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Ceruloplasmin levels in β-thalassaemia major: therapeutic insights and implications for iron homeostasis

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Abstract

Ceruloplasmin (CP) is an enzyme that has ferroxidase activity and is important in maintaining iron homeostasis and serving as a copper-transporter in the bloodstream. Beta-thalassemia major (BTM), a common hereditary disorder in Iraq, can affect CP activity in patients with iron overload resulting from frequent blood transfusions in order to sustain haemoglobin levels. The current study is a single-center observational research. CP activity was measured in 304 patients (120 females and 184 males). Anthropometric indices were recorded and the iron load status was determined by measuring serum ferritin. Ninety-two healthy individuals were also included as a control group. Our findings revealed no relationship between age, body mass index, or plasma ferritin and CP activity in BTM patients. No significant influence of sex on CP activity was observed. The outcomes provide insight into assumed pathways regulating CP, and add to the growing body of research on CP's contribution to iron metabolism in BTM patients. To our knowledge, this work is the only study of its kind in Iraq, provides the groundwork for upcoming studies and potential therapeutic lines by generating insightful data on the multifaceted relationships between iron homeostasis, CP, and BTM.

KEYWORDS
thalassaemia, iron overload, ferritin, ceruloplasmin, ferroxidase

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1. INTRODUCTION

Ceruloplasmin (CP) is the main copper-transport protein with serum ferroxidase activity. Like several inflammatory biomarkers including C-reactive protein, tumour necrosis factors, interleukin-1, uric acid, and others, the blood levels of CP will rise in inflammation owing to its well-known positive hepatic acute-phase reactant [1]. CP adjusts the iron homeostasis of the body by its oxidizing ability of ferrous to ferric iron; a rather safer form. Additionally, the activity of ferroxidase has a major contribution in stimulating the loading of iron from body stores and blood toward transferrin. Moreover, CP

participates in several physiological functions like angiogenesis, coagulation, and acts as an antioxidant [2]. The existence of remnants of copper within the lysosomes and in the cytoplasm may be linked to the stronger premise of interaction of the metabolic pathways of the two metals (copper and iron), as CP is a copper-dependent oxidase protein. Thus, CP is essential for the effective release of iron from body cells.

Beta-thalassaemia major (BTM), the most commonly inherited disorder universally, affecting approximately 280 million individuals and causing 16,800 mortalities in 2015. BTM is defined by the lack or decreased synthesis of the β-globin chain, and constitutes a crucial health burden in Iraq [3]. Regular regimens of blood transfusions so as to preserve the haemoglobin levels are critical for the BTM patients' survival. Thus, persistent iron overload exceeding the capacity of the human body to bind free iron, causes its deposition in vital body organs, especially if improper iron chelators have been administered. Earlier articles from tertiary referral centers have demonstrated that the ferroxidase activity and CP levels were correlated with iron overload, thereby suggesting a modulatory influence of iron on the CP genetic expression [4]. The normal value of blood CP levels for adults is around 22 to 40 mg/dL for a male, or 25 to 60 mg/dL for a female [5]. Though the CP ferroxidase activity plays a main role in iron homeostasis, the data about its precise role concerning the status of ferritin in BTM patients are limited, and not well recognized. Hence, this study aimed at estimating the CP activity in patients with multi-transfused BTM, so as to assess the efficiency of the iron chelation therapy.

2. PATIENTS AND METHODS

Study design and sampling: During this single-center observational study, 304 BTM patients (184 males and 120 females) aged between 2.3 and 17.9 years were included throughout the period from May to December 2020. Patients were recruited from those documented and followed up at the Babylon Hereditary Blood Disorders Center in Babylon. The diagnosis of BTM was already established for registered patients. Those who were not fully-compliant with the blood transfusion regimens, or were severely incapacitated, were excluded from the study. A uniform and approved study formulary was filled by the interviewer so as to collect data from all BTM patients. Ninety-two healthy volunteers (60 males and 32 females) constituted the control group, and were included while performing a systematic medical examination in the Babylon Hospital for Maternity and Childhood. Demographic parameters, age at diagnosis, family history, rate of blood transfusions received per month, and types of chelating therapy received were recorded. Blood samples were collected for serum ferritin and CP analysis.

CP (ferroxidase) activity assay: The CP activity was measured by a spectrophotometric assay (*p*-phenylenediamine-method) [6].

Ethics approval: An ethics approval was obtained by the hospital's local ethical committee. A verbal consent was obtained from all participants.

