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The key role of vitamin E supplementation on secondary Iron overload and the oxidative hassle in transfusion dependant beta thalassemia major children

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Abstract

Children with β- thalassemia major experience significant cellular oxidative injury predominantly due to excessive breakdown of uncoupled globin chain and secondary iron overload. These processes contribute to cellular oxidative hassle damage affecting platelets & RBCs, potentially exacerbating ineffective erythropoiesis, the increase of risk of thromboembolic actions. Present study aimed to judge malondialdehyde (MDA), iron, vitamin E, Erythrocyte superoxide dismutase (ESOD), and consequence of 24 weeks of vitamin E supplementation in transfusion dependant β- thalassemia major. A prospective cross sectional before and after comparison study was conducted among 120 subjects were between age group 4 to 11 years. Six homozygous beta thalassemia cases were assessed at baseline and afterwards with vitamin E tablet (10U/kg/day) for 24 weeks with regular standard treatment, and they were compared with 60 age and sex matched controls. The intensities of MDA, ESOD and iron in serum were found to be significantly elevated ($p<0.001$) and the vitamin E level was significantly reduced ($p< 0.001$) in the cases as compared to controls. After the supplementation of the vitamin E orally for 24 weeks, significantly lowered ($p<0.001$) mean standards of MDA, iron and ESOD in blood, non- significantly Hb although vitamin E was significantly advanced ($p<0.001$), as compared to baseline. Recurrent blood transfusions consequence in undue free iron load in the blood, which indications to substantial generation of huge free radicals, ROS. Vitamin E orally can be harmless effective supplement to recover oxidative harm in thalassemic children. Besides it appears that a extended period of consuming antioxidant supplementations prerequisite to mark medical haematological expansion in β- thalassemia major children.

KEYWORDS: Homozygous β- thalassemia; Vitamin E; Iron overload; Oxidative hassle.

Introduction

β-thalassemia major is one of the most common inherited single gene disorders which affecting thousands of people's worldwide [1]. About 240 million peoples have clinically seeming to this disorder worldwide. In India alone, it is estimated almost 30 million individuals with the mean prevalence of 3.3% are affected [1-5]. The disease result from mutation in the gene which synthesize β globin chain of haemoglobin, results in ineffective erythropoiesis, reduced Hb production, abnormality in organ function and causes anaemia at early age [2-5]. In its most severe form children with thalassemia major require frequent blood cell transfusion to maintain adequate haemoglobin levels to survive. This causes economic, mental and social burden for the patient, their families and health care system [6].

This oxidant - antioxidants balance is necessary to achieve typical functioning and to preservation cellular homeostasis [4-6]. In individual with beta Thalassemia major, unpaired global chain and elevated intracellular iron attention may endorse oxidative impairment. These factors promote oxidative damage to RBCs, with corresponding

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reduced RBC survival in the circulation. Although iron is essential for metabolic progressions, an excessive iron itself be able to generate reactive oxygen species (ROS), leads produce oxidative stress and lipid peroxidation [2, 4, 7].

To mitigate this, Iron chelation treatment is routinely administered to patients to avoid the adverse effects of surplus iron [7]. Nevertheless, excessive iron accumulation may still occur in several vital organs such as liver, pancreas, pituitary gland, heart. Ultimately accumulated with iron and over all effects on depleted total antioxidant defence can leads to the cognitive heart failure, and this is the main and major cause of the death in beta Thalassemia major children [5, 7-9]. Previous studies have showed an association between increased susceptibility of thalassemic RBCs to oxidative stress and subnormal levels of antioxidant vitamins [5, 7-10].

In the present study, it is aimed to evaluate malondialdehyde (MDA) formed as a degradation of lipid peroxidation, erythrocyte superoxide dismutase (ESOD) makers as antioxidant defense activity, serum iron levels, and vitamin E as adjunct therapy to reduce oxidative tension and iron overload in cases of β- Thalassemia major patients.

