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Status of Hepcidin and SOD in a Sample of Jordanian β-Thalassemia Patients

Dana A. Alomari[1] and Hamed R. Takruri[1]✉

Abstract

Background Aims: Hepcidin is low in β-thalassemia patients, suggesting a critical role in iron accumulation. Hepcidin has not been assessed in Jordanian thalassemia patients. There is variation in the response of superoxide dismutase (SOD) to iron overload and it has not been correlated with hepcidin. Thus, the aim of this study was to assess serum hepcidin and SOD in patients diagnosed with β-thalassemia intermedia and major, and to examine the correlation between hepcidin and SOD in those patients.

Subjects and Methods: Sixty patients with β-thalassemia aged 2–25 years were recruited from the Thalassemia Unit of the Zarqa New Governmental Hospital. The patients were classified according to two forms of thalassemia and assessed for serum levels of hepcidin and SOD. Statistical analysis was performed to investigate the differences between the patients of the two forms, compare the levels of hepcidin and SOD with the normal reference ranges, and calculate their correlation coefficients.

Results: The study sample included 31 thalassemia intermedia patients and 29 patients with thalassemia major, who are 33 females and 27 males. All patients had significantly deficient hepcidin (2.21±0.16 ng/ml) and increased serum SOD (3.13±0.14 ng/ml) levels compared to normal values. Ferritin was very high (3036.9±309.7 ng/ml) in all patients and highest in adult patients and those with thalassemia major. No significant correlations were found between hepcidin or SOD and ferritin (p> 0.05). Only in child patients was hepcidin positively correlated with SOD (r=0.848, p=0.033).

Conclusions: Jordanian β-thalassemia patients had hepcidin deficiency, contributing to iron accumulation. SOD had a significant protective role against oxidation in β-thalassemia. Chelation therapy is inadequate to treat iron overload, which is still the predominant contributor to health complications in thalassemia. Therefore, the use of hepcidin agonists could be a beneficial treatment of excess iron.

Keywords: β-Thalassemia, thalassemia intermedia, thalassemia major, hepcidin, superoxide dismutase

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INTRODUCTION

β-Thalassemia is an inherited β-globin disorder characterized by the inability to make normal hemoglobin, resulting in anemia [1,2]. Abnormal and/or low hemoglobin molecules lead to red blood cell malformation, hemolysis, and microcytic anemia [2,3]. β-Thalassemia is also called Mediterranean anemia because it occurs mostly in people of Mediterranean ancestry (2–18%) [4], but its prevalence in Jordan is 2–4% [5], and the registered number of cases is 1,228 [6].

β-Thalassemia is classified according to the number and degree of defective genes and severity as: trait or minor, intermedia, and major [1,2,7]. Patients with thalassemia intermedia and major forms need a regular blood transfusion and have severe symptoms [1]. Recurrent blood transfusion is the main contributor to iron overload, which is the hallmark of β-thalassemia and is also caused by ineffective erythropoiesis, increased dietary iron absorption, and suppressed hepcidin levels [8]. Excess iron forms harmful reactive oxygen species, developing into active oxidative stress [8–10]. Oxidative stress in thalassemia causes serious health complications that threaten life quality, accelerating morbidity and subsequent mortality [9,10]. Globally, about 50,000–100,000 thalassemia patients die every year [11].

Hepcidin is a hepatic hormone that controls dietary iron absorption and the release of iron stores

[1]Department of Nutrition and Food Technology, School of Agriculture, The University of Jordan, Amman, Jordan.
✉Corresponding author: htakruri@ju.edu.jo

[12]. In thalassemia, its deficiency causes iron overload due to enhanced gastrointestinal absorption of iron [8,12,13] and increased iron release by macrophages and hepatocytes [12]. Hepcidin was suggested as a target for iron overload treatment in thalassemia due to its significant role in raising iron more than that required for erythropoiesis [14]. Hepcidin assessment in β-thalassemia began in the early twenty-first century and continues, with a few studies recommending the need for more findings.

Cytosolic superoxide dismutase (SOD) is a very important antioxidant enzyme against free radicals and oxidative stress, especially in erythrocytes [15]. There is well established evidence on the importance of SOD in different red blood cell disorders as an antioxidant [15]. SOD dismutates the superoxide anion (O^{2-}), which is the first oxidant produced in the body, into hydrogen peroxide (H_2O_2); this can be metabolized by other antioxidant enzymes [16,17].

