International Journal for Pharmaceutical Research Scholars (IJPRS)

V-4, I-1, 2015 ISSN No: 2277 - 7873

REVIEW ARTICLE

Relation of Oxidative Stress with Serum Antioxidant Enzymes Level in Thalassemic Subjects

Choudhary M*[1], Vyas RK[2]

[1,2]*Department of Biochemistry Sardar Patel Medical College, Bikaner (Raj.), India.*

Manuscript No: IJPRS/V4/I1/00016, Received On: 23/01/2015, Accepted On: 27/01/2015

ABSTRACT

The association of oxidative stress with serum antioxidant enzymes are not uncommon encounter in thalassemic patients. Patients with this disease need repeated blood transfusion for survival. This may cause oxidative stress and tissue injury due to iron overload, altered antioxidant enzymes levels. The levels of antioxidant enzymes in thalassemia patients revels a significant change. The aim of this review is to scrutinize the relationship between oxidative stress and serum antioxidant enzymes level and degree of damage caused by oxidative stress. Studies published on the antioxidant enzymes level in thalassemia patients also showed variable results. The reports are controversial for the relation of oxidative stress with serum antioxidant enzymes status. Hence the present study is undertaken to determine the correlation of oxidative stress with these enzymes in thalassemia patients.

KEYWORDS

Thalassemia, Serum Catalase, Superoxide Dismutase and Glutathione

INTRODUCTION

Thalassemia is the genetically inherited blood disorder passed down through families in which the body makes an abnormal form of hemoglobin, the protein in red blood cells that carries oxygen. It results in excessive destruction of red blood cells, which leads to anaemia. It is not infectious and cannot be passed from one individual to the other by personal or any other contact, or through blood transfusion, food or air.[1]

Recent data indicate that about 7% of the World's population is a carrier of a hemoglobin disorder and that 3,00,000-5,00,000 children are born each year with the severe homozygous states of these diseases.[2]

***Address for Correspondence:**
Dr. Mamta Choudhary
Biochemist, Department of Biochemistry
Sardar Patel Medical College
Bikaner (Raj.), India.
E-Mail Id: mamtasharad7@gmail.com

Thalassemia is one of the major hemoglobinopathies among the population all around the world. It has been reported that now a day's approximately 1 out of 14 peoples are carriers for different sub types of thalassemia. Each year about 400,000 infants born with serious hemoglobinopathies and carrier frequency is about 270 million.[3] In developing countries like in India, hemoglobinopathies increases in alarming rate due to lack of proper health care and knowledge. About 10% of total world thalassemic patients belong to Indian subcontinent, among them 3-4% are carrier. In India, 32,400 infants are born with hemoglobinopathies.[4]

Patients with this disease need repeated blood transfusion for survival. Recurrent blood transfusions in beta thalassemia major lead to accumulation of excess iron in the body tissues. This secondary iron overload is responsible for peroxidative damage by increased production of

reactive oxygen species within the erythrocytes leading to oxidative stress. This oxidative stress will cause growth failure as well as liver, cardiovascular, endocrine and neurological complications in thalassemia. Production of free radicals by iron overload, alteration in serum trace elements, and antioxidant enzymes status play an important role in the pathogenesis of thalassemia.[5] Use of iron chelatory agents in combination with antioxidants can be helpful in the regulation of the antioxidant status in patients with these patients. Oxidative stress and disturbance in antioxidant enzymes balance in thalassemia has been studied extensively.[6]

Oxidative Stress

Oxidative stress is defined as the interruption of balance between oxidants and reductants within the body due to the excess production of peroxides and free radicals. This imbalance will cause damage to cellular components and tissues in the body leading to oxidative stress. Oxidative stress occurs when the generation of free radicals and active intermediates in a system exceeds the system's ability to neutralize and eliminate them (Figure).[7]

