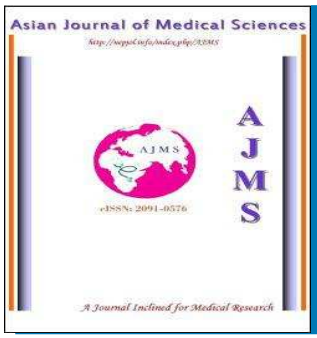

VITAMIN E SUPPLEMENTATION AND MARKER OF OXIDATIVE STRESS IN INDIAN ACUTE MYOCARDIAL INFARCTION PATIENTS

ORIGINAL ARTICLE,Vol-5 No.2

Asian Journal of Medical Science, Volume-5(2014) http://nepjol.info/index.php/AJMS

[1]Ijen Bhattacharya [2]Rahul Saxena, [3]Raj Saxena and [4]Alok Milton Lal. [1]Associate Professor, Department of Biochemistry, Meenakshi Medical College, Hospital & Research Institute, Enathur, Kanchipuram Tamilnadu, India. [2]Assistant Professor, Department of Biochemistry, SMSR, Sharda Hospital, Sharda University, Greater Noida, U.P. India. [3]Senior Research Fellow, Department of Clinical Research, Sikkim Manipal University, Manipal, India. [4]Associate Professor, Department of Biochemistry & Biochemical Engineering, Jacob School of Biotechnology & Bioengineering, SHIATS, Allahabad, U.P. India.

CORRESPONDENCE:

Dr. Rahul Saxena
C/o Prof. G.R.K. Rao
Central Lab, Department of Biochemistry,
School of Medical Sciences & Research
Sharda Hospital, Sharda University
Plot No. 32-34, Knowledge Park III
Greater Noida, U.P. 201036;
Email ID:
rahul.saxena@sharda.ac.in
(M) +91-9839351748

"Amelioration of modifiable indexes in the blood of AMI patients by Vitamin E"

ABSTRACT

Background: Reactive oxygen species have been identified as mediators of cell injury in a variety of cardiovascular complications including Myocardial Infarction (MI). It is conceivable that vitamin E supplementation can be used therapeutically due to its role in ameliorating antioxidant status and free radicals scavenging activity.

Aim: Therefore, the present study was undertaken to assess the markers of oxidative stress i.e. erythrocyte glutathione peroxidase (GSHPx) & malondialdehyde (MDA); plasma vitamin C, E, A and uric acid level in the blood samples of MI patients and to investigate the effect of in-vitro vitamin E supplementation in ameliorating the levels of these antioxidants in the blood sample of MI patients.

Material & Method: 60 MI subjects (age group 30-60 years) were taken for the study and 60 healthy individuals served as controls. In-vitro vitamin E supplementation in the blood samples of MI subjects were performed and above mentioned parameters were estimated by using standard methods. Data was compared statistically by using student t-test. **Result:** Vitamin E supplementation brought about an improved antioxidants status with significantly raised vitamin C, E, A and GSHPx levels ($p<0.05$, $p<0.001$), and simultaneously depleted level of erythrocyte MDA ($p<0.001$) in blood samples of MI subjects. However, plasma uric acid levels remain unaltered ($p<0.1$).

Conclusion: These findings further support the preventive and cardio protective role of vitamin E supplementation in reducing oxidative stress levels in the blood samples of MI patients.

Key words: Oxidative stress, Glutathione peroxidase, uric acid, malondialdehyde.

INTRODUCTION

Biomolecular deterioration caused by reactive oxygen species including those associated with altered plasma antioxidant reserve and lipid peroxidation are now accepted to be related with a wide range of pathological processes including cardiovascular disease, cancer, rheumatoid arthritis and aging process etc.[1]. Increasing interest has been focused on the role of oxidative stress in the etiopathogenesis of Myocardial infarction (MI), which are convincingly linked to the altered antioxidant defense system and major interrelated derangements of cell metabolism including DNA strand breakage, damage to membrane ion transporters, specific proteins and lipid peroxidation. [2]

Most common radical and non radical derivatives of oxygen includes superoxide free radical anion ($O_2^{\cdot-}$), hydroxyl radical ($OH^{\cdot}$), lipid peroxide (LOOH), hydrogen peroxide (H_2O_2) and singlet oxygen. These agents are indiscriminate and if not promptly neutralized they can inflict damage to cell membranes leading to development of disease.

