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Trace elements in patients with p-thalassaemia major

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Abstract. In this study, 82 patients with (5-thalassaemia major, and 142 apparently healthy control subjects were investigated for: haemoglobin, blood indices, serum iron, total iron binding capacity, transferrin saturation, serum ferritin, zinc, copper and copper/zinc ratio. Patients with (5-thalassaemia major had significant growth retardation as their BMI equal to 15.99±2.97 kg/m^2 compared to 20.61 ±4.28 kg/m^2 for the controls (p<0.001). Haematological investigations revealed that mean haemoglobin level was 6.6 ± 1.6 g/dl for patients while for controls being 12.4± 1.5 g/dl. Other blood indices were severely altered in patients with P-thalassaemia major. In all age groups, patients with (5-thalassaemia major had severe iron overload with about 20-fold increase in the concentration of serum ferritin as compared with normal subjects and nearly 70% saturation of transferrin. Serum zinc was significantly decreased while serum copper and Cu/Zn ratios were higher among patients for all age groups compared to the respective age group in control subjects. There was a significant negative correlation between serum zinc and copper levels in patient's (3-thalassaemia major (r=-0.65, P<0.01). In conclusion patients with homozygous (3-thalassaemia were zinc deficient; as zinc can reduce the copper and iron concentrations and increase growth we recommend that zinc supplementation for these patients is important.

Keywords: p-thalassemia major • iron status • copper • zinc • children

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INTRODUCTION

Beta-thalassaemia is the most important type of thalassaemia because it is common and produces severe anemia in its homozygous and compound heterozygous state[1]. In 1999, WHO has estimated that the carrier frequency of p-thalassaemia in Iraq is about 3% and the annual births of homozygote of p-thalassaemia are about 571 patients/year[2]. In Basrah, a recent premarital study on couples attending the Public Health Laboratory revealed that prevalence of P-thalassaemia trait is about 4.6%'.

Iron, copper and zinc are essential trace elements in human body and all are altered in different types of blood diseases including thalassaemia in which they may play a role in pathogenesis[4-5]. The alteration of these elements combined with excess amounts of haemoglobin subunits enhance the generation of oxygen radicals after a chain of reactions leading to early death of the red cells and haemolysis[2].

It has long been recognised that serum iron is elevated in homozygous |3-thalassaemia and iron binding capacity is fully saturated. but percent transferrin saturation and elevated plasma ferritin are the classical indices for iron overload[6-7]. However, serum ferritin is not always the most accurate indication of iron over-

load because it may be elevated due to inflammation or hepatocellular damage[8,11].

In thalassaemia major, plasma erythrocytes and hair zinc levels were found to be much lower than that in normal subjects and this is also true in sickle cell disease and haemoglobin H disease[5,12,14]. Serum copper levels were found to be significantly increased compared to normal subjects. This is also true in other types of haemolytic anemia[12,14,16].

The aim of the study was to determine the changes in iron status, zinc and copper levels in subjects with thalassaemia major and compare them with levels in normal subjects and to determine the effect of age, chelating therapy and splenectomy on these parameters.

MATERIAL AND METHODS

Subjects

This study was conducted on 82 patients with P-thalassaemia major, aged 1 to 32 years (48 males and 34 females), who attended the Thalassaemia Center in Basrah Maternity and Children Hospital from July 2000 to July 2001. Along side, 142 age-matched healthy subjects, aged 1 to 61 years (69 males and 73 females) were used as a control group.

Patients and controls were categorized into four main subgroups according to their ages, group A (1-6 years), group B (7-12 years), group C (13-18 years) and group D (included subjects aged more than 18 years).

From patients with (5-thalassaemia major, the following data were collected; age, sex, parental consanguinity, age of diagnosis of the disease, complaints, frequency of blood transfusion, data of last transfusion, chelating therapy (dose, route and duration), splenectomy, weight and height. For control group only age, sex, parental consanguinity, weight and height were recorded. Body Mass Index (BMI) was measured as weight (in kilograms) divided by the square of the height (in meters).

Methods

The importance of the procedure was explained to all subjects participated in the study and/or their parents. Fresh venous blood (6 ml) was drawn from patients and control subjects. Two ml of blood was collected into EDTA tubes and immediately tested for haemoglobin variant and haematological parameters. The test was transferred to plain tubes (without anticoagulant), centrifuged and the sera were obtained for the biochemical investigations.

