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PRODUCTION OF REACTIVE OXYGEN SPECIES, ITS EFFECT, DRUGS AND PLANT EXTRACT USED AS AN ANTIOXIDANT, CHELATOR ON THALASSEMIC PATIENT: A REVIEW

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ABSTRACT

β- Thalassemia is an inherited genetic disorder which is caused by different kinds of mutations in the HBB gene in chromosome 11. Due to several types of mutation in β- gene, globin chains cannot synthesise completely and free α-globin is highly unstable and readily precipitates bound heme and iron. In β- thalassemia these precipitated iron, repeated blood transfusion and increased gastrointestinal iron absorption lead to iron overload in the body. The increased free iron in blood is responsible for the formation of Reactive Oxygen Species (ROS). If the production of ROS exceeds the capacity of enzymatic and non-enzymatic antioxidants systems to scavenge these species or if these protective systems are compromised, then oxidative stress occurs. This review summarizes the production of ROS, its effect and different drug and plant extract used as an antioxidant as well as chelating agent in thalassemic patient.

INTRODUCTION: β- Thalassemia is an autosomal recessive disorder characterized by microcytosis and hemolytic anemia, which is a result of the reduced synthesis of the β-globin chains of haemoglobin [1]. The disorder affects about 150 million people in the world [2]. β- Thalassemia is prevalent in Mediterranean countries, the Middle East, Central Asia, India, Southern China, and the Far East as well as countries along the north coast of Africa and in South America. The highest carrier frequency is reported in Cyprus (14%), Sardinia (10.3%), and Southeast Asia [3].

Although there are now more than 180 known β-thalassemia mutations worldwide [4], a smaller collection of alleles accounts for the inactivation of most β-globin genes in each population or ethnic group. The genes involved in thalassemia control the production of a protein in red cells called haemoglobin. The synthesis of haemoglobin is controlled by two developmentally regulated multigene clusters: the alpha-like globin cluster on chromosome 16 and the beta-like globin cluster on chromosome 11. Human haemoglobin is a hetrotetramer protein, compose of two alpha and two beta subunits as shown in **Figure 1**. Each subunit contains a heme group, an iron containing compound that binds to oxygen.

In β-thalassemia, mutations in one or more of the β-globins gene loci that result in reduced β-globin production. In addition to the direct effects of reduced β-globin synthesis, many of the symptoms of this disorder appear to be consequences of the resulting 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.

