

TRACE ELEMENTS AND HEMATOLOGICAL CHANGES IN THALASSAEMIA MAJOR AND MINOR

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Abstract

Background: The Thalassaemias are hereditary hemolytic anaemia due to genetic defect in the DNA or in the messenger RNA. Many studies on B-thalassaemia (major and minor) have reported variation in the level of the trace elements zinc, Copper and Magnesium. These variations may play a role in cellular abnormalities, which characterize this disorder. Thus, these trace elements have been used as indices for following up these patients.

Objective: To assess the level of serum Cu, Zn and Mg in thalassaemic patients (major and minor) and to investigate different hematological parameters as well as serum ferritin in those patients.

Methods: 72 patients with B-thalassaemia major (Th. M.), 17 patients with B- thalassaemia minor (Th.Mn.) and 30 healthy age matched subjects who served as control. Trace elements, Hb, PCV, MCH and MCHC and serum ferritin were estimated.

Results: A significant decrease in Hb concentration and MCHC was found in Th.M. compared to control group whereas a significant increase in MCV, MCH and MCHC was found in Th.M. compared to Th.Mn. In addition, MCV, MCH and MCHC were

significantly reduced in Th.Mn. compared to control, while PCV level was significantly higher when compared to Th.M. patients and control group.

Serum Cu and Zn in Th.M. were significantly increased when compared to control group while serum Mg in Th.M. was significantly reduced when compared to healthy control but not in Th.Mn. In Th.Mn., serum Cu was significantly increase when compared to control and to Th.M. patients.

Serum ferritin was significantly high in B-thalassaemia major and minor when compared to control. In addition, it was significantly high in Th. M. compared to Th.Mn. Furthermore, only serum Zn and Mg showed a significant positive correlation with PCV in B-Th.M. patients.

Conclusion: Serum Cu and ferritin were high in B-Th.M. and Th.Mn., serum Mg was low and serum Zn was high in B-Th.M. Since serum Zn and Mg were closely related to PCV, therefore the therapeutic value of Mg and Zn supplementation should be tested in those patients.

Keywords: Trace Elements, Thalassaemia Major and Minor, Hematological Changes

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Introduction

Copper (Cu), Zinc (Zn) and magnesium (Mg) are biological elements that are called as trace elements because small amount of them are found in human body^[1], however they are essential metals that are required

for growth and proliferation of healthy cells and for normal lymphocytes maturation and regulation of the immune function^[2]. Moreover, changes in the level of these elements may impair cellular and physiological functions through changes in the activities of metalloenzymes which require a small and constant number of metal per mole to attain full activity of these enzymes^[3].

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The thalassaemia syndromes are characterized by an inherited defect in the synthesis of one or more of the peptide chains of haemoglobin^[4]. The biochemical changes especially of the trace elements such as Cu, Zn and Mg had been investigated. Many clinicians had noticed growth retardation^[5-7], various retinal abnormalities^[6,8] and different abnormalities in the RBC of thalassaemic patients^[9], which were attributed to alteration in the level of these trace elements. Therefore, trace elements had been critically examined in the follow up and treatment of patients with B thalassaemia.

Aims of the study

1. Assess the level of Cu, Zn and Mg in the blood of thalassaemia major and minor and compare it with control level.
2. Evaluate the change in different haematological parameters including Hb, PCV, MCV, MCH, MCHC and serum ferritin in B thalassaemia major and minor and compare it with control values.

Materials and Methods

This study was conducted on 72 patients with B thalassaemia major and 17 patients with B thalassaemia minor who were attended Ibn Al-Balady hospital from 2002-

2003 along with 30 healthy age matched control subjects table 1.

All those patients were previously diagnosed as having thalassaemia by clinical, family history, hematological and electrophoresis study.

Blood samples collected from the patients before blood transfusion, in an EDTA tube and in plain tube. Serum Cu, Zn and Mg were estimated by Atomic Absorption Spectrophotometer, Perkin Elmer, model 103, serum ferritin was estimated by Immunotech Kit, using Automatic Gamma Counter (Wallac-Wizard). Other haematological parameters, Hb, PCV, MCV, MCH and MCHC were estimated by multichannel analyser, Coulter S Plus.

All data were analyzed by unpaired student t-test and by linear regression test, taking $p < 0.05$ as the lowest limit of significant.

Results

This study was performed on 72 patients with thalassaemia major, 17 patients with thalassaemia minor, and 30 healthy age matched control subjects, table 1.