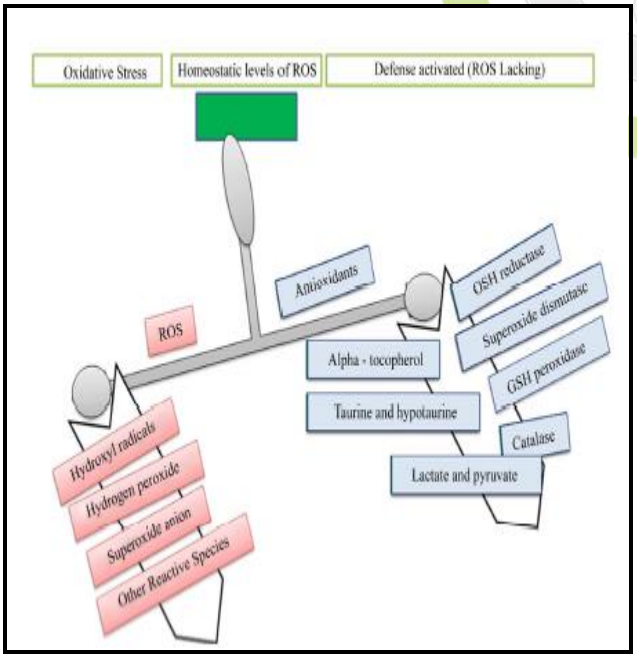

Figure 1: Imbalance between oxidant and antioxidant

To cope with the oxidative stress elicited by aerobic metabolism, animal and human cells have developed a ubiquitous antioxidant defense system, which consists of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase together with a number of low molecular-weight antioxidants such as ascorbate, α-tocopherol and glutathione, cysteine, thioredoxin, vitamins, etc. However, this antioxidant defense system may be overwhelmed by various pathological or environmental factors so that a fraction of ROS may escape destruction and form the far more reactive hydroxyl radicals.[8]

Oxidative Stress and Antioxidant Enzymes

Oxidative stress in thalassemia patients activates various antioxidant enzyme systems to protect the body tissues from its damaging effects. A large number of antioxidant enzymes present in the body, here we are interested to determine the antioxidant status of the following enzymes in thalassemia patients:

1- Catalase
2- Superoxide dismutase
3- Glutathione (GSH)

Catalase

Catalases, H202:H202 oxidoreductase, EC 1.11.1.6, was first discovered by Louis Jacques Thenard in 1818. Bovine liver catalase was one of the first enzymes to be isolated to a high state of purity and the first iron-containing enzyme to be isolated (Sumner and Dounce 1937). The reaction mechanism was initially proposed to be a free radical mechanism by Oppenheimer and Stern in 1939.

Catalase is an enzyme which is present mainly in the peroxisomes of mammalian cells. It is a tetrameric enzyme consisting of four identical, tetrahedrally arranged subunits of 60 kDa, each containing in its active center a heme group and NADPH. (Scibior and Czeczot 2006). Catalase has two enzymatic activities depending on the concentration of H2O2. If the concentration of H2O2 is high, catalase acts catalytically, i.e. removes H2O2 by forming H2O and O2 (catalatic reaction). However, at a low concentration of H2O2 and in the presence of a

suitable hydrogen donor, e.g. ethanol, methanol, phenol, and others, catalase acts peroxidically, removing H2O2, but oxidizing its substrate (peroxidatic reaction). Catalase is responsible for detoxification of hydrogen peroxide in the cells. Alteration in gene expression of this enzyme will lead to increased risk of cancer.[9]

Superoxide Dismutase

Superoxide dismutases, EC.1.15.1.1, are the most important antioxidant enzymes that alternately catalyze the dismutation (or partitioning) of the toxic superoxide (O_2^-) radical into either ordinary molecular oxygen (O_2) or hydrogen peroxide (H_2O_2). Two biochemists Irwin Fridovich and Joe McCord discovered the activity of superoxide dismutase. SOD's were previously known as a group of metalloproteins with unknown function; for example, Cu Zn SOD was known as erythrocuprein and as the veterinary antiinflamatory drug "Orgotein". Likewise, Brewer (1967) identified a protein that later became known as superoxide dismutase as an indophenol oxidase by protein analysis of starch gels using the phenazine-tetrazolium technique.