For thalassaemia major subjects, the samples were collected just prior to blood transfusion. Haemoglobin, PCV, red cell counts, MCV, MCH and MCHC were estimated by using automated Coulter Counter MS9. Haemoglobin typing was performed quantitatively by an automated ion exchange HPLC system using fS- thalassaemia short program on the Bio-Rad VARIENT instrument (Bio-Rad Laboratories, Belgium). The diagnosis of p-thalassaemia trait was based on characteristic elevation of HbA, (>3.8%).

Serum iron and total iron binding capacity were tested within 24 hours according to Geriotti[17], and transferrin saturation % was calculated. Sera were stored at -20°C, for the later estimation of serum Zn, Cu, by using direct method with acetylene and air flame atomic absorbance spectrophotometer (FAAS) after simple dilution[18]. Serum ferritin estimation was carried out by radioimmunoassay technique as described by CIS bio (UK).

Statistical Analysis

The results were expressed as mean ±SD. The data were analysed statistically by one-way analysis of variance (ANOVA). The correlation between the data was tested by simple linear regression, employing SPSS computer program. A P value less than 0.05 was considered to be the lowest limit of significance.

RESULTS

In this study the mean age of all four age groups of pMhalassaemia major patients was 9.9±6.7 years, and mean age of diagnosis was 13.5±12.8 months. Body mass index (BMI) was 15.99 + 2.97 kg/m^2, which was significantly lower than that of control group (P <0.001).

The mean date of last blood transfusion was 30.8±15.9 days and the mean rate of blood transfusion was 11.4±5.8/year. Moreover, 24 patients with |3-thalassaemia major were splenoctomised (29.3%) and 58 (70.7%) were not.

This study also showed that 63 of the fi-thalassaemia major parents were relatives (76.8%).

As illustrated in Table 1, the haemoglobin level (Hb), packed cell volume (PCV), mean cell volume (MCV) and mean cell haemoglobin (MCH) were all significantly decreased in (5-thalassaemia major individuals in comparison to the control group (P<0.001). Red blood cells count and mean cell haemoglobin concentration were also significantly lower in (3-thalassaemia major patients as compared with healthy control (P<0.001).

Table 2 illustrates the biochemical characteristics, which were investigated for both studied groups. The serum levels of iron, ferritin, copper, Cu/Zn ratio and transferrin saturation were all increased in patients with (3-thalassaemia major as compared with controls, and the differences were statistically significant (P<0.001). The serum levels of TIBC and Zn were significantly lower in |3-thalassaemia major as compared with the control group (P<0.001).

The results reported in this study also showed a statistically significant decrease in serum zinc levels for all age groups as compared with control groups (P<0.001), (Table 3). The same table illustrates that there was a significant increase in the levels of serum copper and Cu/Zn ratio in all age groups as compared with the control groups of the same ages (P<0.001). Concerning age groups for each single test there was a statistically significant difference in the Cu/Zn ratio between the first (1-6 years) and last (>18 years) age groups and also between the second (7-12 years) and the last age groups (P<0.001 and P<0.05 respectively), (Table 3).

The study has revealed also that there was a de-

Table 1. Haematological findings in patients and controls

Parameters		Control (n= 142)	p-thalassaemia major (n=82)
Hb gm/dl	Male	12.911.6	6.3H.7 7.011.5
Female		11.9+1.3	
Total		12.411.5	6.611.6***
PCV%	Male	42.5+3.7	20.3+5.7
Female		38.413.8	22.1+6.1
Total		40.4+3.7	21.115. 8***
RBC counts	Male	5.4+0.5	3.110.8
Mil/^1	Female	5.1+0.4	3.310.9 3.3+0.9***
Total		5.310.5	
MCV fl	Male	78.9±5.5	65.4111.5
Female		78.117.4	67.4112.0
Total		78.5±3.6	66.1111.7***
MCH pg	Male	26.8+2.7	20.4+4.2 21.5+4.8
Female		25.812.7	
Total		26.3H.4	20.914.5***
MCHC g/dl	Male	32.911.6	30.6+5.2 29.8+6.0
Female		32.212.2	
Total		32.6H. 9	30.313.3***

Results were expressed as mean 1 SD with healthy subjects
*** Significantly different as compared (P<0.001).

