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Is Vitamin C Supplementation Beneficial on Plasma Levels of Vitamin C and Total Anitioxidants for Pediatric Thalassemic Patients Undergoing Hematopoietic Stem Cell Transplantation?

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ABSTRACT

Background: Thalassemic patients undergoing Hematopoietic Stem Cell Transplantation (HSCT) are faced with cumulative high level of oxidative stress and depletion of critical antioxidants. Administration of antioxidants, is promising towards minimizing oxidative damage in both thalassemic and HSCT patients.

Method: This was a prospective cross-sectional observational study. Patients as a part of institutional protocol were received Vitamin C (Vit C) (all the patients received oral Vit C; 200 mg and 400 mg Vit C, if they were less or more than 20 kg respectively plus 10 mg/kg/day intravenous infusion of Vit C).We measured plasma Vit C and total antioxidant (TAs) levels at four different time points; baseline, transplantation day (0), day +7 and day +14. We calculated mean and standard error for plasma levels of Vit C and TAs.

Results: Fifthy patients enrolled in this study (mean age 7.97± 3.53). In all four time points, means of Vit C and TAs serum levels were under their reference values and their highest means were belong to baseline. Serum TAs and Vit C both depleted significantly from baseline to day 0 (P: 0.00 for both variables), then increased up to day +7 and it keeps rising till day +14 (P: 0.00 from day 0 to day +7 and +14 for both variables). These changes were significant through the measurement time. There is also a significant correlation between baseline Vit C and baseline TAs (P: 0.11). This means the higher level of Vit C is correlated with higher level of TAs and vice versa.

Conclusion: We did not observe any beneficial effects of administering Vit C in thalassemic patients undergoing HSCT in order to increase or prevent depletion of Vit C and TAs serum levels. This could be resolved by further investigations carrying out higher doses or longer duration and having a control group.

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Introduction

Thalassemia is the most frequent hereditary disease in the world (1) and beta thalassemia major is its most severe form (2). Worldwide about 150 million people carry beta-thalassemia genes. Iran is one of the regions with the high gene frequency (4-10%) with the highest ratio (>10%) in population around the Caspian Sea and Persian Gulf (3). Hematopoietic stem cell transplantation (HSCT) is the only available curative approach to thalassemia (4).

Thalassemia major and HSCT both are associated with enhanced oxidative stress (5-7). This toxicity is mainly caused by iron overload in both populations and can play important roles in morbidity and mortality (5, 8). Iron is a catalyst for formation of reactive oxidant species (ROS) and converse free radical intermediates to highly toxic free radicals such as Hydroxyl (8, 9), which is the most hazardous and high potency radicals to biochemical substances (10-12). This will consequently affect Total antioxidants (TAs) and vitamins levels (13) in both conditions and impairs their antioxidant defense system. Different studies observed that almost all thalassemic patients suffer from significant deficiency of vitamin C (Vit C) and total antioxidants (TAs) status (14, 15). In the other hand studies in HSCT also reported that Vit C concentrations have found to be depleted in addition to micronutrient antioxidants (7, 16). As a result, thalassemic patients treated by HSCT procedure will face cumulative and high level of oxidative stress, in which depletion of critical plasma and tissue antioxidants is expected (4).

The body's antioxidant system is an integrated one (14) and includes different agents such as enzymes, large molecules and small molecules (9). Antioxidants mechanisms in protecting cells against oxidative damages are preventive or chain breaking (12).

Vit C is a potent water-soluble antioxidant (7, 17) with the mechanism of reducing radical and nonradical redox reactions (7). It has been shown to scavenge hydrogen peroxide, superoxide anion, hydroxyl anion, aqueous proxy radicals and singlet oxygen. It also protects plasma from lipid peroxidation (7). Vit C concentration is identified to have an influence on γ-glutamyl-cysteine-glycine (GSH) status (16). Antioxidant defense can be evaluated by measurement of either individual antioxidants levels in cells and plasma or TAs capacity (9).

