



STUDY OF SYNTHETIC FORM OF TOMATO LYCOPENE AND ITS EFFECTIVITY OVER NATURAL AND PLACEBO FORM OF LYCOPENE IN PATIENTS WITH OXIDATIVE STRESS

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ABSTRACT

Objective- Lycopene is a phytochemical, found in tomatoes having singlet oxygen quenching ability higher than other antioxidants, participates in most of chemical reactions to protect critical cellular biomolecules. Present study is to analyze effect of tomato's lycopene, synthetic lycopene, its placebo form in patients of oxidative stress.

Methods- Study includes 45 patients having oxidative stress, aged 40-60 years and 30 age and sex matched healthy controls. Blood samples were collected at the time of enrollment, end of 2 weeks of lycopene restricted diet and after 10 weeks of lycopene supplementation. Oxidative stress biomarkers MDA, SOD, vitamin C and E were measured.

Results- Results of study revealed decreased lipid peroxidation and oxidative stress after various forms of lycopene supplementation but comparative analysis showed significant increase in oxidative stress biomarkers except MDA in patients receiving synthetic lycopene in comparison with natural and placebo forms of lycopene.

Conclusion- Dietary intake of tomato lycopene (natural) is beneficial to fight against OS but in the synthetic form it is more bioavailable and directly effective against OS.

KEYWORDS : Oxidative stress (OS); Lycopene; MDA; SOD; Vitamin C; Vitamin E, before supplementation(BS), post supplementation(PS)

Introduction

Oxidative stress represents an imbalance between the production of reactive oxygen species and biological system's ability to readily detoxify the reactive intermediates or free radicals to repair the resulting damage¹. It may be prevented by lycopene supplementation. Lycopene is a 40 carbon acyclic carotenoid containing 11 conjugated double bonds, a phytochemical found in tomatoes and other red fruits, lycopene configuration enable it to inactivate free radicals.

Oxygen derived free radicals are the most reactive species and as an antioxidant lycopene has a singlet oxygen quenching ability twice as high as that of β -carotene and 10 times higher than that of α -tocopherol², lycopene participate in a host of chemical reactions to protect critical cellular biomolecules including lipid, proteins and DNA³. Lycopene from processed tomato products appears to be more bioavailable than from raw tomatoes⁴. Comparative bioavailability of lycopene from diverse tomato products such as paste, juice, ketchup, sauce and soup are not known but lycopene from processed tomatoes was shown to be more bioavailable than from fresh tomatoes⁵. Dietary lipids and heat treatment formulate lycopene which is more bioavailable because of its early release from the food matrix and heat-induced isomerization from all trans to cis conformation enhance its biological activity^{3,6}.

Despite several epidemiological and experimental evidences showing tomatoes lycopene is effective against oxidative stress^{3,4,5,6}, there is no data on the effect of lycopene capsules in patients suffering from OS in India so the aim of the study is to access the comparative analysis of various forms of lycopene in OS by evaluating antioxidant vitamins and enzymes status related to oxidative stress.

Material and Methods

We enrolled 123 patients from outpatient department of NSCB Medical College Jabalpur M.P. for the study in which 54 patients not fulfilling the criteria (Based on their life style) of having oxidative stress 24 patients left the study before its completion, only 45 patients having oxidative stress were involved in the study till the end. 30 age and sex matched healthy subjects having sedentary life style, doing yoga regularly were selected as a control. Patients already on antioxidant supplementation at the time of enrollment

were excluded. After overnight 12 hours fasting; blood samples were collected, under aseptic conditions in 3 rounds. 1st round is for baseline levels soon after enrollment of patients. 2nd round involve collection of samples after 2 weeks of lycopene restricted diet. Final and 3rd round of samples collection involve 10 weeks after lycopene supplementation. Samples were analyzed for biochemical markers within 3 hours of sample collection by the following methodologies: MDA (TBARS)⁷, SOD by Mishra and Fridovich⁸, Vitamin E (alpha tocopherol)⁹ and Vitamin C¹⁰.