Superoxide radical is unstable and either spontaneously or enzymatically by superoxide dismutase, transformed into potent oxidant, H_2O_2. Hydrogen peroxide, either in presence of transition metal (Fe^{2+}& Cu^{2+}) produces highly toxic hydroxyl radical or produces hypochlorous acid (HOCl) by the action of enzyme myeloperoxidase in neutrophils and macrophages which amplify further destruction leading to cardiovascular disease (CVD).[3] Prime targets of these free radicals attack are the PUFA in the membrane lipids causing lipid peroxidation which initiates a complex cascade of events in arterial wall and thereby leads to the development of Atherosclerotic plaques followed by myocardial infarction.[4]

These free radicals are efficiently removed by antioxidant defense system which includes antioxidant enzymes and antioxidants. Among various antioxidant enzymes, Glutathione peroxidase (GSHPx) is selenium containing enzyme and catalyses the decomposition of H_2O_2 with the help of reduced glutathione. It also prevents the oxidation of lipids and phospholipids.[5] Oxidant scavenging role of these enzymes are well supported by cooperative action of other widely recognized non-enzymic antioxidants such as vitamin C, E & A, and uric acid etc, may have a significant role in preventing the cascade leading to the development of MI. It has been shown previously that these non-enzymic antioxidants contribute significantly in scavenging free radicals, inhibiting lipid peroxidation, in repairing endothelial cells and restoring endothelium derived vasodilators etc.[6,7]. Conversely, their role as proxidant and relation of uric acid with vascular injuries reflect the need of further investigation.[8,9]

It is conceivable that vitamin E, mainly α-tocopherol, can ameliorate the modifiable indexes via regulating the free radical production. Previous epidemiological studies and in our recent study on vitamin E supplementation have been found to reduce risk of CVD and age related complications.[10,11,12] However, outcomes of several clinical trials of cardioprotective effect of vitamin E have been proved disappointing and need further investigation.[13,14] Therefore, the overall objectives of present study were to investigate the effect of in-vitro vitamin E supplementation in ameliorating the altered levels of plasma antioxidants (vitamin E, C, & A; and uric acid), erythrocyte GSHPx and lipid peroxidation (via MDA estimation) in the blood samples of MI patients.

MATERIALS AND METHODS

n the present study, 60 patients who were diagnosed clinically and angiographically as MI patients (30-60 years; 45 Male & 15 Female) were selected and 60 age matched normal healthy volunteers were taken as control.

Inclusion criteria: All the patients had their first episode of myocardial infarction with diagnostic criteria: typical chest pain, specific abnormalities of MI on electocardiogram, elevated serum creatine phosphokinase (CPK-MB) and aspartate amino transferase (AST) enzyme activity. A general information or pre-experimental questionnaire regarding anthropometric and clinical data was completed from all the patients after taking their informed consent and approval of protocol by ethics committee of college.

Exclusion criteria: Patients with diabetes mellitus, smoking habit, renal insufficiency, hepatic disease, obese (BMI >25.0), hypertensives (B.P. >120/80 mmHg), taking lipid lowering drugs or antioxidant vitamin supplements were excluded.

Height and weight were measured with subject barefoot and light dressed. The body mass index (B.M.I.) was calculated as B.M.I. = *weight (Kg) / Height (metre*^{2}). Fasting blood samples were collected in Acid Citrate Dextrose vials from the anticubital vein of the patients as well as controls.