It has been stated that administration of selective antioxidants, along with an appropriate, nutritionally balanced diet would represent a promising approach towards counteracting oxidative damage and its deleterious effects on the disease status in both thalassemic and HSCT patients (5, 13, 14). Thus, investigation of the impact of antioxidants in this population seems rational.

Methods

Study design and Setting

This prospective cross-sectional observational study was performed from July 2009 to October 2011, in Hematology-Oncology and Bone Marrow Transplantation Research Center at Shariati Hospital, Tehran, Iran. Our institutional review board approved the study proposal and the study was approved by the Committee for Research Ethics and followed the Declaration of Helsinki.

Participants

Fifty pediatric patients with beta thalassemia major hospitalized for HSCT in pediatric ward were recruited for this study. Patients were excluded if they had acute or chronic infection, hematological diseases other than thalassemia, malnutrition, chronic renal failure (<60 mL/min per 1.73 m square), herbal or antioxidant medication such as Vitamin A, Vitamin E and patients who were assumed to acquire ascorbic acid before hospitalization. Eligible patients were consent by their parents for blood collection and modification. The study commenced from the first day of conditioning regimen for HSCT up to day +90 as follow up.

Conditioning regimen

All the patients Conditioning regimen (CR) included Busulfan (3.5mg/kg for 4 days) and Cyclophosphamide (40 mg/kg in thalassemia major class III and 50 mg/kg in thalassemia major class I and II for 4 days after Busulfan) followed by cyclosporine as acute graft-versus-host disease (GHVD) prophylaxis agent. Anti-Thymocyte Globulin (rabbit ATG, 1.25mg/kg for 2 days before HSCT) was added if the donor was unrelated matched or transplantation source was Peripheral blood.

Supplementation

All the patients received a regular and similar diet and they all consumed food and medicine by mouth during the study. None of the patients went under nasogastric tube feeding or total parenteral nutrition (TPN).

As a part of institutional protocol, all 50 patients took 10 mg/kg/day IV Vit C from day +1 till they discharge. Furthermore, daily oral multivitamin (multi-Sanostol®, Nycomed, Germany) was administered from admission date after collecting baseline sample and it continued throughout their hospitalization. The multi-Sanostol® dosage was based on weight of patients; if their weight was less than 20 kg they would have received 20 ml (200 mg Vit C) and if they were more than 20 kg, they received 40 ml in 2 divided doses (400 mg Vit C).

Variables

We gathered baseline and clinical data during one month of observation. We measured plasma Vit C and TAs at four different time points during admission: just before starting conditioning regimens (baseline), transplantation day (0), day +7 and day +14 (after transplantation).

The patients' data including demographic information,

treatment history, blood transfusion, level of ferritin, chelation therapy, current medication and comorbidities were also collected during the study.

Laboratory measurement

Blood samples were collected from central venous catheters in citrated blood collection tubes. After centrifugation blood at 3000 rpm for 10 minutes, the plasma was separated and divided in eight cryogenic vials. The samples' portions were stored at -80°c till serum concentration analyses. Four vials were used for total antioxidant measurement and the rest were used for total ascorbic acid (TAA) assessment.

Ferric Reducing Antioxidant Power (FRAP) assay is used as a direct measure of total antioxidant activity. In which can be assessed by absorption of blue color resulted from reduction of a ferric tripyridyl-s-triazine (fe III – TPTZ) complex to the ferrous at 593 nm. The change in absorbance is promptly related to the total reducing power of the electron donating antioxidants which is present in the reaction mixture. This reaction was performed in a semi dark room. Final steps were performed in a semi dark room.

For evaluation of TAA in patient's serum, we used the coupling reaction of dehydroascorbic acid with 2, 4-dinitrophenylhydrazine in the presence of DTC solution. During the procedure, ascorbic acid first oxidized to dehydroascorbic acid then produced 2, 3-diketoguloconic acid in presence of sulfuric acid and low PH. This reaction creates an orange-red color which has an absorbance at 520 nm in Cintra 40 spectrophotometer.