Few studies have assessed SOD status in thalassemia and have reported variable results on its response and serum levels. No published study has yet been conducted on Jordanian thalassemia patients to assess hepcidin status; as far as we know, the association between hepcidin and SOD in patients with β-thalassemia remains to be investigated. Therefore, in this study, we examine the levels of hepcidin and SOD in Jordanian β-thalassemia patients, compare these levels between patients with intermedia and major forms, and investigate the correlation between hepcidin and SOD in these patients.

SUBJECTS AND METHODS

A cross-sectional study was carried out at the Zarqa New Governmental Hospital in Zarqa, Jordan. Ethical approval was obtained from the Ministry of Health through authentication of the scientific committee [MBA/ Research Ethics Committee/18681]. Privacy and confidentiality were taken into consideration by assigning codes to the participating patients and obtaining informed written consent.

A convenient sample of 60 Jordanian β-thalassemia patients, with a regular blood transfusion, was recruited following Cochran's formula to calculate the representative sample size. The sample was divided into two groups according to the form of β-thalassemia including both sexes as well as children, adolescents and adults (2–25 years).

The patients were interviewed to communicate the study's purposes and obtain general data. Three venous blood samples were drawn from each patient: two for the routine hospital hematological and biochemical analyses and the third to analyze hepcidin and SOD later outside the hospital, at Smart Labs, Amman. The third sample for each patient was allowed to coagulate at room temperature and then centrifuged at 2,000 rpm for 20 min. to obtain the serum. Samples were saved in cryotubes at $^{-}80^{o}$C in the blood bank of the hospital until the end of data collection.

The hepcidin and SOD were tested using ELISA kits employing the double antibody sandwich principle (My BioSource, USA). In this procedure, plates are coated with an antibody that will attach to the tested protein, and then another antibody is added, followed by a conjugate and color reagent to develop a color able to absorb wavelength. The testing protocols were done following the data sheets of the kits. The absorbance of plates was read at 450 nm. Concentrations of hepcidin and SOD were calculated after developing the standard curves of each depending on the 4PL regression model. Serum levels of hepcidin and SOD were assessed according to the normal reference ranges set by the kits' manufacturer. The other biochemical tests were assessed depending on the hospital reference ranges. SPSS v.20 was used to analyze the data.

RESULTS

General characteristics of the sample classified according to the thalassemia forms are described in Table 1. The sample included 31 patients with thalassemia intermedia and 29 with thalassemia major. There were no significant differences between patients with the intermedia and majors form, except in the number of adolescents (n=19 vs. 8, respectively) and adults (n= 8 vs. 19). A minority of the sample was children (2–9 years, 10%), while adolescents (10–18 years) and adults (≥19 years) numbered equally (27 each, 90%). The number of females was significantly larger than that of males in the intermedia group (n= 21 vs. 10) but not in the whole sample and the major group. Most of the patients in the whole sample (61.7%) used the iron chelatorDeferasirox, whereas only five patients with thalassemia major used the Desferalchelator.

Table 1: Selected characteristics of the sample by thalassemia form

Characteristics		Frequency n (%)		
		Intermedia (n= 31)	**Major (n= 29)**	**Total (n= 60)**
Age (years):[#]	2-9	4 (12.9)[b]	2 (6.9)[b]	6 (10)[b]
	10-18	19 (61.3)[a]*	8 (27.6)[b]*	27 (45)[a]
	19-25	8 (25.8)[b]*	19 (65.5)[a]*	27 (45)[a]
Sex:	Female	21 (67.7)[a]	12 (41.4)[a]	33 (55)[a]
	Male	10 (32.3)[b]	17 (58.6)[a]	27 (45)[a]
Iron chelator:	Desferal	0	5 (17.2)[b]	5 (8.3)[c]
	Deferiprone	12 (38.7)[a]	6 (20.7)[b]	18 (30)[b]
	Deferasirox	19 (61.3)[a]	18 (62.1)[a]	37 (61.7)[a]

Data are presented as percentages of the number of patients out of females, males and total.
Values in columns within each character with different superscripts are significantly different ($P < 0.05$).
*Indicates significant differences between intermedia and major ($P<0.05$).
[#]Age is classified into three groups: children (2-9 years), adolescents (10-18 years) and adults (19-25 years).