Plasma antioxidants, erythrocyte GSHPx and MDA levels were estimated in MI subjects before (non supplemented group i.e. Group I) and after 2 hours of vitamin E supplementation in-vitro (40 µg/ml of blood at 37°C; Group II) and compared it with that of healthy controls. Erythrocyte GSHPx and MDA levels (marker of lipid peroxidation) were estimated by Beutler's method and Sinnhuber et al method spectrophotometrically, after preparation of hemolysate.[15, 16] Plasma vitamin C, E & A, and uric acid levels were estimated by Mc Cormick and Greene method, Hashim and Shuttringer method, Sinha's method and Caraway's method respectively.[17-20] Values were expressed as Mean ± SD. The significance of mean difference between groups was compared by using Student's t-test and distribution of probability (P)

RESULTS

In the present study, the mean blood pressure and anthropometric indices of the study group subjects are depicted in Table 1. The observations made reveal significant changes in antioxidant status and MDA levels in study group subjects before and after in-vitro vitamin E supplementation, as represented in Table 2 and 3. Erythrocyte GSHPx activity was significantly low (37.02 %; $p<0.001$) in MI subjects as compared to healthy controls that was found to be increase significantly (26.5%; $p<0.05$) on vitamin E supplementation. Plasma vitamin C, E & A levels were found to be significantly low in MI subjects ($p<0.001$, $p<0.05$; 58.6%, 48.7% and 31.25% low respectively). On in-vitro vitamin E supplementation, marked amelioration in plasma antioxidant vitamin levels were observed ($p<0.001$, $p<0.05$). Similarly, marked elevated levels of malondialdehyde (MDA) and plasma uric acid were observed in Group I ($p<0.001$; 52.8 % & 79.1 % high respectively) as compared to healthy controls .However, only MDA level was found to be decreased significantly ($p<0.001$; 32.0 %) in Group II whereas plasma uric acid levels decreased insignificantly ($p<0.1$; 8.2 % low) in Group II as compared to Group I.

DISCUSSION

Biomolecular deterioration caused by reactive oxygen species including those associated with altered plasma antioxidant reserve and lipid peroxidation are now accepted to be related with a wide range of pathological processes including cardiovascular disease, cancer, rheumatoid arthritis and aging process etc.[1]. Increasing interest has been focused on the role of oxidative stress in the etiopathogenesis of Myocardial infarction (MI), which are cona Several evidences have been documented regarding enhanced production of ROS as well as for a decrease in the antioxidant reserve in plasma and tissues of MI patients.[2] Theoretically, it is conceivable that the exogenous administration of non-enzymic antioxidants such as vitamin E may prevent the development of cardiovascular diseases although contradictory evidences have been documented.[13] In this context, an attempt is made to control oxidative stress in MI by exogenous vitamin E supplementation. Among various free radicals, superoxide anion, efficiently removed by SOD [as reported in our previous study],[11] significantly participate in the etiopathogenesis of MI not only by inducing structural and functional damage to endothelial cells and vascular smooth muscle cells but also by producing H_2O_2 which amplify further destruction via $OH^{\cdot}$ and HOCl formation.

In addition to catalase, GSHPx plays a crucial role in the final detoxification of H_2O_2. It spontaneously reacts with and scavenges many forms of ROS, prevents oxidation of lipids and phospholipids, maintains intracellular redox milieu, replenishes a number of crucial antioxidants (vitamin E and C) and produces vasodilatory prostacyclin by the endothelium.[1] In the present study, low GSHPx activities were observed in Group I subjects as compared to healthy controls which focused the role of GSHPx not only in the scavenging of H_2O_2 but also in the prevention of progressive deterioration of endothelial cells (via. lipid peroxidation) initiated by highly reactive hydroxyl radical ($OH^{\cdot}$). Kumar and Biswas also observed that erythrocyte antioxidant enzymes activity of RBC including GSHPx had decreased due to augmented oxidative stress in acute myocardial infarction subjects.[21] Reduced levels of free radical scavengers such as vitamin E, glutathione and SOD have been reported in MI patients by Bhargava et al. and suggested it as a result of their consumption during

Table1: Demographic profile of MI patients and healthy controls. (Mean ± SD)

S No	Particulars	Control group (n=60)	Group I (n=60)	Level of significance
1)	Age (years)	44.5 ± 4.0	47.0 ± 6.0	-
2)	M:F ratio	3:1	3:1	-
3)	Height (meter)	1.58 ± 0.07	1.60 ± 0.06	p<0.1
4)	Weight (Kg)	56.3 ± 3.8	63.1 ± 4.2	p<0.05
5)	B.M.I. (Kg/m^2)	22.5	24.5	p<0.05
6)	Systolic blood pressure (mmHg)	102 ± 8.0	124 ± 4.8	p<0.001
7)	Diastolic blood pressure (mmHg)	74.0 ± 3.5	82 ± 4.0	p<0.05