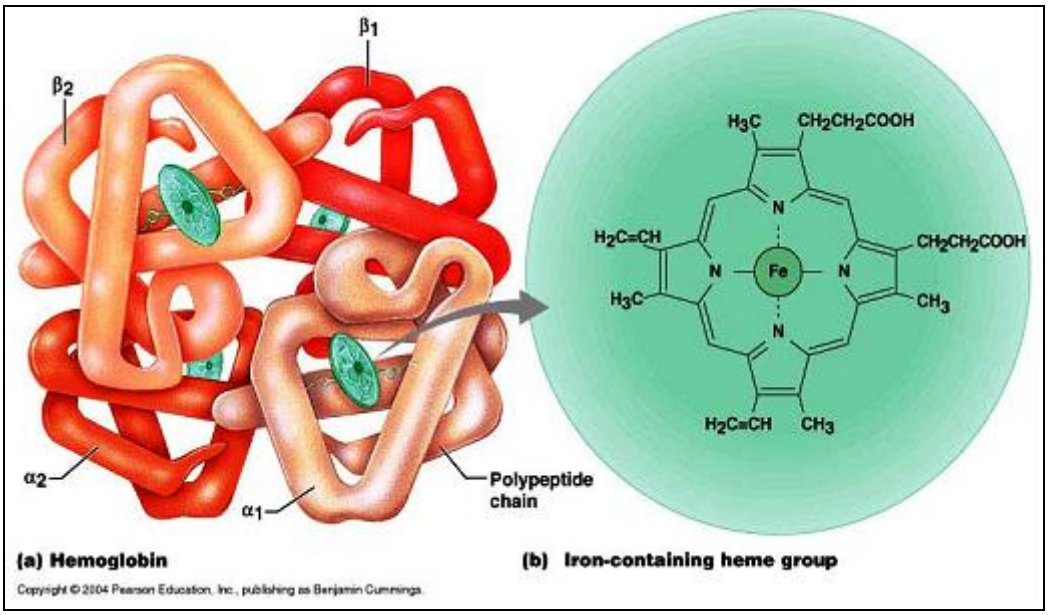


FIG. 1: HUMAN HAEMOGLOBIN

The accumulation of toxic quantities of iron causes tissue damage and leads to complications such as heart failure, endocrine abnormalities like diabetes, hypothyroidism, liver failure and ultimately early death [8, 9, and 10]. 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, or if these protective systems are compromised, then oxidative stress occurs [7, 11]. This oxidative stress and a possible consequential accelerated apoptosis may contribute to shortened life span of erythrocytes, primary or secondary amenorrhoea, hypogonadism, osteoporosis and other endocrine disorders [12].

Biomarkers of oxidative stress included plasma malondialdehyde (a marker of lipid per oxidation [13-16] and plasma protein carbonyls, a marker of oxidation to circulating proteins. Inflammatory biomarkers were cytokines (including interleukin-6) and high-sensitivity C-reactive protein (hsCRP), markers previously found useful in thalassaemia [14, 17, and 18].

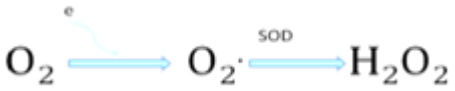
Antioxidant are complex group of protein such as superoxide dismutase's, which convert superoxide to oxygen peroxide, catalase and glutathione peroxidase, which convert hydrogen peroxide to water, and non-enzymatic scavengers such as glutathione, peroxiredoxin, ascorbic acid and carotenoids.

Malondialdehyde (MDA), a product of lipid peroxidation is generated in excess amounts in supporting the fact that large amount of membrane bound iron is present in thalassemic erythrocytes [19, 20].

Chelation therapy reduces iron-related complications and thereby improves quality of life and overall survival [21, 22]. The poor oral bioavailability, short plasma half-life and severe side effects make available chelators suboptimal [22- 26]. Iron chelators mobilize tissue iron by forming soluble, stable complexes that are then excreted in the faeces and/or urine. It was reported that chelating agents are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion [27].

Production of ROS in thalassemic Patient: Reactive oxygen species are partially reduced forms of atmospheric oxygen. They typically result from the excitation of O_2 to form singlet oxygen or from the transfer of one, two or three electrons to O_2 to form, respectively, a superoxide radical (O_2^-), hydrogen - peroxide (H_2O_2) or hydroxyl radical (OH^-). Free α-globins 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]. The deposited iron is responsible for the formation of reactive oxygen species which causes oxidative stress in thalassemic patient.

In mitochondria during oxidative phosphorylation for energy production O_2 molecules accept four electrons to form two molecules of water. The acceptance of a single electron by O_2 generates superoxide O_2^- . So mitochondria are a major source of superoxide. These produced superoxides undergo rapid dismutation both spontaneously and by a family of enzymes, super oxide dismuatase to form H_2O_2 [28, 29.30].



This H_2O_2 reacted with highly reactive Fe^{2+} ions which catalyze the Fenton's and Haber-Weiss reactions [31] these reactions takes place in the presence of hydrogen peroxide (H_2O_2) and superoxide anion radicals ($O_2^{\bullet-}$).

According to Fenton's;

$$Haem\text{-}Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + {}^{\bullet}OH$$

$$Fe^{3+} + O_2^{\bullet-} \rightarrow Fe^{2+} + O_2$$

In general, the O_2 carried by heme proteins is only bound directly to the ferrous iron with an oxidation state of two (Fe^{2+}). In the presence of H_2O_2, ferrous iron and H_2O_2 are going to react to generate ferric iron (Fe^{3+}) and highly reactive hydroxyl radicals ($^{\bullet}OH$). Addition of a reducing agent, such as ascorbate, leads to a cycle which increases the damage to biological molecules.

