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The Effects Of Vitamin E Supplementation In Patients With Beta Thalassemia On Iron Chelation Therapy

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Abstract

Antioxidant vitamins such as vitamin E have been shown to boost the efficacy of iron chelator medicines in patients with ß-thalassemia. The objective of the present study was to evaluate the effects of vitamin E supplementation as an adjuvant therapy on the antioxidant state of beta-thalassemic major patients who were enrolled in the research.Three-month trial including the administration of 400 IU vitamin E orally twice daily was conducted on ß-thalassemic patients who received blood transfusions and oral iron chelator;deferasirox, with a median age of 40 years old were chosen to participate. Measurement of reactive oxygen species (ROS), dinitrothiocyanobenzene reduction were used in conjunction with red blood cell (RBC) reduction; thiobarbituric acid-reactive substances (TBARS) were used in conjunction with serum malondialdehyde were also measured. When comparing patients with thalassaemia to healthy controls, patients with ß-thalassemia exhibited significantly higher baseline levels of serum TBARS and reactive oxygen species (ROS) ($P<0.001$). They also showed a significant decrease in serum levels of TBARS ,ROSand and RBC after receiving vitamin E therapy($P<0.001$). The treatment regimen resulted also in a significant rise in the amount of GSH present in red blood cells. In accordance with our findings, patients with ß-thalassemia experience increased oxidative stress, and treatment with vitamin E specific adjuvant antioxidants may help to prevent this damage. Thus patients may benefit from using vitamin E as a safe and effective supplement to reduce oxidative stress in their bodies. Furthermore, it appears that individuals with beta-thalassemia require a longer period of time to benefit from antioxidant supplements in order to demonstrate clinical hematologic improvement.

Keywords: Beta-thalassemia, Vitamin E, Glutathione Peroxidase, Superoxide Dismutase, Antioxidants

Introduction

The β-thalassemia syndromes are characterized by inadequate or missing β-globin synthesis, which is typically caused by a mutation in the β-globin gene, a blood condition that affects the red blood cells. Genomic changes or a genetic mutation can both cause and contribute to this condition.This results in a significant amount of alpha-globin chains being produced, which are incapable of producing hemoglobin tetramer. Thalassemia syndromes afflict around 200 million people worldwide, a number that has grown in recent years(Trehan et al. 2015). The thalassemia gene can be found in approximately 3% of the world's population, or approximately 3 million people.

According to available information, roughly 15,000 Iranians are known to be thalassemic, while approximately 3,000,000 Iraqians are known to carry the thalassemia gene (Hashemian, Hashemi, and Fateminasab 2012). TRAP (Total Peroxyl Radical Trapping Capacity) decreases as a result of this illness, indicating a decline in the body's antioxidant defense mechanism. Scavenging of toxic oxygen metabolites occurs in the body through the action of antioxidant enzymes such as superoxide dismutase (SOD). Glutathione peroxidase (GPX) is another antioxidant enzyme found in the body (GPX). The first reaction occurs when SOD reacts directly with H2O2, and then enzymes (Catalase and GPX) enter the picture to assist with neutralizing the H2O2. To combat oxidant causes, these three enzymes collaborate on a number of tasks in addition to their unique functions(Lü et al. 2010; Pisoschi and Pop 2015). Vitamin E is considered as the most important fat-soluble antioxidant vitamin, and it is believed to be the first step in the body's antioxidant defense mechanism. Vitamin E is also known as tocopherol, which is short for tocopherol. A number of studies have found that people with beta-thalassemic illness had lower levels of this vitamin in their blood than the general population (Fibach and Dana 2019).

According to a study conducted on patients with beta-thalassemia intermedia in 2020, the administration of vitamin E enhanced the balance of their antioxidant system and prevented the process of lipid peroxidation from occurring in their bodies (Al-Talib and Althanoon 2020). There are a range of profound and biologically relevant tasks performed by vitamin E, including the generation of structural changes in more than 300 metalloenzymes, such as super oxide dismutase, that are critical for human health. It also performs a number of antioxidant actions in the human body, which is beneficial(d'Arqom et al. 2020; Rashidi et al. 2011). In order to lower the intake of iron in the diet, specific dietary guidelines for thalassemic patients are developed. Unfortunately, this reduces the amount of vitamin E consumed as a result of the reduction in iron intake (Mobarra et al. 2016). As a result of their excretion effect on vitamin E, chelator factors, which are

used to prevent the accumulation of iron, have a diuretic effect on individuals who are suffering from anemia (De Sanctis et al. 2020).