Superoxide is the main reactive oxygen species, produced as a byproduct of oxygen metabolism, which react with nitric oxide radical and forms peroxynitrite thereby causing oxidative stress and cellular damage. SOD is the essential antioxidant that decreases the formation of reactive oxygen species and oxidative stress thus protecting the cells from damage. Erythrocyte superoxide dismutase protects the erythrocyte from being damaged during oxidative stress.[10]

Glutathione

The next vital antioxidant enzyme in the body is glutathione which is a tripeptide containing three amino acids. It is present in almost all living cells and is considered to be the most powerful and most important antioxidant produced in the human body. Glutathione is found exclusively in its reduced form (GSH). Glutathione reductase, EC 1.8.1.7, catalyzes the reduction of glutathione disulfide (GSSG) to the sulfhydryl form glutathione (GSH), which is a critical molecule in resisting oxidative stress and maintaining the reducing environment of the cell. Ratio of reduced glutathione to oxidized glutathione can be used to determine the cellular toxicity.

It can act as a scavenger for hydroxyl radicals, singlet oxygen, and various electrophiles. Reduced glutathione reduces the oxidized form of the enzyme glutathione peroxidise, which in turn reduces hydrogen peroxide (H2O2), a dangerously reactive species within the cell. In addition, it plays a key role in the metabolism and clearance of xenobiotics, acts as a cofactor in certain detoxifying enzymes, participates in transport, and regenerates antioxidants such as Vitamins E and C to their reactive forms.[11]

Review of Literature

Preliminary studies have shown that levels of serum antioxidant enzymes might be altered in patients with thalassemia but the results were inconsistent. Now with the more accurate determination by atomic absorption spectrophotometer and spectrophotometer, the oxidative stress in thalassemia was found to be most frequently associated with abnormal serum antioxidant enzymes status in patients attending general medical clinics. A comprehensive and critical review of the work done on the subject of concern provides a sound base for further research.

G.C. Gerli et al. (1980)[12] revealed increase levels of antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase in red blood cells of beta thalassemia minor and nearly normal values of these enzymes in beta thalassemia major patients.

S.Ponnazhagan et al. (1992)[13] analysed glutathione reductase and glutathione peroxidase in 25 cases of homozygous beta thalassemia,20 heterozygous beta thalassemia and 10 controls. The results indicate significant elevation in these enzymes.

Mei-ling Cheng et al. in (2004)[14] studied on 182 alpha thalassemic patients and 50 healthy controls and analysed for redox status. They found the plasma level of Catalase, Superoxide

dismutase and Glutathione were significantly lower than controls.

S.Filiz et al. (2005)[15] revealed higher levels of superoxide dismutase and glutathione peroxidase in thalassemia major patients as compared to healthy controls.

AL-Mudalal et al. (2005)[16] also studied superoxide dismutase and catalase activities in RBCs in thalassemia major and minor. They observed that the SOD activity was increased in both the cases compared to healthy controls but thalassemia major patients have much higher SOD activity than in thalassemia minor. This correlation was not found in case of catalase activity.

Cheng ML et al. (2005)[17] studied that redox status of patients with thalassemia. The overall antioxidant capacity is plasma was inversely correlated with the severity of alpha-globin gene defect. The erythrocytes isolated from alpha thalassemia patients were found to had lower levels of glutathione, catalase and superoxide dismutase.

Veena Dhawan et al. (2005)[18] done a similar study and analyzed superoxide dismutase in 41 thalassemic and 20 healthy individuals and found that SOD enzyme activity in thalassemics, was at least 1.5 lower in comparison to the activity documented in controls ($p<0.05$). The observations indicates that thalassemics have enhanced oxidative stress. Administration of selective antioxidants and a balanced diet may preclude oxidative damage.

Rahul Naithani, Jagdish Chandra et al. (2006)[19] studied on 50 transfusion-dependent β-thalassemics, subjected to analysis of superoxide dismutase(SOD), glutathione (GSH), along with serum iron and ferritin, liver functions and uric acid and all these parameters in 30 non-anemic healthy controls attending the child health promotion clinic of hospital were also studied. They found that antioxidant enzyme SOD were significantly elevated in thalassemic children while mean GPx levels were decreased in patients compared to controls ($P < 0.001$).

Pavlova LE. et al. (2007)[20] studied on 22 thalassemia major patients and concluded that the activity of antioxidant enzyme SOD in the blood of thalassemia major patients is decreased by more than 30% and total antioxidant activity is dimished by about 70% compared to controls.

