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Research Article

IN VITRO EFFECT OF LYCOPENE ON OXIDATIVE STRESS IN THALASSEMIA MAJOR PATIENTS

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ABSTRACT

Background: The aim of the present investigation was to study the *in vitro* effect of lycopene on oxidative stress by measuring the MDA level which is the marker of oxidative stress in thalassemia major patients. This study also meant for measuring the enzymatic and non enzymatic antioxidant level in thalassemic blood sample incubated with lycopene.

Methods: Blood samples of thalassemic patients after two months of blood transfusion were taken and incubated with lycopene at concentration 1.5µmol/l, 5µmol/l, and 10µmol/l .Then analysed for effect of lycopene on oxidative stress as well as for antioxidant levels.

Results: From the obtained result it was cleared that plasma MDA levels was higher whereas erythrocyte SOD, GPx, Catalase and vitamin C levels were decreased in thalassemic patients as compared to the normal one. With increasing the concentration, lycopene restored antioxidant level of SOD, Catalase, GPx and Vitamin C significantly and decreased the MDA level also.

Conclusion: Lycopene might have quenched the superoxide and other free radical anions which are released in thalassemia major patients due to iron overload caused by regular blood transfusion. It leads to increase in concentration of SOD, GPx and CAT and decrease in concentration of MDA. Therefore, lycopene reverses the disturbed balance to the antioxidant enzyme side, and decreases the oxidative stress.

Keywords: Oxidative Stress, Lycopene, Antioxidant, Free radical.

INTRODUCTION

Thalassemia, a common genetic disorder characterized by impaired biosynthesis of the globin chain, resulting in ineffective erythropoesis and premature hemolysis[1]. β-Thalassaemia is characterized by reduction or absence of β-globin production[2] . The disorder affects about 150 million people in the Mediterranean, West Africa, and large parts of Asia[3]. Although there are now more than 180 known β-thalassaemia mutations worldwide[4], a smaller collection of alleles accounts for the inactivation of most β-globin genes in each population or ethnic group. In addition to the direct effects of reduced β-globin synthesis, many of the symptoms of this disorder appear to be consequences of the cytotoxic build up of free α-globin. Free α-globin is highly unstable and readily precipitates, and release iron in reactive form.[5,6] In addition to this, repeated blood transfusions and increased gastrointestinal iron absorption lead to iron overload in the body[7]. Humans are unable to eliminate the iron, and the excess iron is deposited as hemosiderin and ferritin in the liver, spleen, endocrine organs and myocardium. The deposited iron are responsible for the formation of reactive oxygen species such as superoxide anion (O_2^-), hydroxyl radical (OH^-), singlet oxygen and hydrogen peroxide (H_2O_2). If the production of ROS exceeds the capacity of enzymatic and non-enzymatic antioxidants systems to scavenge these species, then oxidative stress occurs.[7,8] This oxidative stress may contribute to shortened life span of erythrocytes, primary or secondary amenorrhoea, hypogonadism, osteoporosis, heart failure, endocrine abnormalities like diabetes, hypothyroidism, liver failure and ultimately early death[9,12] Biomarkers of oxidative stress included plasma malondialdehyde (a marker of lipid peroxidation)[13,16]

Antioxidants are protective agents that inactivate ROS and play an essential role in protection of the cells from oxidative damage[17]. They include several agents such as enzymes (glutathione peroxidase, superoxide dismutase, catalase), large molecules (ferritin, albumin), and small molecules (uric acid, glutathione, bilirubin, ascorbic acid, α-tocopherol, and vitamin E). Their defense mechanism in biological system involves chain breaking (SOD) and preventive (Vitamin E) mechanisms.[18,21]

Lycopene is a natural pigment synthesized by plants and microorganisms but not by animals. It is carotenoids, an acyclic isomer of β-carotene. It is a highly unsaturated, straight chain hydrocarbon containing 11 conjugated and two non-conjugated double bonds. Recent interest in lycopene has focused on its antioxidant properties[22,24]

The aim of the present investigation was to study the *in vitro* effect of lycopene on oxidative stress by measuring the MDA level which is the marker of oxidative stress in thalassemia major patients. This study also measures the enzymatic and non enzymatic antioxidant level in thalassemic blood sample which is incubated with lycopene to observe its possible antioxidants properties.

