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Role of L-Carnitine in Treatment of β -Thalassemia Major Disease

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Abstract:

β -thalassemia syndromes are a group of hereditary disorders characterized by a genetic deficiency in the synthesis of β -globin chains. L-Carnitine helps reducing oxidative stress; hence it may have a beneficial effect on β -thalassemia syndromes. The aim of this prospective study was to evaluate and compare the effect of different doses of L-Carnitine on clinical status and laboratory investigations of β -thalassemia major patients. The study was carried out on 30 β -thalassemia major patients (15 females), aged from 1.3 to 14 years old and their weights ranged from 7kg to 32kg; from hematology clinic at Beni Suf University Hospital. Patients were divided into three equal groups. Group A received dose 100mg/kg/day, group B received dose 50mg/kg/day and control group did not receive L-Carnitine. Patients were treated and followed for 6 months. They were represented to full history taking, clinical examination, laboratory investigations and determination of frequency of transfusion on admission and every month for six months. There was an improvement in general health of children after therapy and pallor, decrease in the elevated serum ferritin level, decrease in the frequency of transfusion, increase in blood transfusion intervals and increase in hemoglobin level. The increase in Reticulocyte count was in all groups, more in control group, but still with no significant difference between the three groups. Dose of 100mg/kg/day of L-Carnitine showed more improvement than dose 50mg/kg/day in pallor and laboratory findings except Reticulocyte count and more effective in reduction of frequency of transfusion.

Keywords: L-carnitine, β -thalassemia, Human erythrocytes, hemoglobin level, serum ferritin

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Introduction

β -Thalassemia major is characterized by an imbalance between the synthesis of α and β globin chains (1). RBC destruction is increased in β -thalassemic patients due to excess, unmatched α -chains (1), leading to decreased red blood cell (RBC) counts and hemoglobin (Hb) levels. The exact mechanism leading to RBC destruction in β -thalassemia is still not clear (1). RBC quality may also be altered; increased oxidant damage (2), impaired mechanical properties (3, 4), and altered membrane composition (5) have been reported. Intracellular calcium levels of RBC in β -thalassemia major patients were higher than those of normal patients (5, 6). Such alterations in RBC would increase in-vivo destruction and reduce their life span (7). Hematopoietic stem cell transplantation provides curative treatment for β -thalassemic patients (8), however regular RBC transfusions still remain to be

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the main therapeutic method. Additionally, there are new therapeutic approaches by increased synthesis of γ chain to reduce oxidative damage and stimulate fetal hemoglobin production to reduce the imbalance between α and β chains (9, 10). L-Carnitine as a treatment was also reported to be effective in such patients to prolong transfusion interval (10).

L-Carnitine is essential for intermediary metabolism of fatty acids (11). Additionally, L-Carnitine have demonstrated effects on membrane stability and function that are not related to this mitochondrial function (12), such as altered sodium-potassium pump activity and activation of enzymes for lipid incorporation in RBC membranes (13, 14). L-Carnitine also stabilized membrane fluidity alterations and morphological changes in RBC under various conditions (15). These membrane stabilizing actions of L-Carnitine may also contribute to the observed protective action in β -thalassemic RBC. RBC mechanical properties are closely related to membrane properties and function (16-18), and these regulatory actions of L-Carnitine may play role in maintaining normal RBC deformability.

Material and methods

A local hospital research ethics committee approval was obtained for the patients study. 30 patients were included in the study (15 males and 15 females); their ages ranged from 1.3 to 14 years old and their weights ranged from 7 kg to 32 kg, they were collected from hematology clinic at Beni Suf University Hospital with β -thalassemia major. The study was conducted during the period of February to October/ 2009. An informed consent was obtained from parents or legal guardians.

Inclusion criteria:

Patients diagnosed as β -thalassemia major.

Exclusion criteria:

Individuals with seizures, severe kidney disease and any allergies to L-Carnitine.

All patients were evaluated by:

1- Detailed history and thorough clinical examination which include Name, age, sex and Present history, with concentration on Pallor, Jaundice, Dark urine, Family history (As positive consanguinity and similar

condition in the family), Frequency of blood transfusion, painful crises, hospitalisation, medications, Weight, Splenomegaly and Hepatomegaly.

2- Laboratory investigations in the form of CBC (Smear Air Dried 2 -Blood on EDTA kept at room temperature), Retics count % (Blood on EDTA at room temperature), serum ferritin level (ELISA method).

