Effect of Lycopene Supplementation on Osteoblastic Cells

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Summary

Lycopene is a carotenoid phytochemical antioxidant found in tomatoes. Oxygen derived free radicals are the most reactive species cause oxidative stress. It is one of the major causes of osteoporosis with increased lipid peroxidation, distorted osteoblastic cell activity and antioxidant status. Supplementation of lycopene improves antioxidant status by maintaining osteoblastic cell activity because it has a singlet oxygen quenching ability twice as high as that of β-carotene and 10 times higher that of α-tocoferol therefore believe to improve the condition of osteoporotic patients.
The present study included 60 clinically diagnosed osteoporotic patients, age between 40-60 years. 60 patient matched healthy subjects were taken as control. After estimation of base line osteoblastic markers including ALP, Pi and Ca^{++}, lipid peroxidation product MDA, oxidative stress biomarkers like SOD, GPX, GR, GSH, antioxidant vitamins A, C and E, patients supplemented 180gm of tomato products. After 90 days of lycopene supplementation patients were reassessed for all biochemical parameters included in the study.
The results of study revealed increased ALP while decreased Pi and Ca^{++} in osteoporotic patients but after lycopene supplementation levels were improved without any significant change in inorganic phosphorus. Decreased lipid peroxidation and oxidative stress were confirmed by MDA and recovered levels of SOD, GPX, GR, GSH, Vitamin A, Vitamin E and Vitamin C respectively in patients.
The results of present study concluded that dietary intake of tomato lycopene is beneficial for bone health in osteoporotic patients as it acts as an external antioxidant to fulfill the body need. It is confirmed by improved levels of osteoblastic markers and antioxidants after lycopene supplementation.

Key Words: - Osteoporosis; Oxidative stress; Lycopene, Alkaline Phosphatase (ALP); Inorganic Phosphorus (Pi); Free or ionic calcium; MDA; GSH; SOD; Vitamin C; Vitamin E.

Running title: "Osteoprotective effect of lycopene"

Introduction

Bone is a dynamic tissue that is continuously renewed all over the life by the process of bone remodeling, which involves the coupled events of removal of old bone by osteoclasts and formation of new bone by osteoblasts.[1,2] The remodeling process is the result of interactions, involving these cells and multiple molecular agents like hormones, growth factors, and cytokines. Disturbances in metabolic process due to oxidative stress and free radical generation alter the process of bone remodeling and lead to bone diseases.[3,4] Osteoporosis is a major metabolic bone disorder characterized by low bone mass and micro architecture deterioration of bone tissue causing enhanced bone fragility lead to increased risk of fracture therefore it is known as "silent disease"[5] and affects 1 in 4 women and 1 in 8 men.[6] Osteoporotic fractures are a major cause of morbidity and disability in the elderly, these fractures also contribute to a considerable economic burden on health services. Only in India about 60 million adults have osteoporosis and approximately 2-3 million cases are being added annually[7]. There are several risk factors for osteoporosis including non modifiable and modifiable risk factors in which non modifiable risk factors are asian race, old age, female sex, inactivity, lack of weight bearing exercise. Modifiable risk factors include smoking, excessive alcohol consumption, excessive caffeine consumption, deficiency of calcium, lack of sunlight exposure, diabetes mellitus, hyperparathyroidism and oxidative stress.[8,9]

Of these risk factors oxidative stress represents an imbalance between the production of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage.[10,11,12,13] 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 fruit, lycopene configuration enables it to inactivate free radical.

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 that of α-tocoferol[14], lycopene participate in a host of chemical reactions to protect critical cellular biomolecules including lipid, proteins and DNA.[15,16] Lycopene from processed tomato products appears to be more bioavailable than from raw tomatoes[17]. Comparative bioavailability of lycopene from diverse tomato products such as paste, juice, ketchup, sauce and soup are not known but lycopene from tomato paste was shown to be more bioavailable than from fresh tomatoes[18]. Dietary lipids and heat treatment formulate lycopene more bioavailable and after processing lycopene release from the food matrix. Heat-induced isomerization from all trans to cis conformation enhance lycopene bioavailability[15, 19]

Despite several epidemiological and experimental evidences showing antioxidant status in osteoporotic patients, there are no data on the effect of lycopene in patients suffering from osteoporosis in India so the aim of the study is to access the consequences of lycopene supplementation in osteoporotic patients by evaluating antioxidant vitamins and enzymes status.