According to the Haber–Weiss reactions

$$O_2^{\bullet-} + H_2O_2 \rightarrow {}^{\bullet}OH + OH^- + O_2$$

$${}^{\bullet}OH + H_2O_2 \rightarrow O_2^{\bullet-} + H_2O + H^+$$

The above reaction is catalysed by Fe^{3+} and is a possible source of $^{\bullet}OH$.

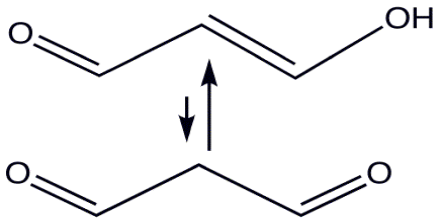
It was demonstrated that the release of Fe^{3+} from the porphyrin ring increased as a function of O_2 concentration as shown below

$$Heme\text{-}Fe^{2+} + O_2 \rightarrow Heme\text{-}Fe^{3+} + O_2^{\bullet-}$$

$$Heme\text{-}Fe^{3+} \rightarrow Fe^{3+} + \text{iron-free Heme}$$

When Fe^{3+} is released, it could generate reactive oxygen species that would, in turn, damage cellular compartments [32]. The accelerated ROS production also causes the expression of several specific metal-binding proteins (transferrin, cerruloplasmin, ferritin, lactoferitin, etc.) and subsequent progression of oxidative stress [33].

MDA a Marker of oxidative stress: Malondialdehyde (MDA) is a three carbon dialdehyde which is widely produced in mammalian organisms as a side product of prostaglandin biosynthesis and an end product of polyunsaturated lipid peroxidation [34-36]**.** MDA is generated in excess amounts in thalassemia, supporting the fact that large amounts of membrane bound iron are present in thalassemic erythrocytes. MDA is a bi functional reagent and has been reported to crosslink several cell constituents including membrane components [37, 38].



Increased plasma MDA level was found in beta-thalassemia patients [39, 40]. Plasma MDA was studied as a marker of tissue injury and oxidative stress. In oxidative stress condition peroxidative damages to tissues and depletion of endogenous antioxidants may be expected [41]. Peroxidative damage to lipids and protein is indicated by the increase of about two fold of the serum MDA, conjugated diene lipid hydro peroxides and protein carbonyls [42].

Effect of oxidative stress on Enzymatic and Non-enzymatic antioxidant: Antioxidant capacity is an important determinant of tissue injury, especially in patients with increased oxidant stress [43]. Researchers were found significant differences in antioxidant capacity in terms of tocopherol level. Plasma α-tocopherol was decreased in thalassemia [44-46]. These low levels probably contribute to increased MDA levels. In addition to this all others antioxidant level markedly modified except albumin and glutathione. Concentrations of ascorbate and vitamin E (α-tocopherol), the major lipid soluble antioxidant in human blood, decreased by 42%, other lipid soluble

antioxidants such as vitamin A**,** and carotenoids such as carotene and lycopene, which are part of the unmeasured compounds in TEAC determination, were also markedly reduced [42]. Serum levels of aspartate transaminase were inversely correlated with vitamin E, vitamin A, and lycopene, suggesting that liver damage may play a major role in the extent of depletion of these lipid soluble antioxidants. Levels of alanine aminotransferase were similarly inversely correlated with vitamin E**,** vitamin A, and lycopene [(42)]. Then, despite a mean decrease of 14% in the serum total antioxidant potential, a dramatic fall in the amount of ascorbate (44%) and lipid soluble antioxidants, vitamin E (42%), vitamin A (44%), *p* carotene(29%), and lycopene (67%) is observed in all patients [42].