Where, p< 0.001: Highly significant; p< 0.05: Significant; p< 0.1: Non- significant

Table2: Antioxidants and Malondialdehyde levels in MI subjects before vitamin E supplementation. (Mean ± SD)

S.N	Particulars	Control group (n=60)	Group I (non supplemented) (n=60)	% Decrease	% Increase	p-value
1.	GSHPx (IU/gm Hb)	36.2 ± 6.5	22.8 ± 4.0	37.02 %	--	p<0.001
2.	Ascorbate level (mg%)	0.75 ± 0.13	0.31 ± 0.07	58.6 %	--	p<0.001
3.	Tocopherol level (mg%)	1.42 ± 0.23	0.73 ± 0.14	48.6 %	--	p<0.001
4.	Vitamin A (µgm%)	121.32 ± 27.0	83.4 ± 10.5	31.25 %	--	p<0.05
5.	Uric acid (mg%)	4.36 ± 1.18	7.81 ± 1.70	--	79.1 %	p<0.001
6.	Malondialdehyde (µmolMDA/ml)	3.30 ± 0.61	5.04 ± 0.83	--	52.8 %	p<0.001

Where, p<0.05: Significant; p<0.001: Highly significant

metabolism of oxygen free radicals.[22] On in-vitro vitamin E supplementation, significant increase in GSHPx activity (p<0.05) was observed in MI blood samples which could be explained as a glutathione sparing action of vitamin E by inhibiting lipid peroxidation and thereby replenishes GSHPx activity.[23] Consisting findings were reported by Garg et al in their studies on vitamin E supplementation on diabetic rats.[24] However, Li et.al observed no any effect of vitamin E supplementation on GSHPx activity.[13]

Above mentioned observation is well supported by marked reduction in MDA levels (p<0.001) in supplemented samples which was 63.20 % high in Group I subjects. These findings clarify the chain breaking antioxidant property of vitamin E by which it protects the membrane bound lipids and nascent LDL against free radical mediated lipid peroxidation and thus plays a significant role in the reduction of MI. Furthermore, Chan observed that vitamin E administration retards LDL oxidation, inhibits smooth muscle proliferation, synthesis of leukotrienes, platelets adhesion, aggregation and expression.[24] It also potentiates the release of prostacyclin through up-regulation of cytosolic phospholipase A_2 and cyclo-oxygnase .

In addition to antioxidant enzymes, free radicals are efficiently removed by co-operative action of other widely recognized non enzymatic antioxidants such as vitamin C, E & A, and uric acid. Vitamin C, an exogenous water soluble antioxidant functions as primary defense against free radicals in plasma and disappeared more quickly. It has a significant role in protecting plasma lipids against peroxidation (i.e. anti atherosclerotic effect) and positive correlation with HDL-cholestrerol.[6] Another possible mechanisms through which ascorbate plays a significant role in reducing MI includes its protective effect on Na^+-K^+ ATPase against peroxidative damage and thereby in maintaining electrolyte balance enhancement of NO bioavailability and synergistic

Table 3: Antioxidants and Malondialdehyde levels in blood samples of MI subjects after vitamin E supplementation. (Mean ± SD)

SNo.	Particulars	Group I (n=60)	Group II (supplemented) (n=60)	% Decrease	% Increase	p-value
1.	GSHPx (IU/gm Hb)	22.8 ± 4.0	28.84 ± 6.5	--	26.5 %	p<0.05
2.	Ascorbate level (mg%)	0.31 ± 0.07	0.40 ± 0.06	--	29.0 %	p<0.001
3.	Tocopherol level (mg%)	0.73±0.14	1.15 ± 0.13	--	58.2 %	p<0.001
4.	Vitamin A (µ gm%)	83.4 ± 10.5	100.4 ± 17.0	--	20.4 %	p<0.05
5.	Uric acid (mg%)	7.81 ± 1.70	7.16 ± 0.20	8.2 %	--	p<0.1
6.	Malondialdehyde (µmolMDA/ml)	5.04 ± 0.83	3.42 ± 0.57	32.0 %	--	p<0.001

where, p<0.05: Significant; p<0.001: Highly significant; p<0.1:Non-significant

action to regenerate α-tocopherol and urate from their radicals. [26- 28]

