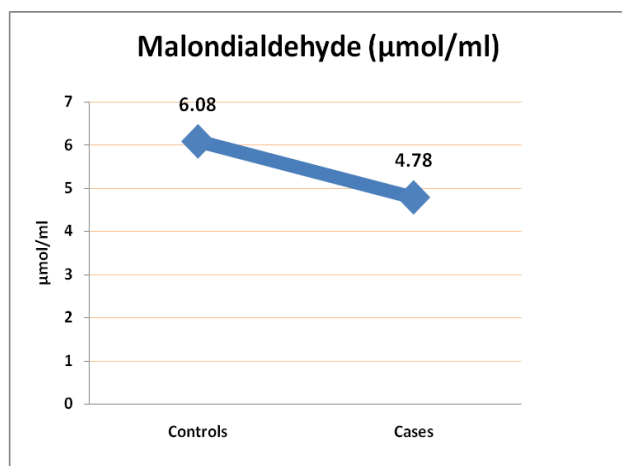

Graph 3. Mean serum CAT in controls and cases

Graph 2. Mean serum GPX in controls and cases

Graph 4. Mean serum GSSH in controls and cases

Graph 5. Mean serum MDA in controls and cases

DISCUSSION

The present observational case control study evaluated effect of oral α- tocopherol supplementation on the MDA, SOD, GPX, CAT, GSSH and bilirubin in type 2 diabetic patients. Subjects were age and gender matched (p<0.05). The null hypothesis that there is no effect of oral α- tocopherol supplementation on the MDA, SOD, GPX, CAT, GSSH and bilirubin was rejected as statistical significant differences were observed (table I). The controls and cases showed statistically significant differences for the systolic BP, diastolic BP, Random blood glucose, fasting blood glucose, fasting insulin and HbA1c (p<0.05) with oral α- tocopherol supplementation for 3 months. HOMA-IR% of controls and cases was noted as 5.47 and 5.11 respectively (P >0.05 Non-significant). Serum SOD, GPX, CAT, GSSH and bilirubin were found raised in cases (type 2 diabetics fed oral α- tocopherol) compared to controls as shown in table I (p≤0.014). Malondialdehyde (MDA) was elevated in controls compared to cases 6.08±2.16 vs. 4.78±2.85 μmol respectively (p=0.012) as shown in table I and graph II. MDA showed positive correlation with HbA1c (r=0.505, p=0.0001) and serum creatinine (r=0.296, p=0.001). Serum bilirubin showed negative correlation with HbA1c (r= -0.305, p=0.001) and serum MDA (r= -0.0.312, p=0.001). Pearson's correlation was significant at the 0.01 level (2-tailed) as shown in table II. A recent study[18] reported that the hyperglycemia and hyperlipidemia resulted in the vascular cell proliferation in a diabetic rat model. Diabetic vasculopathy is directly associated with degree of hyperglycemia and dyslipidemia because of accelerated free radical formation, non-enzymatic glycation and loss of antioxidants. Diabetes produces oxidative which forms the Ox-LDL, which is a surrogate culprit of atherogenesis.[18] Shirpoor et al reported that α-tocopherol supplementation reduced hyperlipidemia, CRP, blood lipids and oxidized LDLc in male Wistar rats. VSMC proliferation was significantly improved. Effects of α- tocopherol supplementation on glycemic has shown controversial results. Previous studies[11,19,20] showed no significant changes in HbA1c were observed. It might be due to use of high dosage of vitamin E in other studies that could cause toxicity. A previous study[11] used α- tocopherol supplementation of 800 IU/day for 6 weeks showed no improvement on blood glucose and HbA1c in type 2 diabetics. The findings of above studies are in contradistinction to present and previous reported studies.[21-23] The previous studies reported[21-23] anti oxidative and anti peroxidative effects of α- tocopherol but its effects on SOD, GPX, CAT, GSSH, MDA, HbA1c, cholesterol, TAG, LDLc, HDLc, serum bilirubin and serum creatinine are not well studied in clinical practice. The α-tocopherol supplementation produced significant reduction in risk factors of cardiovascular disease. The findings are in agreement with previous studies.[24-27] A previous study reported that the high doses of α- tocopherol supplementation improved insulin activity, fasting blood glucose and oxidative stress.[13] Manzella reported reductions in HbA1c, plasma insulin, HOMA and oxidative stress indices,[13] the findings are in agreement with present study except for the HOMA-IR which was improved in our diabetic subjects but was statistically non-significant. The findings of HOMA-IR of present are in agreement with previous studies.[20-24] Blood lipids were improved in present study and findings are in keeping with previous studies.[14,24] Such controversial findings might be due to i) different study population, ii) differences of geographical location, iii) dietary and environmental factors, iv) different drug dosing and duration and more over researcher bias. Previous studies[13,28] have shown α-tocopherol supplementations reduced triglycerides, total cholesterol and LDL similar to the present study, but other have not observed significant changes in blood lipids.[14,26] A previous study showed inverse association of α- tocopherol supplementation and systemic blood pressure.[29] In present study, α- tocopherol supplementation showed significant reduction in systolic and diastolic BP as shown in table I. The findings of present study are in agreement with previous studies[14,25,26] Nweke et al[18] reported that there was a significant increase in SOD, GPX, CAT, GSSH and a reductions in HbAIc and lipid peroxidation (MDA) levels after α- tocopherol supplementation in streptozotocin-induced diabetic animals. The findings of majority of studies support the findings of the present study.

CONCLUSION

The present control reports that the oral α- tocopherol supplementation reduces systemic blood pressure, malondialdehyde, blood glucose, glycated HbA1 and HOMA-IR. Three month oral α- tocopherol supplementation increased the superoxide dismutase, glutathione peroxidase, catalase, reduced glutathione and bilirubin.

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