Role of Antioxidant in thalassemic patient: The body's antioxidant system is an integrated one, in which some components may interact to spare or replace each other. However, the deficiency of individual antioxidants observed in thalassemia is such that no effective compensation could be brought about [(42)]. Antioxidant capacity is a result of the overall effect of water-soluble antioxidants, lipid-soluble antioxidants and antioxidant enzymes such as superoxide dismutase [31].

Dehydroascorbate cannot be regenerated to its reduced form, as its regenerating system involves erythrocyte glutathione, most of which in thalassemia patients can be oxidized. Moreover, as vitamin C is essential to maintain vitamin E status and function, depletion of vitamin C, in turn, contributes to further exacerbate the depletion of vitamin E. Although efficient antioxidants such as uric acid and bilirubin are high, they cannot compensate for lipid-soluble antioxidants, so that tissue lipid compartments are not suitably preserved [44].

Antioxidant activity depends on the kind of oxidative stress and on oxidative substrate. According to Packer *et al*., 1995 [47], when we evaluate the antioxidant potential of a compound, criteria such as.

a) Specificity of free radical scavenging,
b) Interaction with other antioxidants,
c) Metal-chelating activity,
d) Effects on gene expression,
e) Bioavailability,
f) Location (in aqueous or membrane domains, or in both),
g) Ability to repair oxidative damage [48]

LA/DHLA are considered ideal therapeutic antioxidant because they are naturally existing, low molecular weight compounds with very powerful antioxidant effective in both aqueous and lipid domains. Their effects include free radical quenching [49], metal chelation [50] and regeneration of other antioxidant such as ascorbic acid, vitamin E and glutathione [51]. The antioxidant role of vitamin E is attributed to its ability in quenching highly reactive lipid peroxide intermediate by donating hydrogen and this prevents extraction of hydrogen from PUFA. This assists in restricting self perpetuated lipid peroxidation chain reaction [52, 53]. Erythrocyte SOD scavenges superoxide radicals to form hydrogen peroxide and protects the cell membrane from its damage.

Increased Erythrocyte SOD activity may be due to blood transfusion and increase in the proportion of younger erythrocytes, as a compensatory mechanism after increased oxidative stress [54]. Since antioxidants seem to act co-operatively *in vivo*, the evaluation of TAOA in blood plasma could provide a more comprehensive assessment than the evaluation of individual antioxidants. TAOA is a parameter, summarizing the overall content and activity of the water-soluble antioxidants. The depletion of TAOA induced by oxidative stress in β- thalassemia major patients is probably eliminated by the release of stock organ antioxidants and the induction or activation of antioxidant enzymes [55].

Chelators: As the body has no effective means for removing iron, the only way to remove excess iron is to use iron binders (chelators), which allow iron excretion through the urine and/or stool. As a general rule, patients should start iron chelation treatment once they have had 10-20 transfusions or when ferritin levels rise above 1000ng/ml [56]. Since till date there is no effective drug available in the market for the treatment of thalassemia, only chelation therapy is the way by which a patient can live long life. So different researcher used certain types of drugs and plant extracts for the inhibition of oxidative stress in thalassemic patient. These drug and plant extract work as chelator as well as antioxidant.

Drugs used as iron chelator in thalassemic patient: The first drug available for treatment of iron overload was *deferoxamine* (DFO), an exadentate iron chelator that is not orally absorbed and thus needs parenteral administration [56, 57]. The use of DFO decreases morbidity and mortality among those who are able to comply with regular prolonged infusions [58]. However, because of the side effects and the inconvenient parenteral administration, a consistent proportion of patients are non-compliant, limiting the usefulness of this chelator [59].

According to Yasser Ali *et al*., [60] *deferiprone* (DFP) is an effective drug which can be used safely for iron chelation in thalassaemia major patient with iron overload. This drug is very effective in cardiac protection. The orphan drug DFP is an orally active iron chelator which has emerged from an extensive search for new treatment of iron overload [61]. Retrospective and prospective studies have shown that DFP monotherapy is significantly more effective than DFO in decreasing myocardial siderosis in thalassemia major [62-64].