Patients were divided into three equal groups A, B and C. Each group consisted of (10) patients. Group (A): Represented patients who received 100 mg/kg/day L-Carnitine. Group (B): Represented patients who received 50 mg/kg/day L-Carnitine. Group (C): Represented (Control group) patients who did not received L-Carnitine.

All these patients were subjected to:

1- Thorough clinical examination every month for six months, which includes:

- General examination: Pallor, weight, dark urine and jaundice.
- Local examination (abdominal examination) for detection of improvement in organomegally (splenomegally or hepatomegally).

2- Laboratory investigations in the form of CBC, Retics count %, serum ferritin level every month for six months. Samples were taken early in the morning in the same day of every month for six months.

Statistical analysis

A two-way analysis of variance (ANOVA) test was used to compare the effect of different doses of L-Carnitine on clinical data and laboratory investigations using SPSS V15.0 (SPSS Inc., Chicago, IL). Data was summarized as mean and standard deviation (SD). p-value was calculated to compare between the groups, it is considered significant if < 0.05 .

Results

The study was conducted on 30 (15 females) β -thalassemia major patients, collected from Hematology Clinic at Beni Suf University Hospital. The patients' ages ranged from 1.3 to 14 years old. Their weights ranged from 7 to 32 kg. Group A mean

Table I. Clinical data of all patients before inclusion in the study

	Groups		
	A (n)	B (n)	C (n)
Splenomegaly	3	3	4
Hepatomegaly	2	2	1
Consanguinity (positive)	1	2	0
Splenectomy	1	1	1
Mongoloid face	1	1	0
HCV infection	0	2	0
Growth hormone deficiency	1	0	0
Pallor	5	3	3
Dark urine	0	0	0
Jaundice	1	1	2

(SD) age and weight are 5.5 ± 3.5 years and 15.5 ± 7.4 kg respectively. Group B mean (SD) age and weight are 5.4 ± 4.1 years and 16.1 ± 6.8 kg respectively. Group C mean (SD) age and weight are 6.3 ± 4.1 years and 18.4 ± 6.9 kg respectively.

In terms of age, weight and sex, all groups were statistically homogenous. Also data analysis of all three groups before the beginning of study showed no significant differences concerning total hemoglobin concentration, reticulocytic count percentage, serum ferritin level and number of packed red cells transfusion.

Clinical data of all patients before inclusion in the study are shown in Table 1. Means and SD of the collected data after therapy of the three groups are shown in Table 2.

The study showed that the effect of the high dose in group A has a better effect than that in group B which has better effect than the control group C on total hemoglobin concentration, reticulocytic count percentage, serum ferritin level and number of packed red cells transfusion.

In serum ferritin level, there was an insignificant increase after the L-Carnitine treatment in group A ($p=0.210$) and there was significant increase in group B ($p=0.047$) and C ($p=0.030$).

In reticulocytic count percentage, there was no significant increase after the L-Carnitine treatment in group A ($p=0.416$) and B ($p=0.440$) however there was a significant increase in C ($p<0.001$).

Table II. Means and SD of the collected data before and average of after 6 months therapy of the three groups after therapy

	Group A			Group B			Group C		
	Before	After	p	Before	After	p	Before	After	p
Ferritin (ng/ml)	727.5 ± 234.8	878.9 ± 284.0	0.210	720.5 ± 178.4	949.9 ± 207.2	0.047*	816.0 ± 317.1	1251.9 ± 491.2	0.030*
Reticulocyte (%)	3.4 ± 2.8	4.5 ± 3.6	0.416	3.4 ± 2.8	4.9 ± 3.9	0.440	3.4 ± 1.3	6.6 ± 1.5	<0.001***
Hemoglobulin (g/dl)	7.0 ± 1.2	8.4 ± 1.0	0.014*	7.3 ± 0.8	7.6 ± 0.8	0.267	7.4 ± 1.0	6.9 ± 0.7	0.257
Number of packed red cells transfusions per month	4.1 ± 1.0	1.7 ± 0.67	<0.001***	4.1 ± 1.3	2.5 ± 1.27	0.012*	4.7 ± 0.9	5.1 ± 0.6	0.271

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

Table III. p values of the comparison of Group A vs Group C and Group B vs Group C

	Group A vs Group C	Group B vs Group C
Ferritin	0.05*	0.061
Reticulocyte (%)	0.101	0.106
Hemoglobin (g/dl)	0.002**	0.041*
Number of packed red cells transfusions per month	0.251	>0.001***

* p < 0.05 ** p < 0.01 *** p < 0.001

In total hemoglobin concentration, there was a significant increase after the L-Carnitine treatment in group A (p=0.012) and there was no significant increase in group B (p=0.267) and C (p=0.257).