Outcome measures

Main outcomes of the study were to observe changes of Vit C and TAs serum concentration levels in pediatric beta thalassemia patients undergoing HSCT before and during Vit C supplementation, to evaluate their effect in prevention of TAs reduction, especially Vit C in this population. Our reference value for Vit C serum concentration was 14.41±6.93 µg/cc and FRAPS reference levels in plasma considered to be 1017±206 µmol/L.

Bias

In the study there was no control group and all the patients baseline status used as their controls. Based on expert opinion, the case mix of admissions in our study period were comparable to other periods. Standardized measurement protocols and calibration methods were developed and tested in order to diminish possible measurement errors.

Statistical analysis

Descriptive statistics were reported by frequencies and percentages for categorical variables and by Mean ± Standard Error (SD) for quantitative variables. For analysis of categorical variables, Chi-square test was used and for quantitative variables, both T test and Mann-Whitney-U test were utilized.

For comparison of Vit C and total antioxidant status (TAs) within subjects in different measured time points (i.e. baseline, day 0, day +7 And day +14), we conducted repeated measure analysis of variance (ANOVA) with pair-wise comparisons. Pearson correlation coefficient was calculated to compare levels of TAS and Vit Clevels at different two-by-two times. P-values less than 0.05 were considered as being significant. All the statistics were analyzed by SPSS software version 16.0 (formerly SPSS Inc., USA, Chicago).

Results

Participants/ Outcome data

Division of male and female were 26 (52%) and 24 (48%) respectively and their age was ranged between 2 to 14 years (mean 7.97± 3.53). Five patients (10%) were deceased. Detailed baselines and clinical data are summarized in Table 1.

Main results

We calculated mean and standard error for plasma levels of Vit C (Table 2) and TAS (Table 3).

In all four time point's means of Vit C and TAs serum levels were under their reference values and their highest means were belong to baseline.

Serum TA and Vit C both depleted significantly from baseline to day 0. This was followed by gradual improvement up to day +7 and it keeps rising till day +14 (Figures 1 and 2). These changes were significant through the measurement time.

For TAs serum level significant differences exist within study subjects between baseline and day 0 with day 0 (P: 0.000), day +7 (P: 0.000) and day +14 (P: 0.001). Also day 0 found to have a significant differences between day +7 (P: 0.000) and day +14 (P: 0.000). Vit C serum concentration only shows significant difference in transplantation day (day 0) with baseline day (P: 0.000), day +7 (P: 0.001) and day +14 (P:0.000).

Neither oral nor parenteral Vit C intake was correlated significantly with serum Vit C and TAs levels in different time points.

There is also a significant correlation between baseline Vit C and baseline TAs. This means the higher level of Vit C is correlated with higher level of TAS and vice versa (table 4).

Discussion

As it was expected, plasma levels of Vit C and TAs in most of our patients were abnormal at baseline (80.0% and 94.0% respectively) which confirms the results of other studies (14, 15). As it was mentioned before, this can be explained according to our study group underlying disease;

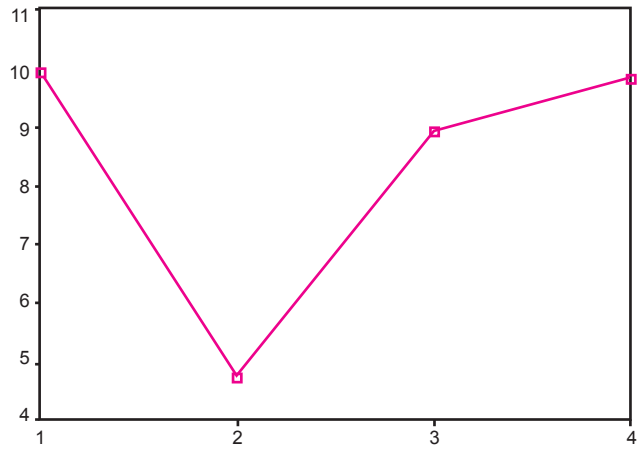

Figure 1. Vit C plasma level changes.
1; baseline day, 2; HSCT day or day 0, 3; day +7, 4; day +14.
Vit C plasma level; µg/cc