Table 2. Basic Biochemical characteristics in the studied groups

Parameters	Control (n=142)	p-lhalassaemia major (n=82)
Iron (ng/dl)	112.7±21	160.5±21.1***
TIBC (ng/dl)	279.2+33.8	233.2±31.51*** 69. 216.
Trans. Sat %	38.2±7.1	81***
Ferritin (|ig/l)	63.5±48	1571.9±273.31***
Zn (ng/dl) Cu	93.9±27.4	47.7±14.11*** 154.1 +
(ng/dl) Cu/Zn	89.7±28.2	28.51*** 3.45±1.47***
ratio	1±0.38	

Results were expressed as mean ± SD
*** Significantly different as compared with healthy subjects (P<0.001).

Table 3. Zinc and copper status in (3-thalassaemia major in different age groups

Age/year	Groups	Zinc (ng/dl)	Copper (ng/dl)	Zn/Cu ratio
1-6	Control (n=20)	93.8±17.77	98.35±21.63	1.16±0.27
	Major (n=28)	40.1±8.08***	158.9±4.4***	4.06±0.85***
7-12	Control (n=23)	90.53±21.39	92.52±23.42	1.02±0.3
	Major (n=32)	40.8+9.31***	147.8±24.58***	3.7+1.01"*
13-18	Control (n=24)	98.0±30.87	77.44±27.47	0.83±0.31
	Major (n=11)	50.36±24.3***	146.3±33.2***	3.31+1.66***
>18	Control (n=75)	93.94+30.22	88.04±30.1	0.98±0.32
	Major (n=10)	63.4±27.56**	163.0±35.97***	2.77±1.55***

Results were expressed as mean ± SD
, * Significantly different as compared with healthy subjects (P<0.01 & P<0.001 respectively).

crease in the levels of serum iron, and transferrin saturation in non-splenectomised patients compared with splenoctomised ones. However, this decrease was statistically insignificant. On the other hand, there was an increased serum levels of TIBC, zinc, copper and ferritin in the non-splenoctomised patients compared with splenoctomised patients but this increase was statistically insignificant (Table 4).

The values of biochemical variables of patients on regular, irregular and without desferrioxamine therapy (DFO) are presented in Table 5. The levels of serum transferrin saturation and serum copper are higher in the group that takes the DFO therapy and it is slightly higher in those on regular therapy than in those on irregular therapy. These differences were statistically not significant.

On the other hand, serum iron, TIBC, serum ferritin and zinc were lower in the group that takes the DFO therapy. All the above variations were statistically insignificant except for serum zinc level, which was significantly lower in patients on regular and irregular DFO therapy as compared with those not taking chelating therapy ($P<0.05$).

Correlation analysis between different biochemical parameters (serum ferritin and transferring saturation; zinc and copper) were illustrated in Figures 1 and 2 respectively.

Table 4. Biochemical changes in splenectomised and non-splenectomised pMhalassaemia major

Thalassaemia major		
Parameters	Splenecto-mised (n = 24)	Non-sple-nectomised (n = 58)
Iron (ng/dl) TIBC ([ig/dl)	162.2±21.3	160±21.1
Transferrin saturation %	226.3±33.2	236.8±30.6
Ferritin ([ig/dl) Zinc	71.9±4.16	68±4.32
(ng/dl) Copper (ng/dl)	1550±253.7	1584.4±263
	46.3±13.4	48.9±14.5
	147.6±23.8	155.8+30.1

Results were expressed as mean ± SD

Table 5. Biochemical changes in correlation with DFO therapy

Parameters	With DFO therapy		Without therapy (n = 30)	
	Regular (n = 24)	Irregular (n = 28)		
Serum iron (|ig/dl) TIBC	163±20.8	159.8±20.6	227.6±27.3	165.3±23.1
(ng/dl) Transferrin saturation	231±33.7	70.8±4.8	70.4±6.1	1606±211
% Ferritin (|ig/dl) Zinc	1505±244.6		47.3±12.8	152.6±21.8
(ng/dl) Copper (ng/dl) Cu/Zn	43.5±12.6	157.2 +	3.8±1.1	
ratio	26.4	3.4±1.8		

233.8±35.6	67.6±9.1	1639±243.7
55.3±17.7*	143.5±31.9	2.8±1.5

Results were expressed as mean ±SD

'Significantly different as compared with patients taking therapy ($P<0.001$).