Faiza Waseem et al. (2011)[21] studied on 48 diagnosed patients of beta thalassemia major and 30 age and sex matched healthy subjects and glutathione peroxidase and superoxide dismutase were estimated. The levels of these enzymes were found significantly lowered ($p<0.001$) in thalassemia patients compared to control groups.

Maher Y. Abdalla et al. (2011)[22] analyzed the antioxidant status and oxidative damage in 40 children with Beta thalassemia and 40 health controls. The results showed that there was compensatory increase in Superoxide dismutase activity and decrease in Catalase activity, suggesting that iron overload is involved in the oxidative stress in cells.

M.M.A. Attia et al. (2011)[23] also observed in their study that the activities of Glutathione and SOD decreased significantly, also the activities of catalase and glutathione reductase significantly increased in thalassemic patients after treatment compared with their activities before treatment and control subjects.

A.B.Patne et al. (2012)[24] studied on 50 beta thalassemia major and 50 healthy controls and found that serum superoxide dismutase and glutathione peroxidase activities were significantly decreased in beta thalassemia major patients as compared to healthy individuals.

Elham Abed Mahdi et al. (2014)[25] studied on 100 subjects (50 beta thalassemia patients and 50 healthy controls) in the range of age 4-18 years and investigate the relationship between oxidative stress and serum antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), glutathione in thalassemia and result obtained that these enzymes level were decreased in thalassemic patients as compared to the normal controls.

CONCLUSION

This comprehensive review of literature indicates that oxidative stress in patients with thalassemia is mainly caused by peroxidative injury due to secondary iron overload. Production of free radicals cause alteration in serum antioxidant enzymes status which play an important role in the pathogenesis of thalassemia. There is limited data available concerning oxidative stress, antioxidant status, degree of peroxidase damage, and role of antioxidant enzymes in thalassemia patients. Studies on antioxidant enzymes like catalase, superoxide dismutase and glutathione reveal significant change in plasma concentration. The administration of selective antioxidant along with essential antioxidant enzymes, in order to reduce the extent of oxidative damage and the related complications in thalassemia still need further evaluation.

ACKNOWLEDGEMENT

We thank all members of staff at the faculty of medical Biochemistry department of Sardar Patel Medical college, Bikaner, Rajasthan for their help and support, and colleagues at Laboratory Unit of P.B.M. Hospital Bikaner for excellent technical assistance in conducting the antioxidant enzymes status analysis.

REFERENCES

1. Wikipedia.(2008).URL:http://en.wikipedia.org/w/index.php?title=Thalassemia&action=edit.
2. WHO. (2006). *March of Dime*.
3. Sarnaik, A. S. (2005). Thalassemia and Related Hemoglobinopathies. *Indian Journal of Pediatrics, 72*(4), 319-24.
4. Sinha, S., Black, M. L., Agarwal, S., Colah, R., Das, R., Ryan, K., Beegard, M., Bittles, A. H. (2009). Profiling â- thalassemia mutations in India at state and regional levels implications for genetic education, screening and counselling programmes. *Springer Science*, 3, 51-62.
5. Shazia, Q., Mohammad, Z. H., Rahman, T. and Shekhar, H. U. (2012). Correlation of oxidative stress with serum trace element levels and antioxidant enzyme status in beta thalassemia major patients: A review of the literature. *Anemia*, 7.
6. Suryakar A. N., Katkam, R. V. and Joshi, N. G. (2008). Oxidative stress and disturbance in antioxidant balance in beta thalassemia major. *Indian Journal of Clinical Biochemistry. 23*, 337-340.
7. Sies, H. and Cadenas, E. (1985). Oxidative stress: Damage to intact cells and organs. Philosophical Transactions of the Royal Society of Lord. *Biological Sciences, 311*, 617-631.
8. Halliwell, B. and Gutteridge, J. M. C. (1999). *Free radicals in biology and medicine*. 3rd Edition, Clarendon Press, Oxford.
9. Kirkman, H. N., and Gian F. Gaetanit Catalase, G. F. F. (1984). A tetrameric enzyme with four tightly bound molecules of NADPH *Proceedings of the National Academy of Sciences USA*, Vol. 81, 4343-4347.
10. Rakhit, R., Avijit Chakrabartty, A., (2006). Structure, folding, and misfolding of Cu, Zn superoxide dismutase in amyotrophic lateral sclerosis *Biochimica et Biophysica Acta* 1762, 1025–1037.
11. Marcel Deponte. (2013). Glutathione catalysis and the reaction mechanisms of glutathione-dependent enzymes; *Biochimica et Biophysica Acta*, 1830, 3217-3266.
12. Gerli, G. C., Beretta, L., Bianchi, M., Ellegatta, P. and Agostoni, A. G. (1980). Erythrocytesuperoxide dismutase, catalase, and glutathione peroxidise activities in beta thalassemia(major and minor). *Scandavian Journal of Haematology*. 25(1), 87–92.
13. Ponnazhagan, S., and Sarkar, R. (1992). Enzymes of the pentose phosphate pathway in glutathione-regulated membrane protection in b-thalassaemia. *European Journal of Clinical Chemistry and Clinical Biochemistry*. 30(8), 481–484.
14. Mei-ling Cheng, Hung-yao Ho, Hsiu-chuan Tseng,Chien-Hong Lee, Lee-yung Shih and Daniel Tsun-yee Chiu.(2004). Antioxidant