MATERIALS

Blood sample

Blood samples were obtained from 16 β-thalassemia major patients (10 male and 6 female subjects, age 5–18). The patients selected were undergo blood transfusion therapy two months before. 5 ml of venous blood was taken in heparinized tubes (Li Heparin 500 U/10 ml) after taking informed consent. Blood from healthy individual was used as normal control for this study. In these patients average hemoglobin concentration ranges from 4.3 to 6.8 g/dL.

Lycopene solution

Lycopene powder (Sigma-Aldrich, USA) was purchased and kept at –70°C. Lycopene (1mmol/L) stock was prepared in tetrahydrofuran (THF) in the dark room just before use.

Chemicals

Thiobarbituric acid, Phenazine methosulphate, Nitroblue tetrazolium, NADH, 5, 5-Dithiobisnitro benzoic acid, Ascorbic acid purchased from Himedia and all other chemicals used were obtained from Merck and Himedia were of analytical grade.

METHODS

Lycopene Treatment

Heparinized blood sample of thalassemic patients were incubated with lycopene at concentration of 1.5µmol/L, 5µmol/L and 10µmol/L. The mixtures were incubated at 37^{0}C for 1 hrs.

Preparation of Hemolysate

Incubated blood and blood of healthy person was centrifuged for 10-minutes at 3000 rpm. The plasma thus obtained was used for lipid peroxide estimation which is the marker of oxidative stress. Remaining packed RBCs were washed thrice with normal saline to remove the buffy coat. Hemolysis was performed by pipetting out 1 ml of washed red blood suspension in ice cold distilled water. Erythrocyte ghosts were sedimented in a high speed refrigerated

centrifuge at 12000 rpm for 40-minutes. The cell content was separated out carefully and used for estimation of superoxide dismutase, catalase, and glutathione peroxidase[25].

Estimation of lipid peroxidation

Plasma MDA levels were estimated by the method of Beuge *et al.,*[26] using thiobarbituric acid (TBA).The acid reacts with MDA to form a stable pink color with maximum absorption at 535 nm. Plasma MDA concentration was expressed as nmol/ml.

Estimation of Superoxide dismutase activity

The activity of Superoxide Dismutase (SOD) in hemolysate was estimated using the method of Kakkar *et al.,*[27]. A single unit of enzyme was expressed as 50% inhibition of NBT (Nitroblue tetrazolium) reduction min/ mg/Hb.

Estimation of Catalase activity

CAT was assayed colorimetrically at 620 nm and expressed as µmol of H_2O_2 consumed min^{-1} mg^{-1} Hb as described by Sinha[28].

Estimation of Glutathione peroxidase activity

The activity of Glutathione Peroxidase (Gpx) was estimated using the method of Rotruck *et al.,*[29]. The activity of Gpx was expressed in nmoles of GSH oxidised/min/g protein.

Estimation of Vitamin C

The activity of Vitamin C in plasma was estimated using the method of Omaye *et al.,*[30]. The activity was expressed in g dL^{-1}.

STATISTICAL ANALYSIS

All data are expressed as mean ± standard deviation. Result were analysed by student t test.

RESULTS AND DISCUSSION

The level of oxidative stress and antioxidant status in the healthy control, thalassemic control and the thalassemic blood sample incubated with lycopene shown in Table 1.