Figure 1. Correlation between ferritin and transferring saturation in (5-thalassemia major.

Figure 1. Correlation between ferritin and transferring saturation in p-thalassemia major.

The present study, Basrah (2001) n=82 Pootrakul, 7.2±1.7 Thailand, (1980)[24] n=115 Winfred, USA, (1986)[25] n=91	6.6±1.6	160.5±21.1	233.2±31.5	182±78	69.2±6.8	
		233±63.7	170±29		73.8±25.5	90±12 1572±273 3350

DISCUSSION

Red cell genetic abnormalities are chronic diseases with no cure; they often require lifelong care and management strategies. The impact of these diseases may vary with the severity of the disease itself and also varies with individuals and families. In general these conditions are leading cause of increased morbidity in children and adults and of increased children mortality. Within these genetic abnormalities thalassaemia constitutes a major public health problem in many parts of the world, particularly in the Mediterranean region, Middle East and Southeast Asia[2].

In Iraq, |3-thalassaemia is one important public health problems. This is because of the considerable burden on the children and their families as well as on health services. From data of this study, 76.8% of children with (5-thalassaemia major were from consanguineous marriage, which is predominant in our country. Also, only 29.3% of patients with p-thalassaemia major were splenectomised and the other 70.7% were not, while desferrioxamine therapy was given regularly to 29.3% of patients, irregularly to 34.1% and the rest were not receiving chelating therapy at all.

The present study showed significant growth retardation in (3-thalassaemia patients compared with controls (P<0.001). It was found that not only under weight was prominent in |3-thalassaemia major but linear growth and variable grades of wasting were also common. Growth failure had been attributed to; chronic anaemia, zinc deficiency, growth hormone deficiency, secondary to desferrioxamine therapy and many other causes". Some studies have shown that nutrition intervention might result in a significant improvement in mean weight for height with increase in zinc plasma levels and a decrease in plasma copper levels[20]. Other clinical trials showed that treatment with zinc supplementation and/or growth hormone may enhance growth velocity and may manage growth retardation of the treated group[19-21-22].

In the present study, it is clear from the serum iron, total iron binding capacity, transferrin saturation and serum ferritin data that patients with |3-thalassaemia major show progressive iron overload. These patients also have severe anemia due to ineffective erythropoiesis, which is a major cause of the pathophysiological findings in these patients[23]. A positive correlation between transferrin saturation and serum ferritin (r=0.51 and P<0.01) was observed (Figure 1), indicating that these parameters are metabolically linked in their functions. Wangruangsathit et al[24] also report similar finding. This could be due to the effect of iron overload and increased iron mobilization from the storage sites.

Table 6 presents the indicators of iron status in our study and other two studies in different countries. Our findings confirm the results observed by Pootrakul et al[25] and Winfred et al[26] except for the serum ferritin level, which was twice higher in the last one.

With regard to the effect of splenectomy on iron status in p-thalassaemia major, this study found a higher level of serum iron and transferrin saturation in splenectomised patients as compared with non-splenectomised patients. Other researchers also demonstrated similar observations[25-27]. Serum ferritin level was lower in splenectomised patients than the non-splenecto-mised group, which could be due to that splenectomised patients take less blood transfusion than the non-splenectomised patients. This was not in agreement to Pootrakul et al[25] who noticed an increase in serum ferritin level in splenectomised patients, although the

Table 6. Comparison of iron status in p-thalassaemia major in the present study and other studies

Study, Country, (Year)[Ref]	Hb g/dl	Serum Iron ug/dl	TIBC ng/dl	Transferrin. saturation %	Serum ferritin ng/dl

Results were expressed as mean ±SD

reason of this increase was not clear.