After estimation of base line antioxidant profile in patients and control, patients were divided in to 3 different groups [group-1 BS/PS (before supplementation/post supplementation), group-2 BS/PS, group-3 BS/PS] each group includes 15 patients.

Group-1 BS (n=15) supplemented 15 mg of lycopene capsule, 200 gm of tomato lycopene (products like soup, paste, ketchup) contain 15 mg of lycopene supplemented to group-2 BS (n=15). Group-3 BS (n=15) supplemented in placebo form (capsules filled with wheat flour). Patients blood samples were reassessed for the same parameters after follow up of 10 weeks of various variety of lycopene supplementation.

Statistical analysis was performed by using SPSS 14.3, which involves paired and unpaired t-tests. Mean values and $\pm$ SD were calculated for each group and were compared between patients before and after different forms of lycopene supplementation. p value <0.05 were taken as point of minimal statistical significance.

Results

In our study mean age of control was 50.4 $\pm$ 5.7 and 52.4 $\pm$ 4.8 of patients, ratio of male/female was 16/14 and 25/20 for control and patients respectively. At the time of enrollment lipid per oxidation product MDA was found to be increased significantly 6.3 $\pm$ 0.69 nmol/ml (p<0.001) where as SOD and vitamins showed significant decrease as SOD 3.13 $\pm$ 0.74 Units/ml (p<0.001), Vitamin E 0.35 $\pm$ 0.18 mg/dl (p<0.05) and Vitamin C 0.27 $\pm$ 0.12 mg/dl (p<0.001) in patients of oxidative stress as compare to control. After washout period the estimated levels of different Antioxidants in each group was compared with the post supplementation levels as in group-1 PS lipid per oxidation product MDA was 3.71 $\pm$ 0.69 nmol/ml, in group-2 PS 3.31 $\pm$ 0.80 nmol/ml p value is (p<0.001) highly significant for

both group whereas in group-3PS it is not significant (5.87 ± 0.82 nmol/ml) as compare to group-1BS, group-2BS and Group-3BS respectively. SOD and vitamins showed significant difference in group-1PS and group-2 PS as in group 1PS SOD 6.01 ± 0.70 U/ml ($p < 0.001$), Vitamin E 0.92 ± 0.31 mg/dl ($p < 0.001$), Vitamin C 0.77 ± 0.32 mg/dl ($p < 0.05$) and in group-2PS SOD 5.7 ± 0.56 U/ml ($p < 0.001$), Vitamin E 0.93 ± 0.39 mg/dl ($p < 0.001$), Vitamin C 0.70 ± 0.32 mg/dl ($p < 0.05$) as compare to group-2BS. Antioxidant levels of group-3PS are not significant as compare to group-3BS.

Discussion

In present study selection of lycopene dose in natural and synthetic form is based on the daily recommended and effective levels of lycopene against OS. Serum MDA is significantly increases in patients suffering from oxidative stress as compared to control while there is a significant decrease in the antioxidant status of the body, reflected by low levels of superoxide dismutase (SOD), Vitamin E and C.

If we compared different groups of patients before and post lycopene supplementation we found that serum MDA is significantly decreases in group 1-PS, 2-PS (post lycopene supplementation) as compared to group 1-BS and group 2-BS except group 3-PS (placebo), it is in accordance to previous findings that OS resulting in excessive free radical formation and these reactive oxygen species degrade polyunsaturated lipids, forming malondialdehyde. This compound is a reactive aldehyde, production of MDA is used as a biomarker to measure the activity of cells in OS patients¹¹. Serum SOD activity is significantly decreased in patient groups 1BS, 2BS, 3BS simply confirmed; SOD outcompete damaging reactions of superoxides released by cells thus protecting them from superoxide toxicity¹².

Antioxidant and Vitamins work with the synergy of antioxidant enzymes; Vitamin E is a most important chain breaking antioxidants and protects polyunsaturated fatty acids from peroxidative damage by donating hydrogen to the lipid peroxyl radical¹³, because of the lipophilic property of the tocopherol, it is the major free radical chain terminator in the lipophilic environment and proven protective against oxidative stress. Vitamin C acts as a reducing agent directly reacts with superoxides, hydroxyl radicals, and various lipid hydroperoxides¹⁴.