Materials and Methods

The present study includes 60 patients having osteoporosis (irrespective of etiology), age between 40-60 years, nonsmokers, with no history of chronic systemic illness and 60 patients matched healthy subjects were taken as control. All subjects were selected from outpatient department of NSCB Medical College Jabalpur M.P. Patients already on antioxidant supplementation at the time of enrollment were excluded. After overnight 12 hours fasting; blood sample (10 ml whole blood) was collected, under aseptic conditions, from both the patients and control. Samples were analyzed for biochemical markers within 3 hours of sample collection by the following methodologies: ALP[20], Pi[21], Ca^{++} [22], MDA (TBARS)[23], SOD by Mishra and fridovich[24] and Erythrocyte reduced

glutathione (GSH) by Beutler et al[25], GPX by Paglia D.E. et al[26], GR by Carlberg I[27], Vitamin E (alpha tocopherol) [28], Vitamin A(Retinol) [28], Vitamin C[29].

After estimation of base line osteoblastic markers and antioxidant profile in patients and control, we supplement 180 gm of tomato (products like soup, paste, ketchup) contain 12 mg of lycopene to patients and their blood samples were reassessed for the same parameters after follow up of 90 days lycopene supplementation period.

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

Results

The main result of the study revealed significant changes in osteoblastic markers i.e. ALP, Ca^{++}, and Pi. ALP showed significant increase 93.3±5.41 IU/l (P<0.001), ionic calcium showed significant decrease 1.05±0.39 mmol/l (P<0.001) while no significant change in Inorganic Phosphorus 3.44±0.61mg/dl (NS). Lipid per oxidation product MDA was found to be increased significantly 6.54±0.71nmol/ml (p<0.001) where as other antioxidant enzymes and vitamins showed significant decrease as SOD 3.11±0.72 Units/ml (p<0.01), GPX 47.7±5.24 Units/gHb (p<0.01), GR 40.04±6.03 Units/L (p<0.001), GSH 8.17±1.10Units/gHb (NS), Vitamin A 18.0±5.72 µg/dl (p<0.002), Vitamin E 0.35±0.19mg/dl (p<0.001) and Vitamin C 0.28±0.13 mg/dl (p<0.01) in osteoporotic patients as compare to control. After supplementation of lycopene osteoblastic marker ALP achieved normal range 90.3±3.9IU/l (p<0.001), ionic calcium increased significant to the level of 1.29±0.46mmol/l (p<0.001) while no significant change in Inorganic Phosphorus 3.8±0.60 mg/dl(NS) was found, lipid per oxidation product MDA significantly decreases 3.89±0.41 nmol/ml (p<0.001) while other antioxidant enzymes and vitamins showed significant increase as SOD 5.8±0.68U/ml (p<0.01), GSH 9.15±0.75U/gHb (p<0.001), Vitamin C 0.69±0.21mg/dl (p<0.001), Vitamin A 52.01±10.64µg/dl (p<0.002), Vitamin E 0.59.±0.14mg/dl. Levels of GPX 72.9±7.61 U/gHb (NS), GR 54.9±6.02Units/L (NS) were not found to be significant. Results of present study shows improved osteoblastic markers, antioxidant profile and decreased lipid peroxidation product in patients after lycopene supplementation.

Table:-1 Levels of Osteoblastic markers, Antioxidant vitamins and enzymes in patients and control.