Vitamin E, a universal lipophilic, chain breaking antioxidant and a stabilizer of biological membranes, prevents accumulation of free radicals and decreases lipid peroxidation. Vitamin E quenches and reacts with superoxide anion, hydroxyl and peroxyl radical to provide protection against oxidative stress; and increases NO bioavailability.[29]

In addition, β- carotene, mainly carried on LDL particle, is also an important fat soluble antioxidant that can quench singlet oxygen, competitively spares selenium in metabolic reactions, inhibits LDL oxidation; and is responsible for immune response, epithelial growth and repair.[30] Alteration in their levels may have a significant role in the development of MI.

In the present study, plasma levels of these antioxidant vitamins (C, E & A) were significantly low (p<0.001, p<0.001, p<0.05) in Group I as compared to control. Decreased levels of these vitamins could not be only due to their free radical scavenging action but also in maintaining the body antioxidant reserve and in normalization of vascular superoxide formation. Pahwa and Seth also observed reduction in antioxidant vitamin levels with increased levels of lipid peroxides in MI subjects and concluded it as a contributory event in the development of CVD.[31] Ugle et al also observed a marked reduction in vitamin A levels in CVD patients with smoking habit and suggested that CVD events can be prevented by improving the blood beta carotene and LDL-beta carotene status.[32] Despite data linking antioxidant role of these vitamins, their pro-oxidant properties also play a controversial role.[33]

On in-vitro vitamin E supplementation, these levels were increased significantly in Group II (p<0.05; Table 3) as compared to Group I subjects. Our findings were in agreement with those of Prasad et al who observed that α-tocopherol treatment increases antioxidant reserves and cardiac contractility.[34] However, Keith et al. found no any significant effect of vitamin E supplementation on other marker of oxidative stress.[35] The increased concentration of vitamin C, E and A, as observed in the present study may be linked to a series of reactions which include synergistic action of vitamin C, E and A (Fig 1.0) i.e. inhibition of lipid peroxidation by lipid peroxyl radical scavenging action of vitamin C and A to produce their radicals. α-tocopherol regenerates vitamin C and A from their radical forms by reducing them and itself being converted into α-tocopheroxyl radical.[36] In addition, erythrocytes and neutrophils take up ascorbate radicals rapidly and convert it back to ascorbate at the expense of GSHPx and in presence of Glutathione–semidehydro ascorbate reductase enzyme.[6] Furthermore, increased vitamin E level could also be explained on the basis of its direct association with vitamin E supplementation.[35]

Moreover, uric acid is an endogenous, preventive and chain breaking antioxidant which contributes about 65% of free radical scavenging action, stabilizes ascorbate, protects DNA and erythrocytes from oxidative damage.[7] Increased production of superoxide anion reduces NO bioavailability by reacting with NO to form toxic product peroxynitrite anion ($ONOO^-$) and thereby enhances blood pressure and platelet