3. RESULTS

The mean age was 14.6±7.3 years, with the dominance of males (n=184; 60.5%). Of the total contributors, 40% were on regular transfusions once monthly, and 47% were on bimonthly transfusions. Moreover, 194 (63.4%) patients were on deferasirox (Exjade®) oral chelators and 104 (34%) were on injectable chelators. There were significant variations in terms of the age, CP, ferritin, and body mass index (BMI) between the thalassaemia patients and the normal subjects. No significant alterations were observed in terms of the CP activity between those with serum ferritin levels above and those below 2.500 ng/mL, as well as between the serum CP levels and serum ferritin levels, age, or BMI of the patients.

Table 1. Comparison of the main study variables between the two groups assessed.

Variables	Group	Mean	Standard deviation	*P*-value
Age (y)	Patients	18.9	9.9	<0.001
	Control	29.6	8.1	
Ceruloplasmin levels (mg/dL)	Patients	42.3	12.6	<0.001
	Control	34.6	5.4	
Ferritin levels (ng/mL)	Patients	2,976.9	2,084.3	<0.001
	Control	180.0	34.7	
Body mass index (kg/m^2)	Patients	18.4	3.2	<0.001
	Control	24.1	3.9	

4. DISCUSSION

Isolating biomarkers involved in the pathogenesis of thalassemia can potentially have a contribution to the linking of the analytical gap and the formation of adapted management regimens. This work aimed to assess the blood CP levels through its ferroxidase activity rather than its concentration, in multi-transfused BTM patients, in an attempt to investigate the role of CP activity in BTM pathogenicity.

Our findings were inconsistent with those of previous reports that have shown that the CP activity was within the normal accepted ranges for both male and female BTM patients. Significantly high levels of CP were reported among thalassaemia patients with certain phenotypes of BTM in a Jordanian study that included 124 BTM patients. Along similar lines, a preceding Iraqi survey included 101 thalassaemic patients who revealed increased CP levels compared to the control group. Turkish researchers have reported significantly higher CP levels among thalassaemic patients aged 6-10 years in a series of 29 patients, whereas CP levels were found decreased in 10.3% of the patients. However, their population sample was small (29 patients) compared to the population of this study.

In the current study, a non-significant correlation between the serum levels of CP and the serum ferritin levels exists. Some authors have attributed the increased serum CP levels detected in thalassaemia patients to several mechanisms associated with iron overload rather than to inflammation. The observed higher CP levels in thalassaemia might be related to the antioxidative property of haptoglobin, which is primarily due to its high serum levels and its elevated affinity to free haemoglobin in the blood of thalassaemic patients. Finally, high CP serum levels might be due to hepatic insufficiency induced by thalassaemia and/or iron chelators.

The CP levels might increase in order to counteract reactive oxygen species through the Fenton pathway, which can be completed by the antioxidant ability of CP by the ferro-oxidation of iron, thereby inhibiting the oxidative injury of cellular proteins, lipids, and DNA [2]. CP is possibly elevated because of the tissue accumulation of iron, therefore increasing the iron efflux into transferrin in blood. Few researchers have delivered evidence to support the physiological ability of CP to mobilize iron molecules from cellular stores or plasma. Furthermore, experimental trials on subjects with haemochromatosis or aceruloplasminaemia have revealed marked iron multi-organ accumulation (including the liver and the brain) [7]. Iron accumulation can result from the inability of the CP ferroxidase capacity to mobilize iron from tissue stores or plasma. Experimental models of animals lacking CP have revealed that CP administration may restore iron homeostasis in these animals [8]. Finally, increased CP levels in thalassemia patients are likely secondary to prolonged hypoxia and defective erythropoiesis, which are shared characteristics among thalassaemia patients.

It is expected for thalassaemia patients to be under oxidative stress, and thus have disturbed iron metabolism besides a high iron-induced oxidative stress. Hence, it is likely that the functional activity of CP, as a main antioxidant, is changed in such a way so as to compete against the sequels of iron overload.

A significantly higher CP activity has been detected among females, although no changes have been observed in female BTM patients when compared with the control group in this regard; moreover, a lower activity in male control subjects has been observed compared to patients [4]. Moreover, serum CP levels have been found to be meaningfully higher in females [9]. To some extent, these incongruous outcomes can be explained from an endocrine perspective. In females, the lack of CP and/or reduced ferroxidase activity might be linked to oestrogen influence and could be responsible for the minor phenotypic expression observed. In any case, the gender differences in serum CP activity should be considered in future works.

To the best of our knowledge, the current study represents the first to evaluate CP activity in BTM patients in Iraq. The authors selected the method of Sunderman and Nomoto [6] employing a spectrophotometric *p*-phenylenediamine oxidase analysis, since it is simple, easy, and reproducible, and it allows us to distinguish the CP's actual activity from the activity of the total serum oxidase.

5. CONCLUSION

This study casts doubt on the idea that BTM patients have elevated CP levels, and emphasizes the necessity of further study into the intricate interactions between CP, iron overload, and BTM pathogenicity.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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