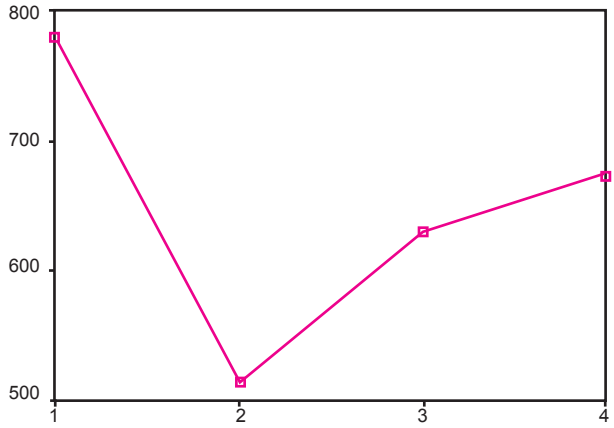

Figure 2. TAs plasma level changes
1; baseline day, 2; HSCT day or day 0, 3; day +7, 4; day +14.
TAs plasma level; µmol/L

patients with thalassemia are faced with considerable increase in lipid peroxidation, and malondialdehyde, thereby they experience significant hyper-consumption of antioxidants at both liver and membrane level (4) which cause significant decrease in the TAs capacity (18). In the other hand studies showed that Patients with thalassemia have reduced intake of many key nutrients and they suffer from dietary inadequacy (19). Besides thalassemic patients with regularly transfusion had deficient circulating levels of Vit C (19).

In our study first we faced significant decrease in Vit C and TAs from baseline and during conditioning regimen (CR) toward day 0, and then an increasing trend from baseline until +7 and +14, which it reaches near to baseline ranges. The similar pattern is reported by another study. They observed that in patients undergoing Busulfan plus Cyclophosphamide (BuCy) CR, plasma Vit C concentrations were significantly lower during CR, days 10 and 20 after BMT compare to healthy volunteer. They also reported that Vit C levels decreased significantly from before CR to CR and to day 10 after BMT then increased until day 20 (7).

It is well-known that CR in patients undergoing HSCT is associated with high oxidative stress followed by formation of reactive oxygen species and depletion of antioxidant enzyme and vitamin levels such as a-tocopherol, b-carotene and Vit C (6, 8, 16), this means that patients will face disturbance of the pro-oxidative/

Table 1. Patient's baseline, clinical and follow-up Data.

Variable	N (%)	Mean	SD
Baseline Data			
Age (years)	50 (100)	7.976	3.5
Gender			
Male	26 (52)	-	-
Female	24 (48)	-	-
Body Mass Index (kg/m2)	50 (100)	14.982	1.6
Clinical Data – Before HSCT			
Type of Thalassemia			
Class 1	14(28)	-	-
Class 2	20(40)	-	-
Class 3	14(28)	-	-
Sickle Thalassemia	2(4)	-	-
Source of Allograft Transplantation		-	-
Peripheral Blood	29(58)	-	-
Bone Marrow	21(42)	-	-
Donor Type		-	-
Sibling	42(84)	-	-
Unrelated	8(16)	-	-
Ferritin status (ng/Lit)	50 (100)	2221.9	1785.56
Transfusion Start Time (months)	46 (92)	17.89	-
Chelation Therapy Start Time (months)	44 (88)	37.09	-
Clinical Data – Conditioning regimen			
Bu, En	13 (26)	-	-
Bu, En, ATG	37 (74)	-	-
Clinical Data – Oral Supplementation			
Vitamin C (mg/d)	50 (100)	-	-
Clinical Data – After HSCT			
WBC engraftment time (days)	50	13.08	3.984