Parameters	Control	Osteoporotics
Alkaline phosphatase IU/l	71.7±6.7	93. 3±5.41**
Free or ionic Calcium mmol/l	1.33±0.48	1.05±0.39**
Inorganic Phosphorus mg/dl	3.9±0.60	3.44±0.61NS
Vit A µg/dl	52.36±8.6	18.0± 5.72*
Vit E mg/dl	1.2±0.37	0.35±0.19**
Vit C mg/dl	0.96±0.28	0.28±0.13*
MDA nmol/ml	3.10±0.69	6.5±0.71**
SOD Units/ml	5.52±0.66	3.11±0.72*
GPX Unit/gHb	64.8±7.46	47.7±5.24*
GR Unit/L	55.61±6.4	40.04±6.03**
GSH Unit/gHb	8.72±0.73	8.17±1.10 NS

**** highly significant * significant NS not significant**

Table:-2 Level of Significance of Osteoblastic markers, antioxidant enzymes, vitamins before and after lycopene supplementation in patients.

Parameters	Baseline levels	After Supplementation
Alkaline phosphatase IU/l	93. 3±5.41	90±3.9**
Ionic Calcium mmol/l	1.05±0.39	1.29±0.46**
Inorganic Phosphorus mg/dl	3.44±0.61	3.8±0.60 NS
Vit A µg/dl	18.0± 5.72	52.01±10.64**
Vit E mg/dl	0.35±0.19	0.59±0.14**
Vit C mg/dl	0.28±0.13	0.69±0.21**
MDA nmol/ml	6.5±0.71	3.89±0.41**
SOD Units/ml	3.11±0.72	5.8±0.68*
GPX Unit/gHb	47.7±5.24	72.94±7.61*
GR Unit/L	40.04±6.03	55.2±5.84**
GSH Unit/gHb	8.17±1.10	9.15±0.75**

