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# Fetal Globin Induction with Oral Butyrates in ß-Thalassemia Major

AMAL EL-BESHLAWY, M.D.*; MONA HAMDY, M.D.*; ILHAM YOUSSRY, M.D.*; MONA EL GHAMRAWY, M.D.*; NAELA OMRAN, M.D.**; HALA GABR, M.D.** and SAUDY HASSANAIN, M.D.**

*The Departments of Pediatrics* and Clinical Pathology**, Cairo University, Cairo, Egypt*

## ABSTRACT

***Background:*** The ß-thalassemias are characterized by insufficient or absent production of ß-globin chains. L-carnitine (L-3-hydroxy-4-N-trimethyl amino butyric acid) is an oral butyrate derivative that can stimulate Hb F production.

***Objectives:*** The aim of this work is to study the hematological effects of short-term therapy of L-carnitine on the induction of fetal hemoglobin (Hb F) production in a group of ß-thalassemia major patients.

***Patients and Methods:*** L-carnitine was given orally (50 mg/kg/day) to 27 patients (mean age 12.26±4.25 years) for 45 days. Complete blood count, hemoglobin electrophoresis and F cell percentages (by immuno-histochemical technique) were estimated before and after L-carnitine therapy.

***Results:*** Fourteen patients (51.85%) were responders showing a significant increase in mean HbF and F cells ($p$=0.00001, 0.00006 respectively) after L-carnitine therapy. The transfusion intervals were significantly prolonged among responders and non-responders ($p$=0.0028 for both).

***Conclusion:*** L-carnitine is a physiologic, well tolerated and safe drug that can stimulate Hb F production in thalassemia patients. Further studies with a larger number of patients and a higher dose of L-carnitine are required to evaluate its overall role in thalassemia.

***Key Words:*** *L-carnitine – Butyrates – HbF induction – ß-thalassemia.*

## INTRODUCTION

The ß-thalassemias are characterized by insufficient or absent production of ß-globin chains leading to imbalanced ß and non ß-globin chain synthesis. This causes accumulation and precipitation of unpaired ß-globin chains and consequently, to ineffective erythropoiesis and hemolysis **[1,2]**. Induction of fetal hemoglobin (HbF) is a novel therapeutic strategy that has been hypothesized for ß-thalassemia, based on the observation that the co-existence of hereditary persistence of fetal hemoglobin (HPFH) in patients with ß-thalassemia reduces the severity of the disease and the need for blood transfusion **[3,4,5]**. This suggested that agents that would mimic the HPFH mutation by preventing binding of "transcription factors" to the ß-globin promoter would favour expression of the ß-globin gene and ameliorate the severity of ß-thalassemia and sickle cell disease **[6]**.

Hb F enhancement would be beneficial especially for ß-thalassemia patients in developing countries that cannot sustain the high cost of maintaining regular transfusion regimen and chelation therapy. L-carnitine (L-3-hydroxy-4-N-trimethyl amino butyric acid) is an oral butyrate derivative that can stimulate Hb F production and stabilize the erythrocyte membrane against oxidative stress. Some studies reported its beneficial effects on physical fitness, cardiac status and pubertal development in thalassemic patients **[7,8,9]**. The aim of this work is to study the hematological effects of short-term therapy of L-carnitine on the induction of fetal hemoglobin production in a group of ß-thalassemia major patients.

## MATERIAL AND METHODS

This is a prospective, single-arm study that included 27 transfusion-dependent homozygous ß-thalassemia patients, after their or their caregivers' consent and approval of our Institutional Ethical Committee. All patients were previously

diagnosed and followed-up at the Hematology Clinic of Cairo University New Children Hospital. Among the recruited patients, 12 were males and 15 females. Their age ranged between 6 and 22 years (mean of 12.26±4.25 years). Twenty-two patients had splenectomy.

The patients' medical records were reviewed and frequency of blood transfusion was recorded. Complete blood count, hemoglobin electrophoresis (by densitometry following cellulose acetate) and F cell percentages (by immunohistochemical technique using monoclonal anti-Hb A antibodies) were estimated before and after 45 days of oral L-carnitine therapy (50mg/kg/day). The Hematology Clinic provided the drug to all patients free of charge on an outpatient basis.