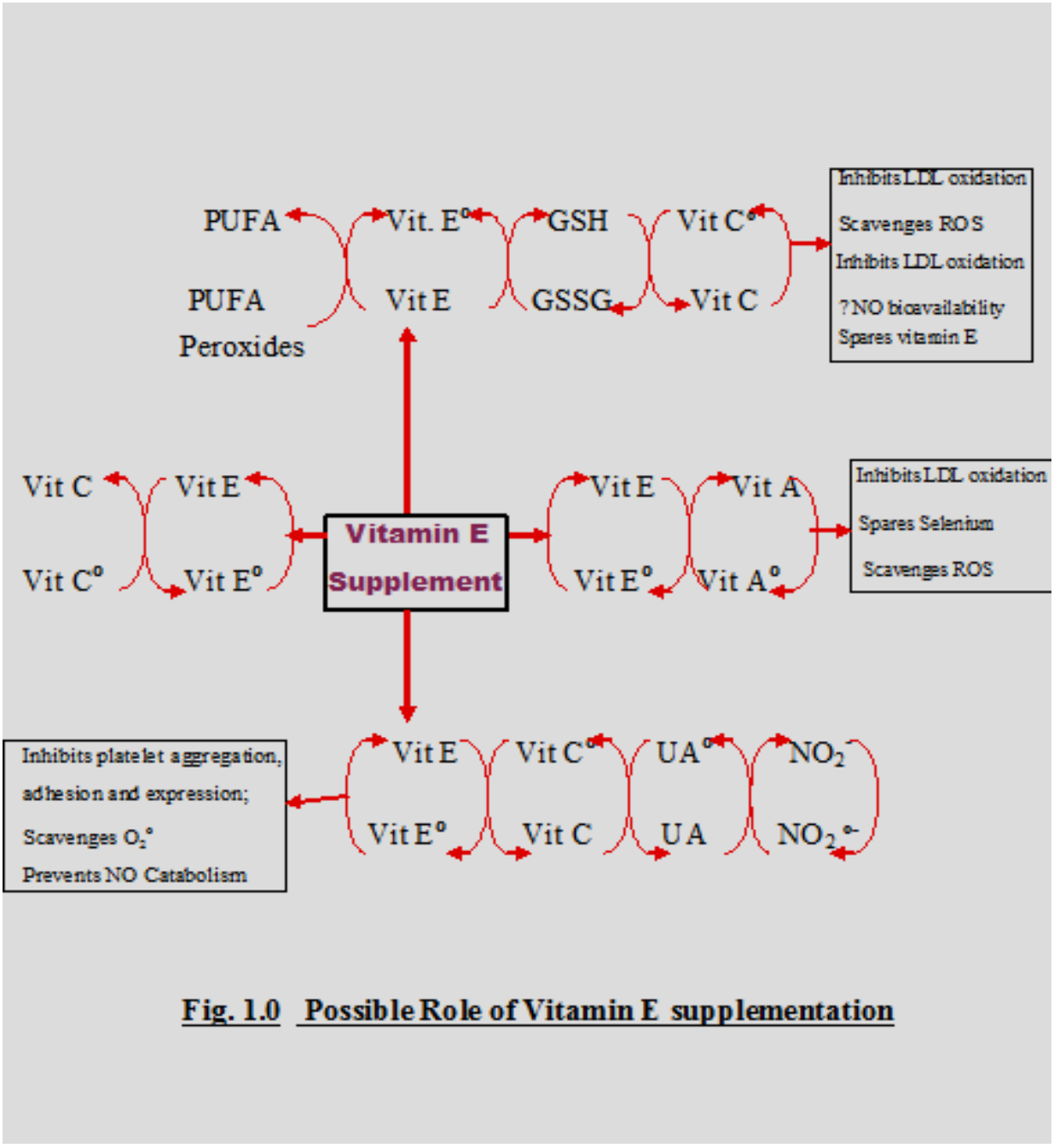

Fig. 1.0 Possible Role of Vitamin E supplementation

aggregation.[29] Plasma uric acid interacts with peroxynitrite anion to form a stable nitric oxide donor, thus promoting vasodilation and restores endothelial function.[37]

In the present study, plasma uric acid levels were found to be significantly high ($p<0.001$) in MI subjects and were in agreement with the findings of Pahwa and Seth which indicates that body is trying to protect itself from the deleterious effects of free radicals by increasing uric acid production.[31] According to Maxwell and Bruinsma, elevation of uric acid production is a secondary event and occur due to disinhibition of xanthine oxidase activity via reduction in vascular NO activity because NO is known to interact with active site of xanthine oxidase and inhibits its activity to produce uric acid.[38] However, on in-vitro vitamin E supplementation, insignificant reduction in plasma uric acid was observed in Group II ($p<0.1$) which may be due to limitation of present study (i.e. in-vitro study), as it is well supported by the fact that uric acid synthesis and the enzymes concerned with its metabolism are present in liver. Therefore, further study is warranted to shed more light on the hidden facts related to therapeutic use of antioxidants.