deficit and enhanced susceptibility to oxidative damage in individuals with different forms of *a*-thalassaemia. *British Journal of Haematology, 128*, 119–127.
15. S. Filiz, O. Gulyuz, K. Sabri, E. Deniz, and H. Alev. (2005). Oxidant and antioxidant status in beta thalassemia major patients. *Journal of Ankara University Faculty of Medicine*, 58, (1), 34–38.
16. Al-Mudalal S. S., Musa, R. J., Al-Duliami N. (2005). Antioxidant status in thalassemic patients. *Iraqi Journal of Medical Sciences, 4* (1), 38-44.
17. Cheng, M. L., Ho, H. Y., Tseng H. C., Lee C. H., Shih, L. Y., Chiu, D. T. (2005). Antioxident deficit and enhanced susceptibility to oxidative damage in individuals with different forms of alpha thalassemia. *British Journal of Haematology, 128*(1), 119-27.
18. Veena Dhawan, Kh. Ratan Kumar, R. K. Marwaha, and Nirmal K. Ganguly. (2005). Antioxidant status in children with homozygous b-thalassemia, *Indian Pediatrics* Volume 42.
19. Rahul Naithani, Jagdish Chandra, Jayashree Bhattacharjee, Priyanka Verma and Shashi Narayan. (2006). Peroxidative stress and antioxidant enzymes in children with β-thalassemia major; *Pediatric Blood & Cancer*, 46(7), 780-785.
20. L. E. Pavlova, V. M. Savov, H. G. Petkov, and I. P. Charova. (2007). Oxidative stress in patients with beta-thalassemia major, *Prilozi*, *28*(1), 145–154.
21. Faiza Waseem, Karira A. Khemomal, Raihan Sajid. (2011). Antioxident status in beta thalassemia major: A single case study, *Indian Journal of Pathology and Microbiology, 54*(4).
22. Maher Y. Abdalla, Mohammad Fawzi, Salem R. Al-Maloul, Nasser El-Banna, Reema F. Tayyem, and Iman M. Ahmad. (2011). Increased oxidative stress and iron overload in Jordanian b-thalassemic children; Informa Healthcare, *Hemoglobin*, 35(1), 67–79.
23. M. M. A. Attia, A. M. Sayed, F. A. Ibrahim, A. S. Mohammed. (2011). Effects of antioxidant vitamins on the oxidant/antioxidant status and liver function in homozygous beta-thalassemia, *Romanian Journal of Biophysics*, 21(2), 93-106.
24. B. Patne, P. J. Hisalkar, S. B. Gaikwad and S. V. Patil. (2012). Alterations in antioxidant enzyme status with lipid peroxidation in b-thalassemia major patients. *International Journal of Pharmacy and Life Sciences*, 3(10).
25. Elham Abed Mahdi. (2014). Relationship between oxidative stress and antioxidant status ib beta thalassemia major patients; *Acta Chimica & Pharmaceutica Indica. 4(3), 137-145.*