Table 1: The demographic characters of the patients and control subjects enrolled in this study

Parameters	Study Groups		
	Thalassaemia major	Thalassaemia minor	Control
Number	72	17	30
Age (years)	4-29	20-33	4-30
Sex (female/male)	20/52	9/5	11/19

This study had revealed that in B-thalassaemia major, serum Cu was significantly increased compared to control individuals and to thalassaemia minor patients ($p<0.01$ and $p<0.05$) respectively (Table 2 and figure 1). In addition, serum Zn was significantly increased in relation to healthy control and to thalassaemia minor patients, ($p<0.05$) {table 2 and figure 1}, while serum Mg in thalassaemia major

patients was significantly decreased in relation to healthy control ($p<0.05$) but its relation with thalassaemia minor was insignificant (Table 2 and figure 1).

In thalassaemia minor although serum Cu was significantly high when compared to healthy control but both serum Zn and Mg were not significantly changed when compared to control group (Table 2 and figure 1).

Table 2: Levels of serum Cu, Zn, Mg and ferritin in thalassaemia major, minor and control group

Parameters	Thalassaemia major n=72	Thalassaemia minor n=14	Control n=16
Cu (nmol/L)	30.094±6.319 ^{***†}	24.58±10.95 [*]	21.38±6.125
Zn (nmol/L)	1.14±0.198 ^{*†}	0.899±0.334	0.858±0.240
Mg (mg/dl)	2.111±0.427 [*]	2.145±0.623	2.428±0.684
S. ferritin µg/L (mean±SE)	1359.5±147.03 ^{****†††}	83.0±30.55 ^{**}	44.0±18.34

* All data are expressed as mean±SD except S. ferritin is expressed as mean±SE.

* $p<0.05$ correlation with control. ** $p<0.001$ correlation with control. **** $p<0.0001$ correlation with control.

† $p<0.05$ correlation with thalassaemia minor. ††† $p<0.001$ correlation with thalassaemia minor.

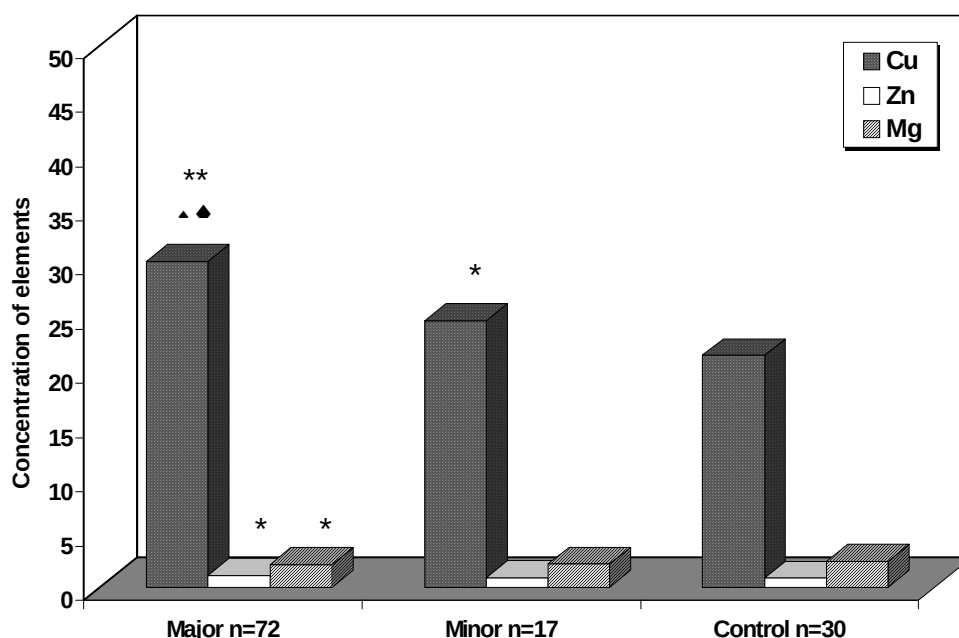


Figure 1: The concentration of serum Cu, Zn and Mg in thalassaemia major, minor and control.

* $p<0.05$ correspondent to control. ** $p<0.001$ correspondent to control.

◆◆ $p<0.01$ correspondent to minor.

The level of serum ferritin was significantly increased in thalassaemia major in relation to its level in thalassaemia minor and control ($p<0.001$, $p<0.00001$). Similarly it

was significantly increased in thalassaemia minor in relation to its level in control ($p<0.001$) {Table 2 and figure 2}.