The fact that beta-thalassemia major patients suffer from micronutrient deficiencies, notably in the area of vitamin E, has been well documented. However, there has been no published research on the use of this nutrient in the treatment of patients with thalassemia major to our knowledge. As a result, we evaluated the combined and independent effects of vitamin E supplementation on antioxidant status in patients with beta thalassemia major, namely glutathione (GPX), superoxide dismutase (SOD), and total antioxidant capacity [TAC].Modern thalassemia treatment involves the administration of blood transfusions and iron chelation, which are both meant to promote growth and prevent bone deformities in patients suffering from the illness. To try to limit the oxidative damage to cells and tissue in thalassaemic patients, antioxidant treatments have been tried both singly and in combinations, according to the researchers. These treatments have attempted to counteract the debilitating effects of reactive oxygen species, which have been demonstrated to induce cell and tissue damage in laboratory animals (Haghpanah et al. 2021) . A low level of plasma vitamin E has been observed in some studies of the thalassaemic population, as well as a reduction in a number of enzymes, including superoxide dismutase, in the thalassaemic population (Maurya et al. 2016). The use of antioxidants in the treatment of thalassaemia patients has, on the other hand, only been investigated in a small number of trials. The results of a recent study revealed that by supplementing with vitamin E for four weeks, the amount of lipid peroxidation in erythrocyte membranes was reduced, and SOD activity in the blood was restored to normal levels. The amount of GSH peroxidase activity did not return to normal levels as a result of this treatment, but it did decrease slightly (Fryer 1993).

The red blood cells (RBCs), platelets, and polymorphonuclear neutrophils (PMN) of people with sickle cell illness were found to have lower levels of oxidative stress when tested in vitro. This suggests that antioxidants may be able to alleviate some of the symptoms of sickle cell disease (Al-Talib and Althanoon 2020). It is possible that the antioxidant vitamin E (gamma-tocopherol) can aid in the prevention of oxidative stress and inflammation. Vitamin E is a powerful free radical chain-breaking antioxidant that may be beneficial in the prevention of oxidative stress and inflammation. Vitamin E (gamma-tocopherol) can be found in a variety of foods and dietary supplements (Haghpanah et al. 2021).Because of this, the objective of this study was to evaluate the role of vitamin E in the reduction of ROS generation as well as the expression of AHSP mRNA.

Material and Methods

A total of one hundred eighty patients with ß-thalassemic major who were hospitalized at the IBN-Atheer Teaching Hospital in the Ninewa district (in northern Iraq). Each of the patients tested positive for beta thalassemia, a complete blood picture by diagnosis. To be eligible for participation, participants had to have a history of continuous therapy involving blood transfusions and the use of deferasirox as anoral iron chelator agent. Tobacco usage, hepatitis B and C, and supplement use for at least three months before to the experiment were all found to be exclusion factors. The patients were divided into two groups using a random allocation process. Patients were randomly assigned to one of two groups (The random allocation method was employing 8 character blocks including the letters A, B, C, and D). To complete the experiment, the first group was required to take a 400 mg vitamin E supplement orally once daily for an additional three months beyond the first three months.