Table 1: Effect of Lycopene on Oxidative Stress and on Antioxidant level

Parameters	Thalassemic Control	Lycopene concentration			Healthy control
		1.5µmol/L	5µmol/L	10µmol/L	
MDA (nmol/ml)	4.18±1.19	3.75±0.76	3.07±0.53	2.94±0.52	2.08±.71
SOD (U/mg Hb)	2.75±0.81	2.85±1.31	3.19±1.27	3.22±1.18	3.39±0.89
Catalase (U/mg Hb)	3.28±0.76	3.16±0.82	3.83±0.81	3.89±0.92	4.8±0.93
Gpx (U/g Hb)	24.81±4.43	25.73±5.56	26.62±7.01	28.36±7.22	36.48±7.88
Vitamin C (g/dL of plasma)	0.89±0.22	0.93±0.21	1.04±0.25	0.91±0.23	1.23±0.27

MDA is a good indicator of oxidative damage. The extent of lipid peroxidation denotes the amount of free oxygen radicals generated, which have not been scavenged by the defense mechanism. In present study, the increased plasma MDA levels in thalassemia patients (4.18±1.19) were found as compared to the normal asymptomatic controls (2.08±.71). The effect of different concentration of lycopene1.5µmol/L, 5µmol/L and 10µmol/L on MDA content was studied in the thalassemic blood sample after one hour's incubation. The results (concentration wise) in the thalassemic blood sample showed significant decrease from 4.18±1.19 to 2.94±0.52 at 10µmol/L. In one of the previous studies, free and total MDA were found to be higher in regularly transfused thalassemia major patients [31-33]. There are increasing evidences suggesting that oxidative stress is a major damaging factor. This is due to the effect of continuous blood transfusions in these patients which leads to peroxidative tissue injury by the secondary iron overload. The extent of lipid peroxidation denotes the amount of free oxygen radicals generated, which have not been scavenged by the defense mechanism. Similarly, it was found that after six months of combined three vitamins (A, C & E) treatment, patients with β-thalassemia major exhibited a significant decrease in the levels of MDA[34]. Also it was found that malondialdehyde concentration in erythrocytes membranes of the patients who were treated with vitamin and β-carotene for 4 weeks, showed a significant decrease in MDA level as compared to the thalassemic control[33].

Erythrocytes are protected from oxidative stress by intracellular enzymes such as superoxide dismutase and several other constituents such as vitamin E[35]. Erythrocyte SOD is a preventive antioxidant. SOD activity is an indirect method for registration of the content of primary ROS (O_2·radicals).

SOD activity in patients with *β*-thalassemia major is decreased (2.75±0.81) as compared to normal healthy control (3.39±0.89).The effects of different concentration of Lycopene results showed maximum increase in SOD activity from 2.75±0.81 to 3.22±1.18 at concentration of 10µmol/L as compare to thalassemic control. SOD activity in patients with *β*-thalassemia major is decreased by more than 30% resulting in pronounced inhibition of the blood antioxidant capacity[36]. The activity of SOD was inhibited by 69% in rotenone treated animals and on lycopene supplementation; the activity increased by 12% when compared to controls. This was accompanied by cognitive and motor deficits in rotenone administered animals, which were reversed on lycopene treatment[37].

CAT, widely distributed in all cells, is present in high amounts in erythrocytes[38] which protect the cell from H_2O_2 generated by various reactions. In present study, the enzymatic antioxidants CAT in the hemolysate were significantly lower in thalassemia patients (3.28±0.76) as compared with healthy controls (4.8±0.93). The maximum significant increase in CAT activity was found (3.89±0.92) at 10µmol/L lycopene concentration as compared to thalassemic control (3.28±0.76), also similar results were found (3.83±0.81) at 5µmol/L lycopene treated blood sample. Decrease in the activity of CAT could be due to increase in the lipid peroxidation product, malondialdehyde which can form cross links, thereby inactivating several membrane bound enzymes.[39,40] It was showed significantly raised CAT activity in presence of lycopene which offers additional protection not only by efficient removal of hydrogen peroxide formed *in situ*[41].