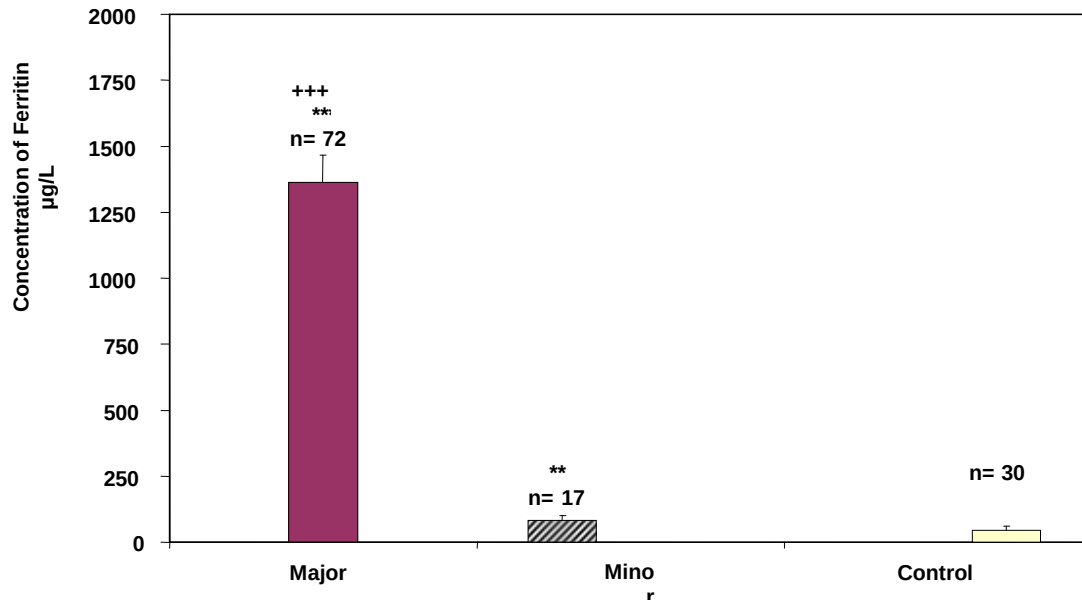


Figure 2: The serum ferritin concentration in thalassaemia major, minor and control.

*** $p<0.001$ correspondent to control.
+++ $p<0.001$ correspondent to minor.

** $p<0.01$ correspondent to control.

This study had revealed a positive correlation between serum Mg and the haemoglobin level in B- Thalassaemia major patients ($r=0.457$) (Figure 3).

Moreover, there was a positive correlation between serum Zn, haemoglobin and PCV ($r=0.278$, $r=0.306$) respectively (figures 4 and 5).

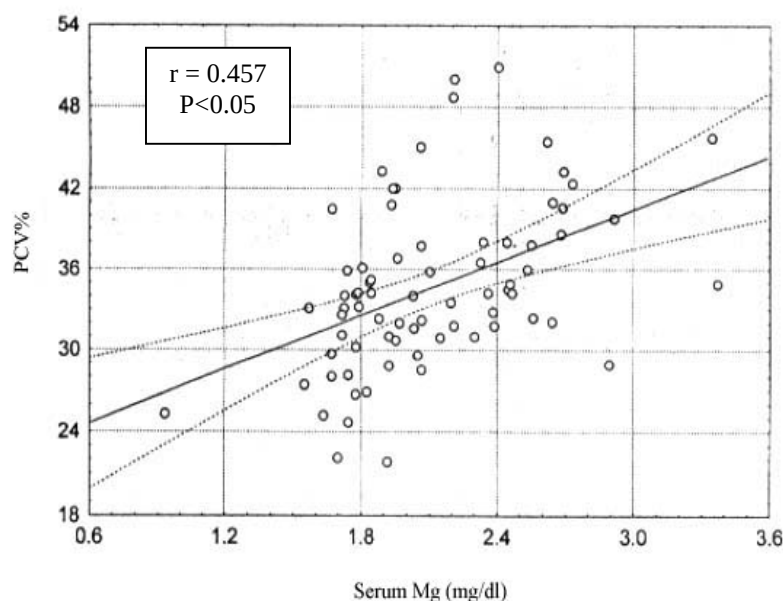


Figure 3: Positive correlation between Serum Mg and PCV in B thalassaemia major patients