The second group does not receive vitamine E supplement .A fifty apparently healthy volunteers were also participitated in this study as a control group of, on the other hand, received no additional nutritional supplements. A questionnaire was used to get information about the participants' demographic characteristics. We considered the subjects' weight and height. The body mass index is calculated by dividing the weight in kilograms by the height in meters (BMI). Following a 12-hour fast, blood samples were taken from patients immediately before and after they received transfusions at the start and end of the study, and they were held at -70 degrees Celsius until the assay could be performed. When thalassemic patients received their initial blood transfusion, vitamin E was promptly administered. The concentration of vitamin E in the blood was determined in this study using HPLC (Knuer Germany) technology(Al-Fartusie et al. 2019). Three times with normal saline 0.9 percent, the full blood sample was cleansed in order to examine the enzyme activity of the SOD and GPX enzymes, which were both quantified. GPX and SOD activities in RBC were determined in this study utilizing the RANCEL and RANSOL (RANDOX, UK) kits. TAC values were established using TAC (RANDOX, UK) testing kits. The protocol for the study has been approved by the ethics council of Mosul University/College of Pharmacy. All patients signed an informed consent form prior to receiving treatment. For statistical analysis, the Statistical Package for Social Sciences (SPSS) software was chosen (SPSS for Windows). The mean and standard deviation were used to present the data (often known as the mean and SD). The paired t-test was used to determine the mean change in various variables between the study's pre- and post-intervention phases. All statistical tests were run on a two-sided basis, in order to ensure fairness and equality of outcomes (Greasley 2007).

Results

There were a total of 180 participants in this study taking part in the research. Overall, the mean (standard deviation) age was 25.2. (10.1) years for all of the groups. In terms of age, the mean standard deviation (mean SD) for males was 21.6 3.8 years, and the mean standard deviation age for females was 22.2 4.6 years, respectively. A significant difference (P<0.001) was seen between the serum levels of vitamin E in groups 1 and 2 compared to those in the control group. When comparing groups 1 and 2, the mean GPX activity fell by a statistically significant amount (P<0.013, P<0.023, and P<0.016, respectively). BMI changes in all groups were statistically significant, indicating that they were losing weight (P<0.001).

A comparison of the mean SOD activity and the serum TAC levels revealed no statistically significant differences between any of the groups under examination.Astatistically significant difference did not exist between the second group (the control group) and the first group, which did not ingest any supplements (Table 1 and 2respectively).

Table 1: Descriptive characteristics of studied groups for cases and controls

Parameter		Cases	Control	Total	P
		N=180 No.(%)	N=50 No.(%)		
Gender	Female	84(43%)	25(50%)	119	0.4
	Male	96(56%)	25(50%)	121	
Agegroup/yr.	5-9	38(48%)	32(64.0%)	70	0.066
	9-15	42(53%)	18(36.0%)	60	
Mean ±SD		8.9±3.1	8.5±2.7		0.8
BMI	5th-84thPercentile*	65(78.8%)	46(80.0%)	111	0.1
	85th-95thPercentile**	8(6.2%)	10(16.0%)	18	
	Below5th Percentile***	14(15.0%)	6(4.0%)	20	
Mean ±SD		16.9±2.2	16.95±2.3		0.2
S.ferritin	Normal(<500ng/mL)	6	-		
	high(≥500ng/mL)	78	-		
Mean ±SD		2995.9 ±2219	-		
Durationoforalchelationmean ±SD		3.5±1.4			

*=NormalBMI,**=Over weight,***=Underweight

Table 2: Comparison of studied parameters for cases (before and after intervention) and controls

Parameter	before	Controls after	Pvalue	before	Case Group 1 after	Pvalue	before	Case Group 2 after	Pvalue
Vit.E (mg/dl)	0.3 (0.2)	0.9(0.5)	0.001 *	0.3(0.2)	0.8 (0.5)	0.001 *	0.4 (0.2)	0.3(0.1)	0.001 *
SOD	1924.8	1759.5	0.5	1839.9	1808.7	0.8	2484.8	1892.1	0.3
(U/grHbg)	(631.1)	(569.7)		(577.9)	(610.4)		(1676.2)	(546.8)	
GPX(U/gr	46.1	38.9	0.03*	46.6	42.3	0.03*	44.7	46.5	0.4
Hbg)	(6.4)	(14.7)		(5.9)	(6.3)		(11.3)	(7.4)	
TAC(mmol/L)	1.2(0.2)	1.1(0.3)	0.4	1.1(0.2)	1.1(0.3)	0.7	1.1(0.2)	1(0.2)	0.9
BMI(kg/m^2)	19.8	20.3	0.001 *	19.6	20.8	0.001 *	19.8	19.5	0.3
	(2.2)	(2.3)		(2.1)	(2.2)		(2.2)	(2.2)	

Case group 1: vitamin E supplemented group. **Case group 2 :**without vitamin supplementation.