** highly significant * significant

Chart 1:-Showing levels of osteoblastic markers in both groups

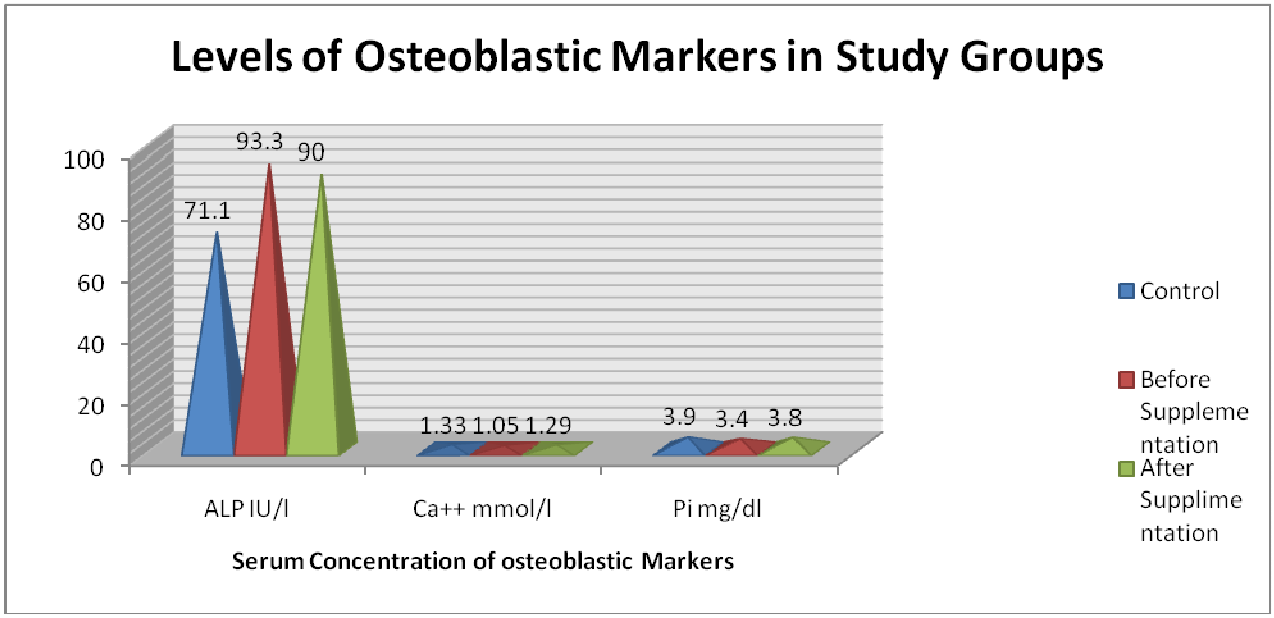
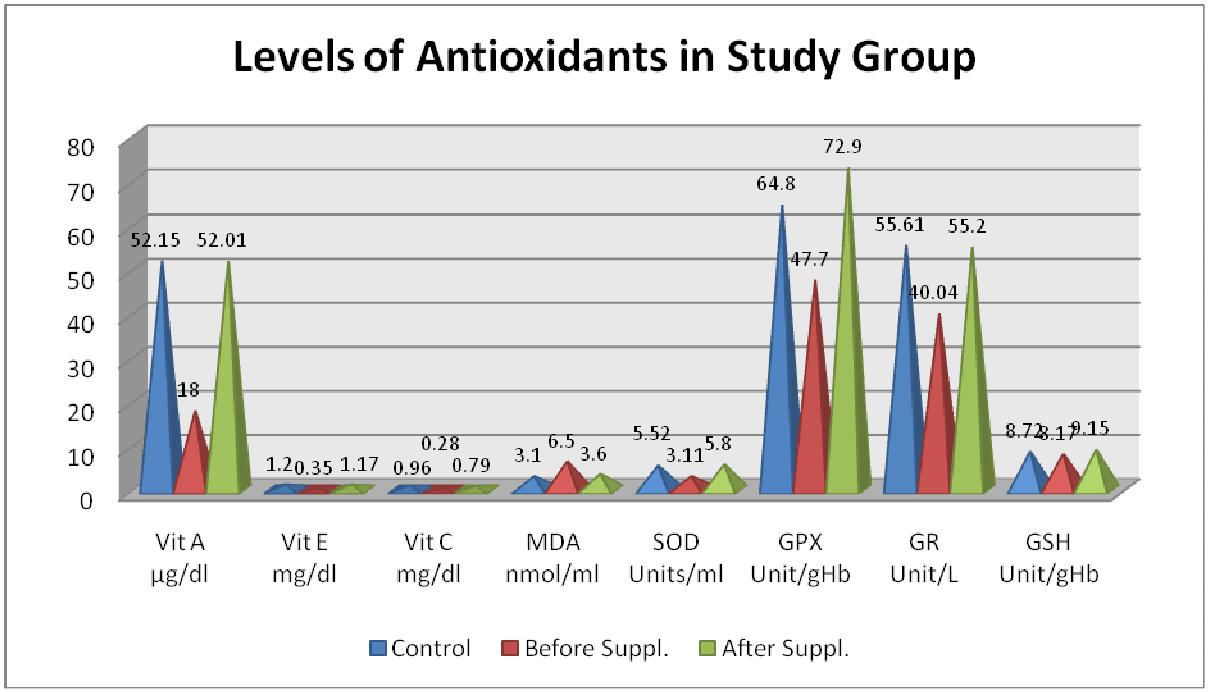


Chart 2:- Showing status of Antioxidant vitamins and enzyme in Control and Patients group before and after supplementation



Discussion

Osteoporosis due to oxidative stress results in excessive free radical formation indicated by increased Malondialdehyde level[30] similar results were found in our study. In present study osteoporotic patients showed increased levels of ALP, decreased levels of Ca^{++} with no significant change in Pi. Apart from this, there is a significant decrease in the antioxidant status of the body, reflected by low levels of reduced glutathione (GSH) glutathione peroxidase (GPx), glutathione reductase (GR), superoxide dismutase (SOD), Vitamin A, E and C.

In this study included markers of osteoblastic activity and antioxidant status, both enzymatic and nonenzymatic. Normally osteoblast secrets cytokines during oxidative stress, these cytokines induce the activity of osteoclast that secretes acid, an enzyme protease which causes lysis of collagen and bone deformation. Deformation of bone is repaired by osteoblast with the release of enzyme alkaline phosphatase which helps in mineralization of bone by deposition of calciumphosphate[31] similar trends were found in present study enhanced activity of ALP and decrease in free calcium in osteoporotics with increased oxidative stress.

