

## Anti-oxidants: Are they important in Thalassaemia? Should they be part of daily management?

### Introduction:

It is well known that metabolic processes in all organisms produce activated oxygen, otherwise known as reactive oxygen species (ROS), such as hydroxyl ( $\cdot\text{OH}$ ), superoxide ( $\text{O}_2^-$ ),  $\text{H}_2\text{O}_2$  and nitric oxide (NO). These free radicals and non-radical oxidants may damage cells and molecules such as DNA, proteins and lipids. They may therefore be responsible for induction of disease or bring about complications.

Their detrimental action is counterbalanced by substances which absorb or neutralize these radicals called anti-oxidants. Some anti-oxidants are endogenous (e.g., albumin, glutathione) and others, particularly Vitamin E, Vitamin A (carotenoids) and Vitamin C (Ascorbate) are taken from the diet.

### In Thalassaemia:

The oxidant/anti-oxidant balance has been shown to be disturbed, i.e. the rate of free radical production is more than the defenses of the cells can cope with. Many complications are attributed to this oxidizing stress.

There are two main sources of activated oxygen in Thalassaemia, the increased red cell destruction and iron overload:

#### 1) Red Cells:

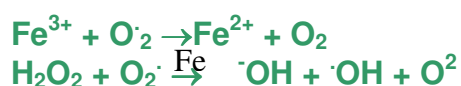
- a) As part of normal red cell apoptosis, haem iron loses an electron, resulting in the formation of ferric methaemoglobin. In the greatly increased red cell death seen in Thalassaemia, there is much more methaemoglobin formation and superoxide release;
- b) The free  $\alpha$ -haemoglobin chains also undergo auto-oxidation;
- c) Thalassaemic red cells have an increased lipid content per unit and so are more susceptible to peroxidation

#### 2) Iron Overload:

Most Thalassaemia patients have iron overload. This means that iron-binding proteins (transferrin and ferritin) are saturated and non-transferrin-bound iron (NTBI) is increased. Much of this unbound iron belongs to the labile iron pool (LIP) which consists of unbound and unprotected metal ions. Normally this is iron which is available for the iron containing proteins. When these ions are available in excess, then more partake in the Fenton reaction, catalyzing the formation of reactive hydroxyl radicals:



In the presence of free plasma iron, hydrogen peroxide is oxidized into hydroxyl ions ( $\text{OH}^-$ ) which are regarded as the main source of oxidative damage.



These superoxide radicals oxidize cell membrane lipids and proteins as well as organelle membranes, leading to cell damage and death. This occurs in vital tissues, including liver, pituitary glands, pancreas and heart. The heart has the least developed anti-oxidant defenses, so iron-induced peroxidative damage is a major mechanism for heart function irregularities.

### Evaluation of the oxidant/anti-oxidant status of Thalassaemia patients

Most of the published literature on the subject is still at the level of research, and there is no firm conclusion as to the value of monitoring patients at clinic level and which are the best tests to utilize. There is however a wealth of literature demonstrating the peroxidative tissue injury in Thalassaemia. The value of clinical monitoring should be considered, especially if supplementary anti-oxidants are to be evaluated.

Oxidative stress is assessed by looking at products of oxidative damage which have been demonstrated to be increased in Thalassaemia (Livrea 1996) as well as some anti-oxidants, the levels of which are decreased in iron overload (Britton et al.).

Products of oxidative stress that have been used in Thalassaemia patients, include malonylaldehyde (MDA), conjugated dienes (CD), protein carbonyls and thiobarbituric acid (TBA) which are all significantly raised in thalassaemia. Of these **MDA is the measurement most frequently used**, as a convenient marker for the extent of peroxidation, since it is formed from the breakdown of polyunsaturated fatty acids,

Anti-oxidant markers include TEAC, urate and bilirubin which may be increased due to haemolysis. Vitamin E, C and A levels have been used, but **superoxide dismutase (SOD), a preventive anti-oxidant is widely used**. Glutathione is usually found at around normal levels in Thalassaemia.

### New information

1. It is known that glutathiones act as anti-oxidants in the important to tissue damage, oxidant/anti-oxidant balance in thalassaemia.

Wu et al (Hemoglobin 2006, 30(6):251-6) have demonstrated that glutathione S-transferases (GSTM), which work as anti-oxidants, are genetically determined. They found that the GSTM genotype was associated with cardiac iron deposition in patients with  $\beta$ -thalassaemia major.

2. Oxidative stress affects cellular immunity: - Alidoost F, et al, of the Isfahan University of Medical Sciences, found a significant reduction of intracellular glutathione levels in peripheral blood mononuclear cells. This may explain abnormalities in cellular immune responses seen in iron overloaded patients. Proliferation of peripheral blood mononuclear cells was also found to be reduced in 28 thalassaemia major patients compared to an equal number of healthy controls.