The oxidative stress was drastically reduced and antioxidant status was improved by supplementation of lycopene in both the forms. As an antioxidant lycopene has a singlet oxygen quenching ability twice as high as that of β -carotene and 10 times higher than that of α -tocopherol¹⁵, it protect critical cellular biomolecules including lipid, proteins and DNA³ and create first line of defense against free radicals so it guard all the antioxidant enzyme and vitamins from oxidative damage thus there is improvement in the levels of SOD, Vitamin E and C in lycopene supplemented groups independent of its natural and synthetic form.

Tomato lycopene is effective against OS, it is established by several studies^{15,16}. Inadequate data is available regarding effect of synthetic lycopene in prevention of OS, Hoppe et al.¹⁷ reported that a 28-day supplementation of 15 mg/day of synthetic lycopene (Lycovit 10% beadlets), or tomato-based lycopene (Lyc-O-Mato) led to identical bioavailability in healthy normal lipidemic subjects. In a recent study, Zhao et al.¹⁸ also showed a significant increase in plasma lycopene levels (from $0.5 \mu\text{mol/L}$ on day 1 to $1.5 \mu\text{mol/L}$ on day 57) in healthy postmenopausal women, following a 56-day supplementation of 12 mg/day of synthetic lycopene. Our study findings showed that synthetic and natural form is effective against OS. Supplementation of natural lycopene is not much effective as compare to synthetic form as natural form is the combined effect of antioxidants present in tomatoes while synthetic form is solely responsible for removing free radicals by lycopene only.

Supplementation of tomato is the combined effect of all carotenoids showed by patients so above studies could not expressed the role of lycopene only in prevention of OS. In our study we also found that lycopene supplementation in the form of tomatoes provide other dietary antioxidants like Vitamin C, E showed by the increased concentration of vitamin E and C in group 2PS as compare to group 2BS.

Conclusion

Tomato is very common form of vegetable utilized by each and every socioeconomic group in daily diet of our population therefore it is the major accessible antioxidant without giving any specific attention and economic burden so antioxidant in the form of lycopene. It is beneficial for peoples of developing country like India where people are less concern with their health. Our study concluded that lycopene supplementation in different forms (natural and synthetic) is protective against OS which is supported by increase in concentration of SOD and decrease MDA in patients suffering from OS. However synthetic form is more effective as it need no heat transformation, directly absorbed by the cells thus more bioavailable in comparison to natural form. Establishment of synthetic lycopene in respect of oxidative stress needs more epidemiological studies.

Table:-1 Levels of Antioxidant vitamins and enzymes in controls and patients.

Variables	Controls (n=30)	Patients(n=45) (Baseline levels)
Age	50.4 $\pm$ 5.7	52.4 $\pm$ 4.8 ^{NS}
Sex(M/F)	16/14	25/20
MDA nmol/ml	3.13 $\pm$ 0.73	6.3 $\pm$ 0.69**
SOD Unit/ml	5.51 $\pm$ 0.67	3.13 $\pm$ 0.74**
Vit E mg/dl	1.15 $\pm$ 0.40	0.35 $\pm$ 0.18*
Vit C mg/dl	0.95 $\pm$ 0.32	0.27 $\pm$ 0.12**

** highly significant * significant

NS not significant

Table:-2: Level of Significance of Antioxidants before and post lycopene supplementation in patients