The present study demonstrates significant reduction in red cell GPx in thalassemic control patients (24.81±4.43), as compared with healthy volunteers (36.48±7.88). Again the concentration wise analysis of lycopene treatment, results show no significant change in GPx activity from thalassemic control (24.81±4.43) to lycopene treated patient's blood sample (25.73±5.56, 26.62±7.01, 28.36±7.22 respectively). It was showed decreased GPx levels is due to inactivation by the increased superoxide anion production leading to an increase in oxidative stress.[42] The activity of GPX was not restored after lycopene treatment of cataract lenses of mice *in vitro*[41].

Lycopene, having good free radical scavenging capacity because of its high number of conjugated double bonds, might have quenched

the superoxide and other free radical anions which are highly released in β-thalassemia major patients thereby significantly restore the activity of SOD, CAT and non-significant at GPx levels. Thus this restoration of enzyme activity reverses the disturbed balance to the antioxidant enzyme side and hence decreases the oxidative stress in thalassemia patients.

Vitamin C, a hydrophilic vitamin, is an important radical scavenger antioxidant, present in all cells which can also act as a reducing agent. Vitamin C was significantly lower in thalassemic control (0.89±0.22) as compared to healthy volunteers (1.23±0.27). The maximum increase in the concentrations of vitamin C was found at concentration of 5µmol/L, from 0.89±0.22 (thalassemic control) to 1.04±0.25 (treated blood sample).

Ray *et al.*[43] showed that vitamin C has a particular role in vitamin E recycling and suggested that the vitamin C deficiency is found in thalassemic patient. In present study, decreased levels of plasma vitamin C may be due to their scavenging lipid peroxides. It was demonstrated that after the period of treatment, vitamin C and vitamin E increased significantly but could not be normalized. This was due to critically excessive oxidative stress[44]. The decrease in the concentration of vitamins C in thalassemic patients is accompanied with a high level of lipid peroxidation product (MDA) and consequently can account for the high percentage of hemolysis of erythrocytes in β-thalassemic patients. The increase in the concentrations of Vitamins C in lycopene treated blood sample can account for the decrease in the level of lipid peroxidation product and membrane damage.

CONCLUSION

From the obtained result it was cleared that plasma MDA levels was higher whereas erythrocyte SOD, GPx, catalase and vitamin C levels were decreased in thalassemic patients as compared to the normal one. With increasing the concentration of lycopene restored antioxidant level of SOD, Catalase and Vitamin C significantly but non-significantly in case of GPx.

The findings clearly suggested that lycopene posses good free radical scavenging capacity due to the presence of high number of conjugated double bonds. Lycopene might have quenched the superoxide and other free radical anions which are released in thalassemia major patients due to iron overload caused by regular blood transfusion, thereby increasing the concentration of SOD, GPx and CAT (the most important cytosolic enzymes involved in antioxidant activities). Therefore, lycopene reverses the disturbed balance to the antioxidant enzyme side, and decreases the oxidative stress.

Abbreviations

ROS: Reactive Oxygen Species, SOD: super oxide dismutase, GPx: glutathione peroxidase, CAT: catalase

Competing Interests

No

Authors' Contributions

All authors read and approved the final manuscript.