Flavonoids are known for their anti-oxidant properties, so the authors tried a flavonoid called Silymarin, derived from herbs (milk thistle seed), which decreases the oxidation of glutathione. They demonstrated, *in*

*vitro*, restoration of glutathione levels as well as the growth of mononuclear cells. Their conclusion is that flavinoids may normalize the immune response in  $\beta$ -thalassaemia major and are progressing to clinical trials of this flavinoid.

Using a different approach, a group from Israel (Amer et al, Br.J. Haematol, 2006, 132:108-13 – Department of Haematology, Hadassah University Hospital), found that red cells, platelets and polymorphonuclear neutrophils of sickle cell patients had a 10-30-fold higher production of reactive oxygen species (ROS) and a 20-50% lower glutathione content than healthy controls. Exposing these cells to anti-oxidants such as N-acetyl-cysteine, Vitamin C and Vitamin E, decreased the oxidative stress.

The same group, looking at neutrophils from  $\beta$ -thalassaemia patients (Br. J. Haemat, 2005, 129:435-41), found the same increase in ROS, but a reduced “respiratory burst” (i.e. producing a burst of ROS in response to bacterial components). This may mean a weaker anti-bacterial capacity. When they treated these neutrophils with anti-oxidants (N-acetyl-cysteine, Vitamins C and E) there was correction, indicating that prophylactic treatment with anti-oxidants may be indicated if there are recurrent infections.

3. The use of flow cytometry in assessing the oxidative status of red cells:- The use of cytometric measurement of reactive oxygen species (ROS) in Thalassaemia was first described by Amer et al.(Eur. J. Haematol., 2003, 70:84-90). Later they described measuring antioxidants like reduced glutathione and peroxidation of membrane lipids - indicators of membrane damage (Cytometry, 2004, 60(1):73-80).

Flow cytometry has also been used by the same team to evaluate labile iron pool (LIP) in erythroid cells in various conditions including thalassaemia. Since flow-cytometry is available in most haematology laboratories, their methods should be considered for adoption for the assessment of oxidant-anti-oxidant balance and LIP in Thalassaemia.

### Anti-oxidant treatment

1. Chelation: Because of the significant role of iron overload in the oxidative stress of thalassaemia, it is expected that adequate iron chelation therapy will contribute significantly to reducing free radicals and their effects. One prerequisite seems to be the presence of the chelating agent in the circulation continuously. DFO has been shown to remove NTBI, but as soon as the infusion is discontinued NTBI reappears rapidly. It is for this reason that continuous infusions through indwelling IV catheters were successful in treating patients at high risk for cardiac complications and even reversing such complications (Davies and Porter, Blood, 2000). Because of difficulties in sustained compliance to such parenteral treatment, it is hoped that oral iron chelation will both improve compliance and at the same time maintain an active, constant presence in both circulation and tissues to be more effective as anti-oxidants. Deferiprone, because of small molecular size, has more ability to permeate cell membranes and remove free iron, decreasing ROS generation. Initial results from the new iron

chelator, Deferasirox, indicate that it has a similar anti-oxidant effect. ROS levels are, however, still increased in well-chelated patients, so that other supplements may be useful in protecting the tissues.

2. Oral anti-oxidants supplements:- At a clinical level the anti-oxidant that has received most attention is Vitamin E which is a lipid anti-oxidant. Tesoriere et al (2001) gave 600mg/day of Vitamin E for 9 months to Thalassaemia Intermedia patients. This resulted in normalization of Vitamin E levels and malondialdehyde and generally improved the anti-oxidant/oxidant balance. Even though improvement is observed in these biochemical indicators, clinical improvement such as increased Haemoglobin levels has not been demonstrated. Rachmilewitz et al (Am. NY. Acad. Sci., 2005) have suggested that Vitamin E alone is probably insufficient to improve RBC survival and other anti-oxidants, acting on proteins may enhance the effectiveness. They suggested N-acetyl-cysteine.

Another group (Dissayabuta et al., 2005) added low dose Vitamin C (100mg/day) to 400mg/day Vitamin E (to avoid the pro-oxidant effect of high dose Vitamin C) and found evidence that this regime was of more benefit than vitamin E alone.

Other anti-oxidants tried in Thalassaemia patients with biochemical benefit include Coenzyme Q-10 (Kalpravidh et al, 2005).

### Conclusion:

Anti-oxidants have the potential of preventing complications, improving clinical status and improving quality of life in Thalassaemia. To date on a clinical level only Vitamin E has been adequately tried. It is possible that other anti-oxidants, used in conjunction with Vitamin E may enhance its action. It is time for clinical teams to test anti-oxidant combinations for both biochemical and clinical effects.

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