Variables	Before Supplementation (after 2 weeks of lycopene restricted diet)			Post Supplementation weeks of (after 10 lycopene supplementation)		
	Group 1BS	Group 2BS	Group 3 BS	Group 1 PS	Group 2 PS	Group 3 PS
MDA	6.6 $\pm$ 0.70	6.4 $\pm$ 0.67	6.2 $\pm$ 0.79	3.71 $\pm$ 0.69*	3.31 $\pm$ 0.80*	5.87 $\pm$ 0.82
nmol/ml						NS
SOD	3.09 $\pm$ 0.64	3.36 $\pm$ 0.83	3.13 $\pm$ 0.59	6.01 $\pm$ 0.7a0*	5.7 $\pm$ 0.56**	3.27 $\pm$ 0.62
Units/ml						NS
Vit E	0.32 $\pm$ 0.14	0.4 $\pm$ 0.21	0.34 $\pm$ 0.19	0.92 $\pm$ 0.31*	0.93 $\pm$ 0.39*	0.40 $\pm$ 0.14 ^{NS}
mg/dl						
Vit C	0.26 $\pm$ 0.11	0.28 $\pm$ 0.14	0.29 $\pm$ 0.13	0.77 $\pm$ 0.32*	0.70 $\pm$ 0.31*	0.32 $\pm$ 0.10 NS
mg/dl						

** Highly significant * significant NS not significant

Group 1BS/PS –lycopenesupplemented in the form of capsule (Synthetic)

Group 2 BS/PS -lycopenesupplemented in the form of tomato products (Natural)

Group 3 BS/PS-plecebosupplementation oflycopenes

Chart:-1 Baseline Levels of Antioxidant vitamins and enzymes in different groups.

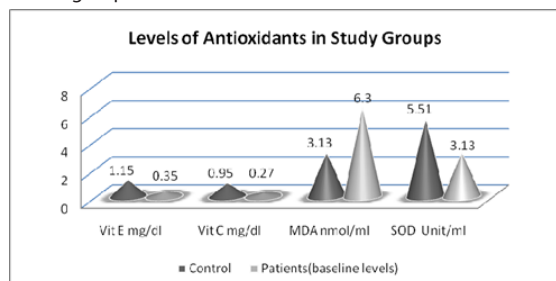


Chart:-2 Levels of Antioxidant vitamins in different patient's groups (group 1- BS/PS lycopene supplementation in the form of capsule, group 2 - BS/PS lycopene supplementation in the form of tomato products, group 3 -- BS/PS placebo supplementation of lycopene to the patients)

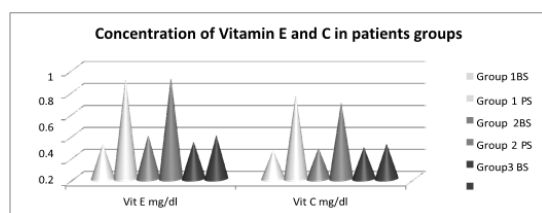


Chart:-3 Levels of MDA in different patient's groups (group 1- BS/PS lycopene supplementation in the form of capsule, group 2 - BS/PS lycopene supplementation in the form of tomato products, group 3 -- BS/PS placebo supplementation of lycopene to the patients)

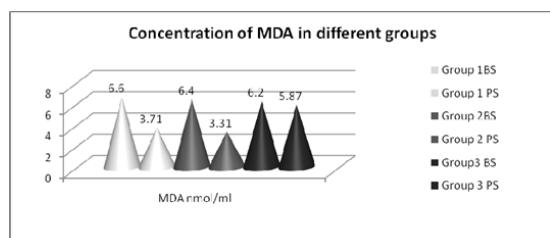
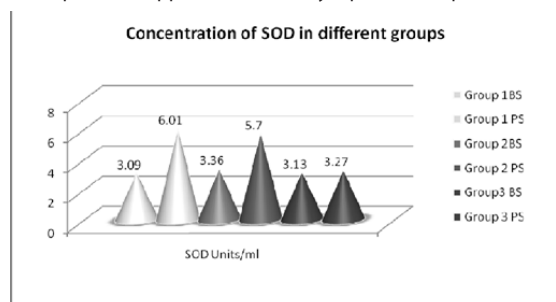


Chart:-4 Levels of SOD in different patient's groups (group 1- BS/PS lycopene supplementation in the form of capsule, group 2 - BS/PS lycopene supplementation in the form of tomato products, group 3 -- BS/PS placebo supplementation of lycopene to the patients)



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