SD: Standard Deviation; HSCT: hematopoietic stem cell transplantation; Bu: Busulfan; En: Endoxan; ATG: Anti-Thymocyte Globulin.

antioxidative balance and plasma antioxidant status deterioration (6, 16). According to recent researches, both acute and chronic iron overload may occur in patients undergoing HSCT and produce free iron-generated ROS (20). Besides the adverse outcomes of CR in HSCT patients, there is extensive evidence of enhanced sensitivity to exogenous oxidant stress in red cells of beta-thalassemia (12) which can worsens the situation in our study population.

Despite the supplementation we could not observe any improvement in the plasma levels of Vit C or TAs or even prevent them from depletion to under their baseline ranges in any of measured time points. Dissayabutra et al., supplemented thalassemic pediatric patients by 100mg daily Vit C for 3 month, consecutively. They reported that TAS was not changed significantly in the corresponding period and although there was an increase in VitC concentration, the figures were still lower than normal population, which is similar to our study (15). These findings can be explained as follow, first, malabsorption due to gastrointestinal tract abnormality in absorption, and second constant consumption of large amount of antioxidants as a whole for trapping reactive oxygen species formed from oxidative stress (4).

In one study plasma TAs status was measured before conditioning therapy and serially at days 1, 3, 7, 10, and 14 after BMT. In this study all patients received 700 mg/day Vit C intravenously. There were no significant

Table 2. Vitamin C plasma level in different time points.

Timepoints	Mean	±Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Baseline	9.944	.865	8.205	11.683
Day 0	4.736	.487	3.757	5.716
Day +7	8.925	1.076	6.763	11.087
Day +14	9.810	.818	8.165	11.454

Table 3. Total Anioxidants plasma level in different time points.

Time points	Mean	±Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Baseline	779.335	22.968	733.178	825.492
Day 0	514.725	19.741	475.054	554.396
Day +7	631.461	24.274	582.680	680.241
Day +14	675.826	25.469	624.644	727.007

Table 4. Correlations between Baseline Vit C status and Baseline TAs status.

Correlations		Baseline Vit C	Baseline TAs
Baseline Vit C status	Pearson Correlation	1	.358
	Sig. (2-tailed)	.	.011
	N	50	50
Baseline TAS status	Pearson Correlation	.358	1
	Sig. (2-tailed)	.011	.
	N	50	50

VitC: Vitamin C, TAs: Total Anioxidants.

changes in Vit C values and TAs immediately after the cytoreductive therapy regimens. Initially Plasma Vit C was at the lower end of the normal ranges before CR and increased significantly during administration. The authors stated that in contrast with similar previous studies with lower Vit C doses, their IV dose of Vit C can keep Vit C concentrations within the normal range. So another possible reason for decline in Vit C ranges during our study could be insufficient supplementation (16).

We observed same pattern as Vit C for TAs plasma levels. We found a significant correlation between baseline Vit C and baseline TAs. This means the higher level of Vit C is correlated with higher level of TAS and vice versa. Therefore we can interpret the reduction of TAs concentration as a result of Vit C depletion. It is quite likely since Vit C has influence on GSH concentrations (8) it may be decreased as a result of Vit C depletion in these patients (16). GSH is known as one of the most important part of the TAs capacity and its depletion can reduce its capacity status (7).

Although up to +14 we observed gradual improvement in both Vit C and total antioxidant concentration but they did not reach to their normal values. This also might be due to underlying diseases in our patients resulting in vitamin deficiency at baseline (4, 15).

We did not observe any beneficial effects of administering Vit C in pediatric talassemic patients undergoing HSCT in order to increase or prevent depletion of Vit C and TAs serum levels. This could be resolved by further investigations carrying out higher doses or longer duration and having a control group.

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