In number of packed red cells transfusion, there were significant decrease after the L-Carnitine treatment in group A (p<0.001) and B (p=0.012) however there was a significant increase in C (p=0.271).

After treatment, there was a significant difference between A and C groups regarding ferritin, and Hemoglobin with p-value 0.05 and 0.002 respectively.

Also, After treatment, there was a significant difference between B and C groups regarding Hemoglobin and Number of blood transfusion with p-value 0.041 and <0.001 respectively.

However, there was no significant difference between A and C groups and B and C groups, after the six months therapy regarding retics count % with p-value 0.101 and 0.106 respectively.

Discussion

The aim of the study was to evaluate the effect of L-Carnitine on the clinical features and the hematological parameters of patients of β -thalassemia and to estimate the dose of choice for better effect, 50mg/kg/day or 100mg/kg/day.

Clinical statistics of patients in all six months of the study showed that groups A and B did not increase the serum ferritin level like the control group with a superior effect by group A. These results showed that serum ferritin level in group A and B increased after therapy but in lower rate than control group and this is an indication to improvement in group A and B than control group. However the increase in group B was not significant, may be due to the low numbers of the patients in the study.

These results are in consistence with an earlier study which revealed that thalassemic children had significant DNA double-strand breaks that was positively correlated to their iron overload reflected by serum ferritin level and can be ameliorated by L-Carnitine supplementation (19).

The mean yearly serum ferritin level in the study by S M. Ragab and R G. Mahfouz, 2010 was lower in those on L-Carnitine therapy than control group (1570 \pm 813 versus 1661 \pm 769 ng/ml, respectively), yet the difference did not reach a significant level (19).

The study also, showed a significant increase in the hemoglobin level and decrease in the number of blood transfusion in groups A and B compared to control group. These indicate an improvement in group A and B patients with a better effect in Group A. AEI-Beshlawy et al 2005 study revealed a similar results. They showed that, L-Carnitine seems to be a good modulator of apoptotic processes in thalassemic patients leading to a decreased frequency of programmed erythroblast death and general improvement of the disease condition. They also showed that patients had a significant decrease in the frequency of transfusions and increase in the pre-transfusion hemoglobin levels after therapy (20).

On the other hand our results did not agree with the study by J Bommer (21) and a study on haemodialysis patients with anemia by J Kletzmayer et al (22). They reported a reduction in the erythropoietin requirement (-36.9 \pm 23%) in 42% of 20 haemodialysis patients treated with L-Carnitine (5 or 25 mg/kg/dialysis I.V. after each dialysis session). 58% of patients did not respond to the L-Carnitine therapy and maintenance of stable hemoglobin necessitated a constant Recombinant human

erythropoietin dose. J Kletzmayr et al did not see a significant increase in erythrocyte survival time in L-Carnitine treated patients (22). However, the insignificant effect found in the above mentioned two studies may be due to the lower and discontinuous dose of L-Carnitine used.

Also, there was an increasing in retics count% in the three groups but only the increase in the control group was significant compared to before the treatment. However, there was no significant difference between the three groups after the treatment. These results are similar to the study by N. Nand et al (23). In this study, reticulocyte count% increased was significantly different in treated and untreated groups with L-Carnitine after 3 and 6 months compared to baseline however the result of both groups was statistically insignificant different when compared to each other.

Finally the present work shows that prescribing L-Carnitine has a very good effect on the β -thalassemia and the 100 mg/kg/day dose of L-Carnitine is better and more effective than the 50 mg/kg/day dose in all laboratory parameters and in reduction of blood transfusion frequency.

Conclusions

The β -thalassemia patients in groups A and B who received dose 100 mg/kg/day of L-Carnitine and dose 50mg/kg/day respectively showed more improvement than control group (in times of blood transfusion, hemoglobin level, serum ferritin, pallor).

The dose 100 mg/kg/day of L-Carnitine is more effective than dose 50 mg/kg/day and L-Carnitine has a significant role in improving general health of patients.

Further wider studies are needed to confirm the results of the presented study.

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