Researchers work on different drug like DFO and DFP and they used in combination and alone they found that, drug is to be most effective in combination rather than alone [65-69]. Similar work has been conducted by Gharagozloo M *et al*., but they used silymarin, a flavovonolignan complex isolated from silybum marianum and DFO. They found that significant improvement in liver alkaline phosphatase and glutathione levels of red blood cells was detected. This is the first report showing the beneficial effects of silymarin in thalassemia patients and suggests that silymarin in combination with DFO can be safely and effectively used in the treatment of iron-loaded patients. Silymarin has a strong antioxidant, hepatoprotective, and iron chelating activities.

Patrick B. Walter's *et al*., research emphasized on MDA level of thalassemic patient. They treated the patient with deferaxasirox and deferaxamine and found that both drugs are equally effective in decreasing iron burden and MDA levels.

Plant extract used as chelation and anti oxidant: Plants are rich sources of natural antioxidants and the antioxidant effect of plant products is mainly attributed to phenolic compounds such as flavonoids, phenolic acids, tannins and phenolic diterpenes [70]. Among the various medicinal and culinary plants, some endemic species are of particular interest because they may be used for producing raw materials or preparations containing phytochemicals with significant antioxidant capacities and health benefits [71]. Plant extracts of various plants are used for the treatment of various diseases, were highly regarded by the ancient civilizations. Even today, plant materials remain an important resource for combating illnesses [72].

It has been documented that flavonoids which contain hydroxyl functional group, show antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of action of flavonoids are through scavenging or chelating process [73-77]. Phenolic compounds are a class of antioxidant compounds which act as free radical terminators [75]. There was a direct relation between chelatory activity and the content of active compounds, phenol and flavonoid. Plant extracts with high phenol and flavonoid contents showed good chelating of Fe^{++} [72] so such type of plant extract now used as chelator antioxidant for thalassemic patient. These are as follows-

According to Olabinri BM, Eniyansoro O.O. *et al*., [78], the aqueous extract of *Tetracarpidium conophorum* (African Walnut) demonstrate dose dependent decrease in chelating ability. They found that the antioxidant activity of plant extract fully depend on their phenolic and flavonoid content. If the plant extract have high concentration of phenol and flavonoid then that plants have maximum antioxidant activity. Similar results were observed research conducted by Phalguni Srimani, Goutam Mandal *et al*., [79] who demonstrated the antioxidant potential of ethanolic extract of leaves of *Piper beetle* Linn. (Pan). Although the extract of *P. betle* contained phenols, chevi, etol, allylpyrocatechol and their respective glycosides [80] but the compound responsible for antioxidant activity is unknown. Effective antioxidant activity, reducing power activity Fe^{++} chelating , nitric oxide and DPPH radical- scavenging property was exhibited by aerial plants extract of Leonurus cardiac subsp. *Grammosciadium platycarpum, Onosma*

demawendicum research conducted by Ebrahimzadeh , M. A. *et al*., 2010 [81].

According to Mohammad Ali Ebrahimzadeh *et al*., 2008 [82], there was direct relation between chelatory activity and the content of active compounds phenol and flavonoid present in plant extracts. They showed that *Epilobium hirsutum*, *Melilotus arvensis, Feijoa sellowiana* showed similar result while corn milk with high phenol and flavonoid content showed very weak chelating activity, although, *Pistacia lentiscus* with low phenol and flavonoid content showed good chelating activity.

CONCLUSION: β-thalassemia is a genetic disorder, symptoms of which appear only in thalassemia major patient. Such type of patient needs regular blood transfusion for survival, resulting in the increase of blood iron concentration which causes oxidative stress leading to the development of other abnormality in the body. Till date, the technique available for the treatment of this genetic disorder are bone marrow transplantation and stem cell therapy but these treatment methods are very expensive and hence people depends on regular blood transfusions throughout their life. The only way to increase the survival rate of such patients is by inhibiting the effect of oxidative stress. So researcher should explore such drugs which may be derive from plant extracts; which can minimize the effect of oxidative stress by removing free radical or remove excess iron from thalassemic patients.

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