Materials and Methods

Study design and patients: This prospective cross sectional before after comparison study was conducted among children diagnosed with β- thalassemia major. A total 120 eligible participants were enrolled in the study. All the β- thalassemia patients were registered at the department of Paediatrics, DVVPF'sMCH and Civil hospital Ahilyanagar Maharashtra. The study approval was obtained from Institutional Ethical Committee (IEC). Prior to commencement of the study. Written informed consent was obtained from the patient's parent or legal guardians.

Inclusion criteria: Sixty children β-Thalassemia major patients between age group 4 to 11 years were included in the study group. The diagnosis β- thalassemia was diagnosed by physician based on clinical history, haemoglobin electrophoresis. The patients further confirmed by ion exchange high performance liquid chromatography and electrophoretic pattern analysis.

All the thalassemia patient received oral Deferiprone (DFO) as an iron chelation in therapeutic dose (75mg/kg body weight/day) when serum ferritin was less than 2500µgm/L and 100mg/kg body weight/day when ferritin was exceeded than 2500µgm/L. The study group were underneath the stern observation of medical experts throughout this study tenure.

The patients were packed transfusion dependent and received regular red blood cell transfusions. The usual haemoglobin levels ranged between 3 to 7.7 g/dL. The children had history of anaemia, low Hb and abnormal complete blood count.

Exclusion criteria: The patient who denied to participate, the patient who had history of Sickle Cell anaemia, hepatitis B, C, HIV infections, asthma, cardiovascular diseases, diabetes mellitus, renal failure etc, antioxidants supplements and herbal medicine takers, and suspected to acquire allergy to Vitamin E were excluded from the study.

Vitamin E supplementation: Patients were assessed before and after supplementation of Vitamin E 10U/kg/day, with a maximum dose of 400 IU/day (Evion 400) orally for 24 weeks along with regular treatment plan. The control group consisted of 60 age and sex matched healthy children.

Blood sample collection: Approx 3cc blood sample was withdrawn from each case in the fasting state prior to transfusion in EDTA and plain vacutainers. The separated serum or plasma was kept at -800C short of preservative until further analysis.

Laboratory examination: Pre-transfusion haemoglobin levels, serum iron concentration, MDA levels, ESOD and serum vitamin E concentrations were assessed at baseline and after 24 weeks of supplementation of vitamin E. Haemoglobin levels were estimated by a calibrated automatic cell counter (Sysmex KX21 corporation Japan) [11]. Serum iron evaluated by means of commercial analytical kit Sigma (Clinical Chemistry Analyser) [12]. Serum MDA evaluated by colorimetric reaction with thiobarbituric acid (TBA) by Kei Satho [13]. ESOD by Kajari Das [14] and vitamin E levels were measured by Baker and Frank method [15].

Statistical analysis- The Statistical Package for Social Science (SPSS) software version 23 was used for analysis. Expressive data were expressed as a mean ± standard deviation. Assessment of quantifiable variable by student Z test among two groups and $P<0.001$ was considered as statistically significant.

Results

Table 1 presents the bassline and post therapy among β-Thalassemia major patients and compared with healthy controls. The base line levels of serum MDA, ESOD and iron were significantly elevated β-Thalassemia major patients, whereas Hb and Vitamin E levels where reduced ($P<0.001$) compared to control. After 24 weeks of Vitamin E supplementation along with consistent treatment protocol, it has been detected that, there were significant reduction in the values of MDA, iron, ESOD compared with the baseline values. The Hb concentration was

increased and non-significant (P<0.05) but not up to the control mark. In contrast, serum vitamin E concentration was determined to be enhanced at the end of 24-week supplementation period, it indicating the improved antioxidant status.