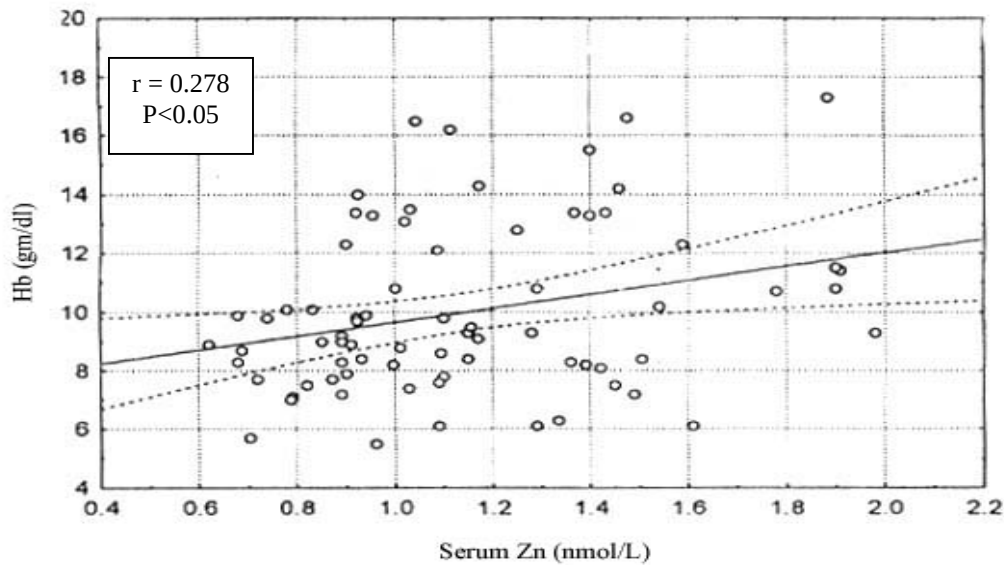


Figure 4: Positive correlation between Serum Zn and Hb level in B thalassaemia major patients

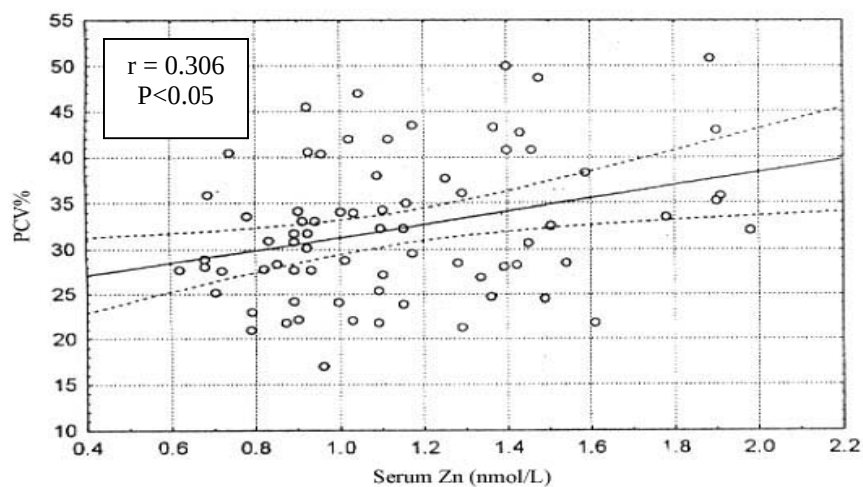


Figure 5: Positive correlation between Serum Zn and PCV level in B thalassaemia major patients

Furthermore the mean haemoglobin level and the MCHC were significantly lower and the MCV was significantly higher in thalassaemia major than in control ($p < 0.05$ and $p < 0.01$) respectively, while the MCV, MCH and MCHC were significantly higher in thalassaemia major compared to thalassaemia minor ($p < p < 0.001$, $p < 0.01$, $p < 0.05$) respectively (Table 3).

In thalassaemia minor the mean MCV, MCH and MCHC were significantly lower than a control mean level ($p < 0.001$, $p < 0.0001$ and $p < 0.001$) respectively while the PCV was significantly higher compared to both thalassaemia major and control group ($p < 0.05$) {Table 3}.

Table 3: The level of Hb, PCV, MCV, MCH and MCHC in thalassaemia major, minor and control group

Parameters	Study group (mean±SD)		
	Thalassaemia major n=72	Thalassaemia minor n=17	Control n=30
Hb concentration (gm/dl)	10.8±3.0*	11.1±1.18	11.5±1.79
PCV(%)	34.6±8.6 ⁺	36.4±7.10 [♦]	34.3±6.02
MCV(fl)	83.0±6.35 ^{**+++}	64.9±1.76 ^{♦♦}	76.3±16.17
MCH(pg)	26.9±3.67 ^{++*}	18.8±1.57 ^{♦♦♦}	28.5±1.57
MCHC(gm/dl)	31.2±3.78 ^{*+}	29.0±2.53 ^{♦♦}	35.5±2.10

* p<0.05 correspondent to control. ** p<0.01 correspondent to control. ♦ p<0.05 correspondent to control. ♦♦ p<0.01 correspondent to control. ♦♦♦ p<0.001 correspondent to control. + p<0.05 correspondent to minor. ++ p<0.01 correspondent to minor. +++ p<0.001 correspondent to minor.