CONCLUSION

On the basis of present findings and consistent findings of previous studies, it can be inferred that oxidative stress plays a crucial role in MI and vitamin E supplementation provide protection against oxidative stress not only by their free radical scavenging action but also by ameliorating antioxidant reserve and by preventing biomolecular deterioration (lipid peroxidation) which are responsible for MI development. Therefore, consumption of diet rich in vitamin E should be increased with suspection of disease, which may prevent or postpone the development of MI.

REFERENCES

1. Sen CK. Oxygen Toxicity and antioxidants: state of the art. Ind J Physiol Pharmacol 1995, 39: 177-196.
2. Chopra K, Singh M. New Biology of Ischemia – reperfusion induced myocardial injury. Pharmacology Update 1992,4: 23-29.
3. Kumar A, Sivakanesan R, Guneasekera S. Oxidative stress and antioxidant status in normolipidemic AMI patients. Ind J Clin Biochem 2008, 23(3): 296-298.
4. Prithviraj T, Misra KP. Reversal of Atherosclerosis – Fact or Fiction ? Cardiology Today 2000, 4: 97-100.
5. Nawrot TS, Staessen JA, Roels HA, Hond DE. Blood Pressure and blood selenium: a cross-sectional and longitudinal population study. Euro Heart Journal 2007, 28: 628-633.
6. Nishikimi M, Yagi K. Biochemistry and molecular biology of ascorbic acid biosynthesis: In subcellular Biochemistry. Plenum press, New York; 1996. p.17-28.
7. Ames BN, Cathcort R, Schwiers E, Hochstein P. Uric acid provides an antioxidant defence in humans against oxidant and radical caused aging and cancer. Proc Natl Acad Sci USA 1981, 78: 6858-6862.
8. Ward JH. Uric acid as an independent risk factor in the treatment of Hypertension. Lancet 1998, 352: 670-671.
9. Schlotte V, Sevanian A, Hochstein, Weithmann KU. Effect of uric acid and chemical analogues on oxidation of human low density lipoprotein in-vitro. Free Rad Bio Med 1998, 25:839-847.
10. Ghatak A, Brar MJS, Agarwal A. Oxygen free radical system in heart failure and therapeutic role of oral Vitamin E. Int J Cardiol 1996, 57: 119-127.
11. Saxena, R. and Jaiswal, G. Influence of vitamin E supplementation on plasma paraoxonase, antioxidants status and nitric oxide levels in hypertensive smokers. S As J Prev Cardiol. 2009, 13(1): 20-29.
12. Bhattacharya, I; Saxena, R. & Gupta, V. Efficacy of vitamin E in knee osteoarthritis management of North Indian Geriatric population. Therap. Adv. Musculo. Dis.2012, 4(1):11-19.
13. Li RK, Cowan DB, Mickle DAG, Weisel RD, Burton GW. Effect of Vitamin E on human glutathione peroxidase expression in cardiomyocytes. Free Rad Biol Med 1996, 21: 419-426.
14. Yusuf S, Dagenais G, Pogue J, Bosch J, Sleight P. Vitamin E supplementation and cardiovascular events in high risk patients. The Heart Outcomes Prevention Evaluation Study Investigators. N Eng Med 2000, 342: 154-160.
15. Beutler E. Red cell metabolism. A manual of Biochemical methods. 3rd ed. New York: Grune & Stratlon Inc; 1971. p.112-113.
16. Sinnhuber RO, Yu TC, Yu TC. Characterization of the red pigment formed in the thiobarbituric acid determination of oxidative rancidity. Food Res 1958, 23: 626-630.
17. Mc Cormick DB, Greene HL. Vitamin: In Tietz Text Book of Clin.Chem. C.A. Burtis and E.R. Ashwood eds. WB Saunders Company USA, 1998. p.1025-1027.
18. Hashim SA, Schuttringer GR. Rapid determination of Tocopherol in Macro and Micro quantities of plasma. Am J Clin Nutr 1996, 19: 137-144.
19. Sinha SN. Estimation of Vitamin A. Practical Chemical Pathology. 1st ed. Sahitya Bhandar Publishers: Allahabad; 1986. p 21-22.
20. Caraway WT. Standard methods of Clinical Chemistry. Academic press, New York: 1963, 4: 239-240.