* Significant differences between before and after intervention.

Discussion

Increased sensitivity of thalassemic red blood cells to exogenous peroxidant danger is caused by morphologic abnormalities in thalassemic red blood cells. Along with low vitamin E levels in the red blood cells and plasma, thalassemia is characterized by diminished activity of a variety of antioxidant enzymes, including SOD and GPX, which act as the body's first line of defense against oxidative stress (Rachmilewitz, Oppenheim, and Shalev 2019).

The results in Table 2 shows that mean serum vitamin E levels in all groups were lower than the normal range at the start of the study, indicating that they were abnormal at the time of the study's initiation.

Consumption of vitamin E increases in thalassemias as a result of increased oxidative stress (Rashidi et al. 2011; Hamdy et al. 2015); Additionally, chronic hepatic iron overloads can result in a significant decrease in blood lipids, as well as a contemporaneous decrease in serum vitamin E levels, as previously indicated (Behera et al. 2014).

A statistical significant increase in serum vitamin E levels in the first group as a result of vitamin E supplementation, were observed whereas there was a non statistical significant changes in controls. Thus, these interventions (particularly vitamin E supplementation) had a beneficial effect on patients' blood vitamin E levels, which was particularly noteworthy given that the baseline vitamin E level in serum in all research groups was below the normal limit of detection.Oxidative stress is caused by an imbalance between the generation of free radicals and the destruction of those radicals (Sultana et al. 2017; Dudzińska et al. 2018).

Cell and organelle membranes are more susceptible to peroxidative damage when there is increased oxidant stress and a lower antioxidant state. The fact that hemoglobinopathies, particularly thalassemia and sickle cell disease, cause abnormalities in the oxidant-antioxidant balance has been well recognized for some time (Rashidi et al. 2011; Sposi 2019; Al-Khyate, Althanoon, and Alkazaz 2020). Antioxidant enzymes such as SOD and GPX are thought to have the role of removing harmful oxygen metabolites from the body. It has already been proven that oxidative stress causes the induction of antioxidative enzymes such as SOD and GPX in the body (Xie et al. 2019; Al-Talib and Althanoon 2020).

Our findings are consistent with those obtained in healthy subjects (Althanoon and Alkazzaz 2020). When beta-thalassemic erythrocytes of beta-thalassemic carriers were studied, it was discovered that they had significantly higher catalytic activity of SOD and GPX than normal (Rashidi et al. 2011; Melo et al. 2019). When beta thalassemia is present, the increased activity of SOD may be implicated in scavenging the superoxide radical (O2-), resulting in the production of more hydrogen peroxide in the erythrocytes. It is possible that the enhanced activity of GPX in beta-thalassemia is involved in the detoxification of hydroxyl radicals (OH-). This finding shows that elevated iron levels cause oxidative stress in cells, which in turn causes cells to increase their antioxidant defenses to combat the stress. It is possible that the increase in intracellular antioxidant enzymes is a direct result of increased intracellular iron on gene expression, as has been proposed (Al-Talib and Althanoon 2020; Charif et al. 2021).

We discovered that the SOD and GPX activities of the participants were significantly greater than normal limits at the conclusion of the study, as shown in Table 1. However, GPX activity was shown to be significantly decreased in all treated groups, which was a surprise to us. As a result, all three forms of interventions had beneficial effects on this enzyme and were helpful in improving the antioxidant status of the individuals. The

findings of this inquiry are consistent with the findings of previous investigations(Charif et al. 2021; Srichairatanakool, Koonyosying, and Fucharoen 2020).

Pfeifer et al reported that Patients with thalassemic disease demonstrated a statistically significant decrease in the levels of erythrocyte RBC-reactive oxygen species (ROS) and serum thiobarbituric acid reactive substances (TBARS), according to Pfeifer and colleagues. As a result, the researchers hypothesized that vitamin E may be effective in lowering oxidative damage in other target organs of beta-thalassemia intermediate patients as well (Pfeifer et al. 2008). Pfeifer and colleagues discovered that administering vitamin E to beta thalassemia patients lowers oxidative damage to low-density lipoprotein (LDL) and erythrocytes in the patients' blood (Pfeifer et al. 2008). Kumru et al., demonstrated Vitamin E may help reduce iron-induced oxidation of lipids (including those found in red blood cells), proteins, and DNA, according to study (d'Arqom et al. 2020; Kumru et al. 2019).