Discussion

Trace elements are increasingly playing a diagnostic, curative and preventive role in many diseases^[10].

Iron overload is one of the major complication in B- thalassaemia which due partly to the ineffective erythropoiesis and the constant hemolysis from early infancy and partly to the frequent blood transfusion required for the treatment of the severe anaemia^[11].

Our study in agreement with other studies found that serum Cu and iron were significantly increased in thalassaemia patients^[12-14]. The close relation between serum Cu and iron can be explained by the importance of copper containing enzymes and co-factors for iron absorption and the effect of copper on the release of iron from the body stores as well as the utilization of iron in haemoglobin synthesis^[15,16]. This will explain the hypercupraemia usually encountered in haemochromatosis which is a principle complication in thalassaemia^[17,18].

In thalassaemia minor serum ferritin and serum Cu level was significantly lower than in thalassaemia major, table 2, figure 1 and

2, which is expected since those patients are non transfusion dependant and have less extend of ineffective erythropoiesis^[11]. Therefore, we expect the increase in serum Cu will be much less than thalassaemia major.

Bashir NA et al^[13] study was similar to our study revealed a significant increase in serum Zn and Cu in thalassaemia major patients, and he had contributed it to impairment in kidney function and to disturbances in the metabolism of Zn and Cu which usually encountered in those patients^[13].

Moreover since Zn concentration in the RBC is approximately 12 time that found in the plasma^[12] and during transfusion of blood a considerable proportion of transfused cells will be destroyed^[11], therefore we may propose that these patients who are transfusion dependant will have higher serum zinc comparing to control subjects and to thalassaemia minor patients who are not transfusion dependant.

It is well known that liver function as major storage organ for zinc^[19] and since iron overload in the liver may generate oxygen free radical and may induce peroxidative

tissue^[20], therefore we may expect an increase in serum zinc from damaged hepatocytes^[19].

Pakarek et al hypothesized that variation of serum Zn level may be due to leukocyte endogenous mediator (LEM) which has the property of mobilizing zinc from its stores in liver and other tissue to the serum^[21].

The present study found significant low serum Mg in thalassaemia major patients which is similar to other studies^[15,16,22]. Magnesium is the second most abundant intracellular metal after potassium and it plays an essential role in the activity of many enzymes involved in cellular metabolism^[23]. De-Franceschi L. et al^[22] had speculated that the hypomagnesemia in B-thalassaemia may be due to chelation by citrate in chronically transfused patients or could just be a consequence of cellular iron overload^[22,23].

Thalassaemia major patients were found to have significant anaemia compared to control, table 2, which was expected since those patients suffer from chronic hemolytic anaemia which is due to ineffective erythropoiesis and the hemolysis of RBC^[11,18].

Generally, thalassaemia minor is a milder disease; therefore, the reduction in haemoglobin did not reach the level of significant. On the other hand, PCV was significantly increased in thalassaemia minor because of the increased RBC count usually encountered in thalassaemia minor patients^[11,18].

In agreement with previous studies, the MCV, MCH and MCHC were significantly reduced in thalassaemia minor^[11,18] who were transfusion independent. While in thalassaemia major, they were significantly increased because all the thalassaemia major

patients were transfusion dependant and the transfused RBCs will affect these haematological parameters. Also the increase in MCV may be contributed to folic acid deficiency usually encountered in B- thalassaemia patients as a result of increased cell turnover in the bone marrow^[11,17,18].

Additionally, this study had revealed a positive correlation between serum Mg and PCV ($r=0.457$) in B- thalassaemia major patients (Figure 3), which was in agreement with a study of De-Franceschi L et al^[22], who had found that supplement of Mg may improve anaemia and reduce dehydration in B- thalassaemia major rats which was attributed to the effect of Mg on membrane stability by decreasing the activity of the K-Cl contrasport system, thus decrease the abnormal erythrocyte K loss and increase the life span of the RBCs^[22].

Many studies had emphasized on the importance of Zn supplementation in the treatment of iron deficiency anaemia particularly in pregnant female^[25] and in children^[26]. This study revealed a positive correlation between serum Zn, haemoglobin and PCV ($r=0.278$, $r=0.306$ respectively) in B- thalassaemia major patients (figure 4 and figure 5). These results were in agreement with many other studies who found that zinc supplementation in B- thalassaemia major had a positive effect on linear growth and anaemia^[25,27].

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