*Statistical methods:*

Numerical data were presented as mean and standard deviation. Student's *t*-test was used to compare numerical data between groups. Statistical significance was considered if *p*-value was <0.05.

## RESULTS

According to the increase in the percentage of Hb F and F cells after treatment, the patients were divided into 2 subgroups; Group 1 (responders) who showed an increase in Hb F and F cells percentage and group 2 (non responders) who showed either decrease or constant Hb F and F cells percentages after treatment.

In group 1 (n=14, 51.85%), mean Hb F increased significantly from 19.3±14.62 % to 45.05±17.45% (*p*=0.00001) and mean F cells increased from 19.72±14.45% to 43.89±16.85% (*p*=0.00006) after L-carnitine therapy. In group 2 (n=13, 48.15%), mean Hb F significantly decreased from 35.92±34.92 % to 32.1±34.16% (*p*=0.012) and mean F cells from 36.53±35.73% to 32.5±33.96% (*p*=0.014) (Fig. 1). The mean intervals between blood transfusions were significantly prolonged in groups 1 and 2 (*p*=0.0028 for both). This was observed in 55.6% (n=15/27) of patients within 2 weeks. Hemoglobin, hematocrit and all red cell indices increased, but non-significantly, after therapy in both groups except for the red cell distribution width (RDW) which decreased significantly in groups 1 and 2 (*p*=0.001, *p*=0.004 respectively) (Table 1). The drug was well tolerated by all patients without any adverse reactions.

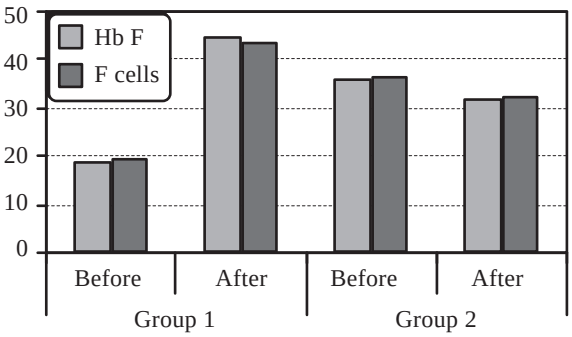


Fig. (1): Mean Hemoglobin F and F cells percentages before and after L-carnitine therapy in group 1 (responders) and group 2 (non-responders) *ß*-thalassemia patients.

Table (1): Clinical and laboratory parameters of *ß*-thalassemia Patients (n=27) before and after 45 days of L-carnitine therapy.

| Parameter | | Group 1 (n=14) | *p*-value | Group 2 (n=13) | *p*-value |
|---|---|---|---|---|---|
| Interval of blood transfusion (weeks) | Before | 3.36±1.22 | 0.0028* | 3.08±1.04 | 0.0028* |
| | After | 4.07±1.27 | | 3.62±1.12 | |
| Hemoglobin (g/dl) | Before | 7.21±0.58 | 0.23 | 7.33±1.01 | 0.73 |
| | After | 7.46±0.96 | | 7.42±0.92 | |
| Hematocrit (%) | Before | 20.79±2.07 | 0.12 | 22.52±5.44 | 0.98 |
| | After | 21.96±2.74 | | 22.57±3.51 | |
| MCV (fl) | Before | 71.01±7.86 | 0.23 | 72.92±7.38 | 0.10 |
| | After | 72.89±7.95 | | 74.65±6.45 | |
| MCH (pg) | Before | 24.58±2.22 | 0.84 | 24.95±2.62 | 0.28 |
| | After | 24.66±2.33 | | 25.45±2.65 | |
| RDW | Before | 29.01±7.10 | 0.001* | 28.82±7.99 | 0.004* |
| | After | 24.54±5.74 | | 23.03±6.35 | |

* Statistically significant. MCV: Mean Cell Volume. MCH: Mean Cell Hemoglobin. RDW: Red cell Distribution Width.