Table 1. Comparison biochemical parameters between healthy controls and transfusion dependant beta thalassemia major cases before and after supplementation of Vitamin E (10U/kg body weight/day) along with regular treatment

Parameters	Controls (n=60)	Beta thalassemia major children (n=60)			$P Value
		Base line	#P Value	Post vit E therapy	
Hb (gm/dl)	13.45±0.8	7.3±0.65	P<0.001	7.8±1.8	P<0.05
MDA (nmol/dl)	0.98 ± 0.31	2.30±0.76	P<0.001	1.78±0.75**	P<0.001
Iron (µg%)	118.58±12.85	239.6±36	P<0.001	198.1±26.24	P<0.05
ESOD (U/ gm of Hb)	1268.26±134	2163.23±223	P<0.001	1703±154**	P<0.001
Vitamin E (mg/L)	1.204±0.14	0.68±0.99	P<0.001	0.98±0.49**	P<0.001

#comparison with control group, $comparison with baseline values
** indicates p<0.001 (greatly significant)

Discussion

Oxidative hassle performs a chief role in the pathophysiology of children with beta thalassemia major. Augmented production of free radicals, reactive oxygen species persuaded lipid peroxidation is the core consequence occurs in these children [4, 16]. Iron burden in thalassemics may provoke oxidative hassle and markedly diminution in the antioxidant defensive mechanism [17]. Our finding sturdily backing to the Haghpanah et al [1], Livrea et al [9], Abed Mahdi [18] that, MDA levels where six folds much advanced in thalassemic children before transfusion when compared with the control groups. Our work shows evidence that oxidative alteration within the cell component can be shown in a serum as a marked increase of conjugated diene lipid hydroxide, MDA and the protein carbonyls [1,8,9]. MDA is an efficient parameter and has existed to cross link innumerable cell membrane elements. An awkward RBC membrane is projected to be rough and inflexible, and increased MDA could be probably explaining the rigidity of thalassemic erythrocytes [9,18]. In the humans the loading of iron is retained inside the assortment of 200 to 1500 mg by an applicable alteration of the bowel iron fascination, as there is a no any excretory mechanism available for iron [1, 5, 6 ,19].

Exhausted Hb levels, instability, repeated blood cell transfusion, boosted oxidative hassle and due to that improved iron absorption from the gastrointestinal track epitomize the major roots for iron overload in the transfusion dependent β-Thalassemia major children [5, 20]. The iron overload leads towards the oversaturation of iron in the organs like liver, heart, spleen, pancreas endocrine gland, eventually this leads near organ dysfunction and failure [20]. The secondary iron overload and continuous blood transfusion can lead to advanced generation of ROS and oxidative tension. In our study there was boosted iron overload expressively (P<0.001) in homozygous beta Thalassemia cases after associated with the normal standard.

RBCs are secured from free radicals by the intracellular first line of defence enzyme erythrocyte superoxide dismutase. Our results are in harmony with former results proposed that, an augmented activity of ESOD was found in patient with beta thalassemia major [1, 5, 9, 20]. The great levels of ESOD point out that, there is a probability of gathering of intracellular H2O2 in incidence of redox activities alteration metal like Cu++ and F+++ could be transformed to the OH- radical, the supreme feared of the ROS [5,6,21].

The principal α tocopherol role in vivo as a chain destruction antioxidant. this one is the most potent peroxyl radical hunter [1, 5,9,19]. It plays a pivotal role to avert auto oxidation of poly unsaturated fatty acids. In alliance with previous studies, our findings sturdily support that, enhanced a production of free radicals by ferritin, secondary iron burden is one of the main reasons to exhaust vitamin E concentration in these children [1,5,9,22].

Our consequences are reinforced by Haghpanah et al [1], Nandita Das [21], Sonali Bhagat et al [5], Nutthida et al [22] that exhausted intravascular haemolysis, oxidative hassle with secondary iron surplus and improved erythropoietin with the supplementation of vitamin E 10U/kg body weight/day for 24 weeks along with regular treatment protocol in transfusion dependent children with beta thalassemia major.

Conclusion

The present study demonstrated elevated oxidative stress markers, including malondialdehyde, erythrocyte superoxide dismutase, and serum iron, along with reduced vitamin E levels prior to antioxidant therapy. Supplementation with vitamin E (10 IU/kg/day) for 24 weeks significantly improved antioxidant status. Regular antioxidant supplementation may help reduce oxidative damage, limit hemolysis, and support improved hematological stability in children with β-thalassemia major. Further, large trials with grater sample size required to validate these results and long-term therapeutic benefits.

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Conflict of Interest: Nil.

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