The effect of DFO therapy on iron status was determined. Patients with regular and irregular DFO therapy were found to have lower serum iron and TIBC levels, and higher transferrin saturation compared to those without chelation therapy, which could be due to the effect of DFO that increases the transferrin saturation due to iron release from the stores. Wang et al[26] also reported same results in which he supposed that the non-transferrin bound iron is elevated only if the transferrin saturation is close to 100%. Another important reason could be that most patients were taking ascorbic acid (vitamin C) inappropriately or even did not take it at all. Ascorbic acid facilitates the release of iron from the storage sites, as well as it can also increase its utilization by the erythroid cells and increase iron excretion by the kidney in the presence of DFO[28]. Some studies showed that administration of 100-200 mg/day of oral ascorbate results in an approximate doubling of DFO-induced urinary iron excretion[21]. Bianco et al[29] noticed that serum iron increases after subcutaneous infusion of desferrioxamine, which probably can be identified with a chelated iron fraction waiting for renal clearance. He also mentioned that stable iron which results from catabolism of transfused red cells constitute the main part of the chelated iron.

Serum ferritin level in patients on regular and irregular chelation therapy were lower than in the group without therapy, it was even lesser in the group with regular therapy which is due to the effect of the DFO on iron stores. Canton et al[30], also noticed this. On other hand, Porter[31] concluded that about half of the iron is excreted in the feces and this proportion increases with higher DFO dose, and that the source of fecal iron is from the liver cells (hepatocytes), whereas urinary iron includes that derived from plasma iron turnover[31].

Zinc is the second most abundant trace element in the body. Our data showed that in all age groups of patients with (3-thalassaemia major there was a significant decrease in the levels of serum zinc. These results are in agreement with several studies published else where in the world[13-14-32-33]. The decreased serum zinc levels in p-thalassaemia major had been attributed to hyperzincuria, which is due to cirrhotic changes owing to hemosiderosis or to an increased rate of glome-rular filtration of zinc seen in chronic hemolysis[33]. Moreover, Milne et al[34], studied the effect of foliate supplementation on some trace elements absorption and excretion and concluded that supplementation of foliate influence zinc homeostasis, perhaps through formation of an insoluble chelate and impairment of absorption. This could add another factor responsible for zinc deficiency in patients with p-thalassaemia major as most of these patients take foliate therapy continuously. Moreover, it is well established that the serum of thalassaemic patients had low zinc levels but increased iron and ferritin levels. These findings implied that zinc and iron levels are associated. Experiments in human and animals have demonstrated that zinc and iron undergo competitive interaction[35-36].

Although splenectomy lowered the serum zinc levels in patients with thalassaemia major, the decrease did not reach the degree of significance. On the other hand, DFO therapy had a significant effect on serum zinc level in f$-thalassaemia major. Serum zinc level was lower in patients with regular DFO therapy, which was also reported in many previous studies[30-32-33]. This was explained by that DFO is associated with increased urinary zinc excretion in pMhalassaemia major as DFO could have an affinity to other metal ions including zinc[37-38].

Copper is widely distributed in the biological tissues where it is present largely in the form of organic complexes, many of which are metalloproteins acting as enzymes. Copper enzymes are involved in a variety of metabolic reactions, such as, utilization of oxygen during cell respiration and energy utilization. They are also involved in the synthesis of complex proteins of connective tissues and in a range of neuroactive compounds[39]. Serum copper level was explored in patients with fi-thalassaemia major and it was found to be significantly higher than the control subjects and for all age groups. These results were in agreement with other studies by researchers in different part of the world[5-15-16-33]. These observations could be explained by the antagonistic effect of the zinc, as zinc deficiency in (3-thalassaemia major could greatly increase copper absorption via the gastrointestinal tract. This negative correlation illustrated in Figure 2 can be attributed to that elements with similar valence-shell hybrid orbital might compete for specific binding sites on proteins involved in their absorption and perhaps also during the de novo synthesis of metal-loenzymes[3,1].

Cu/Zn ratio is a more variable indicator of the body zinc and copper status in haemolytic anaemias[15], and it was also evaluated as a tool to be used as diagnostic criteria in some types of cancers[40]. In our study serum

Cu/Zn ratio was significantly higher in [5-thalassaemia major patients as compared with control subjects. It was also significantly higher in almost all age groups compared with control subjects. These results may indicate the usefulness of using this ratio more efficiently in the estimation of serum levels of these two highly correlated trace elements, than using each one alone. From this study we conclude that patients with homozygous p-thalassaemia were zinc deficient. This conclusion was based on decreased levels of serum zinc and increased Cu/Zn ratio and we recommend that zinc supplementation for patients with (5-thalassaemia major is important as zinc can reduce the copper and iron concentrations and increase growth.

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