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REFERENCES

1. Edward J, Benz J R: In: Principles of Internal Medicine. 15th edn. McGraw-Hill Companies, USA2001: 663-673.
2. Weatherall DJ, Clegg JB: The Thalassaernia Syndromes. 3rd edition, Blackwell Scientific Publications, Oxford, UK 1981.
3. WHO: Community Control of Hereditary Anemias: Memorandum from a WHO Meeting. Bulletin WHO 1983; 61:63.
4. Flint J, Harding RM, Boyce AJ, Clegg JB: The population genetics of the hemoglobinopathies. Bailliere's Clin Hemato 1998; 11:1-50.
5. Brunori M, Falcioni G, Fioretti E, Giardina B, Rotilio G: Formation of superoxide in the autoxidation of the isolated α and β chains of human haemoglobin and its involvement in hemichrome precipitation. Eur. J. Biochem 1975; 53:99–104.
6. Scott MD, Van De Berg JJM, Repka T, Rouyer-Fessard P, Hebbel RP, Beuzard P, Lubin BH: Effect of excess α-hemoglobin chains on cellular and membrane oxidation in model β-thalassaemia erythrocytes. J. Clinic. Invest 1993; 91:1706–1712.
7. Milena R, Branka Z, Biljana S, Vera SR, Elsa R, Martin B: Thalassaemia syndrome in Serbia. Heamoglobin 2010; 34(5):477-485.
8. Bronspiegel-Weintrob N, Olivieri NF, Tyler B, Andrews DF, Freedman MH, Holland FJ: Effect of age at the start of iron chelation therapy on gonadal function in beta-thalassaemia major. New Eng Jour of Medicine 1990; 323:713–719.
9. Taher A, Isma'eel H, Cappellini MD: Thalassaemia intermedia: Revisited. Blood Cells Mol. Dis. 2006; 37: 12-20.
10. Rund D, Rachmilewitz E: Beta-thalassemia. New Eng J of Med 2005; 353(11): 1135-1146.
11. Repka T, Shalev O, Reddy R, Yuan J, Abrahamov A, Rachmilewitz A, Low PS, Hebbel RP: Nonrandom association of free iron with membranes of sickle and beta-thalassemic erythrocytes. Blood 1993; 82: 3204–3210.
12. Cighetti G, Duca L, Bortone L, Sala S, Nava I, Fiorelli G, Cappellini MD: Oxidative status and malondialdehyde in β-thalassaemia patients. Eur J Clin Invest 2002; 32[Suppl 1]:55-60.
13. Walter PB, Fung E, Killilea DW, Jiang Q, Hudes M, Madden J: Oxidative stress and inflammation in iron-overloaded Patients with β thalassemia or sickle cell disease. Braz Jour of Haem 2006; 135:254-63.
14. Kadiiska MB, Gladen BC, Baird DD, Germolec D, Graham LB, *et al.,*: Biomarkers of oxidative stress study II: are oxidation products of lipids, proteins, and DNA markers of CCl_4 poisoning? Free Rad Bio and Med 2005; 38(6):698-710.
15. Kadiiska MB, Gladen BC, Baird DD, Graham LB, Parker CE *et al.,*: Biomarkers of oxidative stress study III. Effects of the nonsteroidal anti-inflammatory agents indomethacin and meclofenamic acid on measurements of oxidative products of lipids in CCl_4 poisoning. Free Rad Bio and Med 2005; 38(6):711-718.
16. Tong PC, Ng MC, Ho CS, So WY, Li JK *et al.,*: C-reactive protein and insulin resistance in subjects with thalassemia minor and a family history of diabetes. Diabet Care 2002; 25:1480-1.
17. Baumgartener TG: Vitamins In: Van Way C.W. ed. Nutrition secrets. Philadephia, Hanley and Belfus, 1999; 13-20.
18. Dhawan V, Kumar KhR, Marwaha RK, Ganguly NK: Antioxidant status in children with homozygous thalassemia. Ind Pedi 2005; 42:1141-5.
19. Van Zoeren-Grobben D, Lindeman JH, Houdkamp E, Moison RM, Wijnen JT, Berger HM: Markers of oxidative stress and antioxidant activity in plasma and erythrocytes in neonatal respiratory distress syndrome. Acta Paediat 1997; 86:1356-62.
20. Simsek F, Ozturk G, Kemahl S: Oxidant and antioxidant status in beta thalassemia major patients. Ankara Universitesi Tip Fakultesi 2005; 58:34-8.
21. Kampa M, Nistikaki A, Tsaousis V, Maliaraki N, Notas G, Castanas E: A new automated method for the determination of the total antioxidant capacity (TAC) of human plasma, based on the crocin bleaching assay. Bio Med Central Clin Path 2002; 2(1):3.
22. Rao AV, Agarwal S: Role of lycopene as antioxidant carotenoid in the prevention of chronic diseases: a review. Nutr Research 1999; 19:305–323.
23. Stahl W, Sies H: Lycopene: a biologically important carotenoid for humans. Arch of Bio and Bio 1996; 36:1–9.
24. Nguyen ML, Schwartz SJ: Lycopene: chemical and biological properties. Food Tech 1999; 53:38–45.
25. McCord JM, Fridovich I: SOD enzyme function for erythropoietin. Jour of Bio Chem 1969; 224: 6049-6055.
26. Buege JA, Aust SD: The thiobarbuturic acid assay. Methods Enzymol 1978; 52:306–7.
27. Kakkar P, Das B, Viswanathan PN: A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophys 1984; 21(2): 130-132.
28. Sinha AK: Colorimetric assay of catalase. Anal Biochem 1972; 47(2): 389-394.

29. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG: Selenium: Biochemical role as a component for glutathione peroxidase. Science 1973; 197: 588-590.
30. Omaye ST, Turnbull JD, Sauberlich HE: Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. Meth Enzymol 1979; 62: 3-11.
31. Meral A, Tuncel P, Surmen-Gur E, Ozbek R, Ozturk *E*, Gunay U: Lipid peroxidation and antioxidant status in beta-thalassemia. Pedia Hema Oncology 2000; 17:687-693.
32. Tesoriero L, D'Arpa D, Butera D: Oral supplements of vitamin E improve measures of oxidative stress in plasma and reduce oxidative damage to LDL and erythrocytes in beta-thalassemia intermedia patients. Free Rad Res 2001; 34(5):529-540.
33. Mahjoub S, Tamaddoni A, Nikoo MZ, Moghadamnia AA: The effects of beta-carotene and vitamin E on erythrocytes lipid peroxidation in beta-thalassemia patients. Jou of Res in Med Sciences 2007; 12(6): 301-307.
34. Attia MMA, Sayed AM, Ibrahim FA, Mohammed AS, Elalfy MS: Effects of antioxidant vitamins on the oxidant/antioxidant status and liver function in homozygous beta-thalassemia. Rom jour of Bio 2011; 2: 93-106.
35. Chiu D, Kuypers F, Lubin B: Lipid peroxidation in human red cells. Sem Hematology 1989; 26:257-276.
36. Pavlova LE, Savov VM, Petkov HG, Charova IP: Oxidative stress in Patient with β- thalassemia major. Sec Biol med and Sci 2007; 145-154.
37. Kaur H, Chauhan S, Sandhir R: Protective effect of lycopene on oxidative stress and cognitive decline in rotenone induced model of Parkinson's disease. Eur Joul of Phys Reh Medicine 2011; 36(8):1435-43.
38. Speranza MJ, Bagley AC, Lynch RE: Cells enriched for catalase are sensitized to the toxicities of bleomycin, adriamycin, and paraquat. Jour Biol Chemistry 1993; 268:19039–19043
39. Kikugawa K, Kousugi H, Asakura T: Effects of MDA, a product of lipid peroxidation on the function and stability of hemoglobin. Arch of Bio and Biophysics 1984; 229: 227.
40. Pfafferott C, Meiselman HJ, Hochstin P: The effect of MDA on erthrocyte deformability. Blood 1982; 59: 62.
41. Gupta SK, Trivedi D, Srivastava S, Joshi S,Halder N, verma SD: Lycopene attenuates oxidative stress induced experimental cataract development: an *in vitro* and *in vivo* study. Nutrition 2003; 19:794-799.
42. Naithani R, Chandra J, Bhattacharjee J, Verma P, Narayan S: Peroxidative stress and antioxidant in children with beta thalassemia major. Pedi Blood Cancer 2006; 46 (7):780-85.
43. Ray SN, Marwaha RK, Sethuraan G, Trehan A. Scurvy in transfusion dependent ß-thalassemia**.** Indian Pedia 1999; 36:504–506.
44. Thasinas D, Piyaratana T, Panya S: The benefits of vitamin C and vitamin E in children with ß-thalassemia with high oxidative stress. Jour Medi Associ of Thailand 2005; 88: 4–8.