Although the precise antioxidant role of vitamin E is still debated, two mechanisms have been proposed: first, vitamin E competitive effect with iron and copper on cell membrane surface proteins involved in preventing of the production of the toxic hydroxyl free radical; and second, the antioxidant activity of vitamin E in the absence of iron and copper. When it comes to proteins, the second one is responsible for binding to the sulphhydryl-–SH groups found on a portion of them and preventing them from getting oxidized (Shu and Dunaief 2018; Raffaeli et al. 2020). Ibuki et al., found that higher levels of GPX, catalase, and lipid peroxidation were seen in rats lower in vitamin E and protein levels (Al-Khyate, Althanoon, and Alkazaz 2020). Our findings corroborated the antioxidant impact of vitamin E as seen in Table 1,which was observed in the vitamin E supplemented group, GPX activity was also dramatically reduced(Talaat et al. 2020). Ibuki et al found that in vitamine E and protein deficient rat the activities of GPX, catalase, and peroxidation of lipids were increased(Ibuki et al. 2020). Our results confirmed antioxidant effect of vitamine E (Table 1), as seen in vitamine E supplemented group GPX activity also reduced significantly.

The results in Table 2 showed that SOD activity in groups did not change significantly after vitamine E supplementation. There was no significant difference in SOD activity between the groups following supplementation, according to the findings (Table 1). Rahul Naithani et al., observed that SOD levels significantly rise in beta-thalassemia major patients, indicating that it may act as a compensatory mechanism to keep superoxide anions under control in order to combat oxidative stress, as previously reported (Naithani et al. 2006). Rashidi et al., discovered that whereas iron, ferritin, SOD, GPX, and TBARS levels were increased in 56 beta-thalassemic individuals, vitamin E and TRAP levels were considerably decreased in the same group (Sposi 2019). Attia, et al reported increase lipid peroxidation levels in beta thalassemic patients associated with compensatory increase in SOD and GPX activity (Attia et al. 2011).

Our findings corroborated those presented before and suggested that patients possessed increased SOD activity, which was previously unknown. Nasseri et al. discovered that, despite increased SOD activity, TAC remained unchanged in transfusion-dependent thalassemics. This is consistent with our findings(Nasseri et al. 2017). TAC did not change over the course of the investigation in a study conducted by Hezaveh et al., despite the provision of vitamin E and vitamin C supplements to beta-thalassemia major patients (Hezaveh et al. 2019).

Numerous strategies have been identified as useful tools for projecting the risk of tissue damage caused by free radicals over the last several years, including total antioxidant capacity (TAC), a methodology developed to assess the total antioxidant capacity of blood. However, it appears as though this method is ineffective in the treatment of thalassaemia patients. Thromboxaemia is associated with an increase in the blood levels of uric acid and bilirubin, which rise as a result of hemolysis and may mask significant changes in the blood levels of other important antioxidants (Al-Talib and Althanoon 2020).

These settings appear to have also contributed to our patients' ability to maintain similar levels of SOD activity and total antioxidant capacity (TAC). Patients with beta-thalassemic disease are more likely than the general population to have a low body mass index (BMI) (Mirhosseini et al. 2013). Following therapy, all individuals' BMIs climbed significantly, showing that they were in good health and that the supplements was having a beneficial effect. Other research reported similar findings (Jilani and Iqbal 2011).

Conclusion

As previously reported, beta-thalassemic people were shown to be suffering from elevated oxidative stress as well as a vitamin E deficiency, according to our findings. Administration of selective antioxidants had positive benefits on blood vitamin E and GPX activity, both of which are components of the antioxidant system, as well as on the overall health and well-being of the persons who received them. It is advised that additional research be conducted on the benefits of antioxidant supplements in beta thalassemic patients, using a variety of doses and durations of the supplements, in order to determine their usefulness.

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Conflict of Interest: None

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