21. Kumar A, Biswas UK. Smoking is associated with reduced serum paraoxonase,antioxidants and increased oxidative stress in normolipidaemic acute myocardial infarct patients. Heart Asia 2011, 115-119.
22. Bhargava R, Kumar A, Singh SK. Status of primary Antioxidant enzymes in Pre and post treated CHD patients by supplementation of certain antioxidant vitamins. Proc ICFR. Lucknow; 2003, p. 48.
23. Costaghiola C, Libondi T, Menzione M, Rinaldi E, Aurrichlo G. Vitamin E and red blood cell glutathione. Metabolism 1985, 49 : 712-714.
24. Garg MC, Chaudhary DP, Bansal DD. Effect of Vitamin E supplementation on diabetes induced oxidative stress in experimental diabetes in rats. Ind J Exp Biol 2005, 43: 177-180.
25. Chan AC. Vitamin E and Atherosclerosis. J Nutr 1998, 128:1593-1596.
26. Yashioka M, Matsushita T, Chuman Y. Inverse association of serum ascorbate level and blood pressure. Internat J Vit Nutr Res 1984, 54: 343-347.
27. Tousoulis D, Davis G, Toutouzas P. Vitamin C increases Nitric oxide availability in coronary atherosclerosis. Ann Intern Med 1999, 131: 156-157.
28. Ford E, Hughes NM, Wardmen P. Kinetics of the reaction of NO_2 with Glutathione, Cysteine and Uric acid at physiological pH. Free Rad Bio Med 2002, 32: 1314-1323.
29. Newaz MA, Nowal NNA. Effect of α – tocopherol on lipid peroxidation and total antioxidant status in SHR. Am J Hypert 1998, 11: 1480-1485.
30. Fairfield KM, Fletcher RH. Vitamins for chronic disease prevention in adults. JAMA- India 2003, 2: 54-64.
31. Pahwa MB, Seth S. Ascorbate lowers the deleterious effect of raised serum uric acid in Cardiovascular Disease. Ind Practitioner 2003, 56: 459-464.
32. Ugle SS, Patel MB, Yadav AS, Deshmukh SR. Low Beta-carotene and elevated lipid peroxides in blood and LDL are major causes of Cardiovascular Disease in smokers. S As J Prev Cardiol 2006, 10: 230-233.
33. Evans RW, Shaten BJ, Day BW, Kuller LH. Prospective association between lipid soluble antioxidants and coronary heart disease in men: the multiple risk factor intervention trial. Am J Epidemiol 1998, 147: 180-186.
34. Prasad K, Gupta JB, Lee JKP. Oxidative stress as a mechanism of Cardiac failure in chronic volume overload in canine model. J Mol Cell Cardial 1996, 28: 375-385.
35. Keith ME, Jeejeebhoy KN, Langer A, Kurian R. A controlled clinical trial of Vitamin E supplementation in patients with congestive heart failure. Am J Clin Nutrition 2001, 73: 219- 224.
36. Niki E, Noguchi N, Tsuchihashi H, Gotoh N. Interaction among Vitamin C, Vitamin E and β – carotene. Am J Clin Nutr 1995, 62 : 1322 S-6 S.
37. Wayner DDM, Burton GW, Ingold KU, Barclay LRC, Locke SJ. The relative contributions of Vitamin, urate, ascorbate and proteins to the total peroxyl radical-trapping antioxidant activity of human blood plasma. Biochem. Biophys. Acta. 1987, 924: 408-419.

Authors Contributions:

IB: Principal investigator, Formulation of study, Facilitate of sample collection.

RS: Biochemical analysis, Data collection and writing of manuscript.

RS^3: Planning, Evaluation and Literature Collection.

AML: Correction of the manuscript and editing.

Conflict of Interest: None

Date of Submission: 12.8.2013
Date of Peer review: 17.8.2013
Date of submission of revised version: 11.9.2013
Date of peer review: 14.9.2013
Date of Acceptance: 15.9.2013
Date of Publication: 10.1.2014