## DISCUSSION

Butyrate is a well-known histone deaceytylase inhibitor (HDACi) that was shown to induce HbF synthesis after intravenous infusion in patients with ß-thalassemia **[10,11]**, and in a majority of patients with sickle cell disease **[12]**. Other butyrate analogues (e.g. phenylbutyrate, isobutyramide and other short chain fatty acids) that also induce HbF expression in human erythroid cells were only used in limited clinical trials in ß-thalassemia **[13]**. Butyrate was shown to displace histone deaceytylase 3 (HDAC3), which might be primarily responsible for ß-globin silencing **[14]**. In addition, exposure to butyrate was shown to alter the DNA methylation status of the ß-globin locus **[15]** and to increase the efficiency of the translation of the globin mRNA **[16]**.

In this study, a response rate of 51.85% was noted among our patients after L-carnitine therapy, with a statistically significant increase in mean Hb F and F cells percentages ($p$=0.00001 and 0.00006 respectively). The oral route of administration of L-carnitine is an additional advantage especially being well tolerated by all our patients. On the other hand, non-responders (48.15%) showed a significant decrease in mean Hb F and F cells percentages ($p$=0.012 and 0.014 respectively). This is difficult to be explained, it might be due to the wide variation in individual responses to pharmacological stimulation of fetal hemoglobin by butyrates. This variability of response can be replicated in vitro when erythroid progenitors from the same patients are cultured in the presence of butyrate **[15]**. It was suggested that the responsiveness of a patient to butyrate may be determined by the epigenetic configuration of the ß-globin gene cluster. The elucidation of the role of this epigenetic variability is a major challenge for the effective use of these compounds in the treatment of patients with hemoglobin disorders. The chromatin structure of the locus is determined by the acetylation state of the histones and the state of its DNA methylation **[17]**. Furthermore, low dose and short duration of L-carnitine therapy in our work may contribute to the reduced response rate.

Even though L-carnitine therapy was administered for 45 days only, it seemed to be beneficial for most homozygous thalassemia patients as shown by prolongation of transfusion intervals in 55.6% of cases within 2 weeks with a significant increase in the mean transfusion interval in both groups ($p$=0.0028 for both). This was previously reported and was explained by the protective effect of L-carnitine on the red blood cells from oxidative stress and the stabilization of their membranes where latent peroxidative damage has occurred **[8,7,18,19,20]**. In ß-thalassemia, increased oxidative stress is probably due to auto-oxidation of globin chains and iron overload **[21,22]**. The counteracting effect of antioxidants on lipid peroxidation and their protective effect against oxidative damage of erythrocytes were previously demonstrated **[23]**.

The non-significant increase in hemoglobin, hematocrit, MCV and MCH values after L-carnitine therapy among our responders group may be due to the low dose and short duration of therapy. On the other hand, improvement of the hematological parameters in the non-responders could be attributed to the other physiologic effects of L-carnitine on the red cells of thalassemia patients. One study demonstrated no alteration in hematological picture after one month of L-carnitine therapy despite of improvement of RBC quality including RBC lipid peroxidation, cytosolic calcium concentrations and RBC deformability **[20]**. Maintaining normal RBC mechanical properties was suggested to be an important objective in therapeutic approach to thalassemia patients **[17]**.

Red cell distribution width (RDW) is a quantitative parameter of variation in the red cell volume; it is equivalent to the microscopic assessment of the degree of anisocytosis. This study showed a significant decrease in RDW in both groups which denotes that red cells became more homogenous. The effect of L-carnitine on red cell membrane may explain this finding. Previous studies supported the effects of L-carnitine on membrane stability and function. Altered sodium-potassium pump activity, activation of enzymes for lipid incorporation in RBC membranes and interacting membrane skeleton proteins were suggested as possible mechanisms **[24,25,26]**.

In conclusion, L-carnitine as an oral butyrate is a physiologic, well-tolerated and safe drug that can stimulate Hb F production in homozy-

gous thalassemia patients. Further longer studies with a larger number of patients and a higher dose of L-carnitine are required to evaluate its overall role in the course and prognosis of thalassemia.

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