After lycopene (a potent antioxidant) supplementation levels of free radicals was found to be decreased in the study, reduced levels of free radical leads to decrease osteoclastic activity with decreased bone deformation, less osteoblastic activity and normal ALP levels.

In our study we found increased Ca^{++} levels after lycopene supplementation, the reason could be, increased levels of ALP nullify the effect of pyrophosphatase (enzyme that prevent the precipitation of calcium) which leads to increased deposition of calcium as calcium phosphate in the bone[32] but after lycopene supplementation decrease in oxidative stress leads to decreased osteoblastic activity, less ALP formation and release therefore there is significant increase in Ca^{++} as compared to before supplementation of lycopene in osteoporotics with no significant change in inorganic phosphate.

In present study serum MDA is significantly increases in patients suffering from osteoporosis as compared to control, it is in accordance to previous findings that in osteoporosis decreased in osteoblastic activity leads to increase in osteoclastic activity (cells responsible for bone resorption) 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 osteoclast in osteoporotic patients.[30,33] Serum SOD activity is significantly decrease in patient group as compare to control simply stated, SOD outcompetes damaging reactions of superoxides released by osteoclast thus protecting osteoblast from superoxide toxicity.[34,35]

Glutathione peroxidase(GPx) and reductase(GR) are two enzymes; GSH is the substrate for them. In this study, lycopene supplementation also increased the levels of reduced glutathione the most important antioxidant metabolite that plays an important role in maintaining significant levels of GPX activity. This is the main enzyme involved in removing the H_2O_2 generated from dismutation of anions by SOD.GSH is also the cofactor of several reducing enzyme such as ascorbate reductase and endoperoxidase isomerase.
The above results suggested that tomato lycopene reduces lipid peroxidation rate by acting as a good chain breaking antioxidant, which reacts with peroxides formed in the propagation phase of lipid peroxidation to form carbon centered radicals. These radical reacts readily and reversibly with oxygen to form new chain carrying peroxile radicals which are stable than.[36]
Antioxidant Vitamins work with the synergy of antioxidant enzymes, Vitamin E and vitamin A are most important chain breaking antioxidants and they protect polyunsaturated fatty acids from peroxidative damage by donating hydrogen to the lipid peroxyl radical.[37,38] Because of the lipophilic property of the tocopherol, it is the major free radical chain terminator in the lipophilic environment and proven protective against hip fracture.[39] Vitamin C act as a reducing and antioxidant agent directly reacts with superoxides, hydroxyl radicals, and various lipid hydroperoxides.[40] it has positive role in bone health as vitamin C is a cofactor in the maturation of collagen.[41]

The oxidative stress was drastically reduced and antioxidant status was improved by supplementation of lycopene. As an antioxidant lycopene has a singlet oxygen quenching ability twice as high as that of β-carotene and 10 times higher that of α-tocoferol,[14] lycopene participate in a host of chemical reactions to protect critical cellular biomolecules including lipid, proteins and DNA.[15,16] lycopene create first line of defense against free radicals so it protect all the antioxidant enzyme and vitamins from oxidative damage thus there is improvement in the levels of all antioxidants after lycopene supplementation.

In addition lycopene supplementation in the form of tomatoes provide other dietary antioxidants like Vitamin C, A, E. These dietary antioxidants contribute for the improvement of bone health and play vital role in the recovery of disease.

Conclusion

The above observations show a significant decrease in oxidative stress with maintenance of osteoblastic cell activity after lycopene supplementation in the form of tomatoes. Lycopene in tomatoes allows for 90% of the total carotenoids and other phytochemicals with other dietary antioxidant vitamins uses for good bone health the study also suggest that body's internal production of antioxidant is not enough to neutralize all free radicals so increased dietary intake of potent antioxidant like lycopene in the form of tomato products is essential to maintain good bone

health. Tomato lycopene is easily for low socioeconomic group which plays an important role in treatment and prevention of osteoporosis in developing country like India.

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