Table 2presents biochemical data of the study groups based on age, thalassemia forms and sex. Adults had significantly higher hemoglobin levels (HGB) than children and adolescents ($p< 0.05$). Mean cellular volume (MCV) was significantly lower in the children group than in the other two age groups ($p< 0.05$). Erythrocyte sedimentation rate (ESR) was significantly higher in adult patients than in adolescents and children ($p< 0.05$). Patients with thalassemia major had significantly higher mean cellular hemoglobin concentration (MCHC) and ESR and lower red cell distribution width (RDW) than those with intermedia form ($p< 0.05$).In the adolescents' group, intermedia form patients had significantly lower levels of red blood cell (RBC) count, MCH, MCHC, and ESR, as well as higher RDW than those patients of the major form (3.2±0.1 vs. 2.9±0.1$10^{12}$/L; 25.9±0.5 vs. 27.6±0.3pg; 33.1±0.4 vs. 34.3±0.3 g/dl; 25.4±4.0 vs. 49.9±7.7 mm/hr; and 21.3±1.2 vs. 17.3±0.9 %), respectively).

Ferritin was very high in all patients (3036.9±309.7 ng/ml) compared to the normal range (15–400 ng/ml). It was slightly but not significantly higher in patients with thalassemia major than those with intermedia (3325.0±481.1 vs. 2767.3±397.4 ng/ml) and in adult patients (3483.3±542.9 ng/ml) than children and adolescents (2103.5±685.3 and 2797.9±389.2ng/ml). It was also not significantly higher in male than female patients (3522.8±534.3 vs. 2639.3±347.4 ng/ml). Hepcidin and SOD were not significantly different considering age, thalassemia form or sex ($p> 0.05$).

Table 2: Biochemical data of the study groups based on age, thalassemia form and sex

Test	Children (n=6)	Adolescents (n=27)	Adults (n=27)	Intermedia (n=31)	Major (n=29)	Females[#] (n=33)	Males (n=27)	Total (n=60)
Selected hospital analyses related to iron status and oxidative stress								
RBC count (10^{12}/L)	3.1±0.1[a]	3.1±0.1[a]	3.2±0.1[a]	3.2±0.1	3.1±0.1	3.1±0.1	3.2±0.1	3.1±0.04
HGB (g/dl)	7.7±0.2[b]	8.2±0.2[ab]	8.3±0.1[a]	8.1±0.1	8.3±0.1	8.3±0.1	8.1±0.1	8.2±0.1
MCV (fl)	74.1±1.8[b]	79.1±0.8[a]	79.1±0.8[a]	77.6±1.0	79.6±0.6	79.2±0.7	77.9±0.9	78.6±0.6
MCH (pg)	25.0±1.0[a]	26.4±0.4[a]	25.7±1.0[a]	25.7±0.5	26.2±0.9	26.6±0.4	25.2±1.0	25.9±0.5
MCHC (g/dl)	33.8±0.6[a]	33.4±0.3[a]	33.6±0.3[a]	33.1±0.3*	34.0±0.2*	33.6±0.3	33.5±0.3	33.5±0.2
RDW (%)	23.4±3.9[a]	20.1±0.9[a]	20.3±0.9[a]	22.1±1.1*	18.8±0.7*	19.7±0.9	21.6±1.1	20.5±0.7
ESR (mm/hr)	20.0±4.5[b]	32.7±4.2[ab]	36.4±5.5[a]	24.9±3.2*	41.9±5.1*	33.9±4.5	32.1±4.5	33.1±3.2
Ferritin (ng/ml)	2103.5±685.3[a]	2797.9±389.2[a]	3483.3±542.9[a]	2767.3±397.4	3325.0±481.1	2639.3±347.4	3522.8±534.3	3036.9±309.7
Tests performed outside the hospital								
Hepcidin(ng/ml)	2.28±0.60[a]	2.25±0.22[a]	2.16±0.25[a]	2.15±0.21	2.28±0.24	2.33±0.20	2.06±0.25	2.21±0.16
SOD(ng/ml)	3.03±0.59[a]	3.08±0.21[a]	3.19±0.21[a]	3.13±0.20	3.12±0.21	2.94±0.16	3.36±0.25	3.13±0.14

Data are presented as mean ± standard error of mean (SEM).
Values in rows within different tests and age groups with different superscripts are significantly different ($p<0.05$).
*Indicates significant differences between intermedia and major ($p<0.05$).
[#]Indicates no significant differences between females and males for all tests ($p>0.05$).
RBC: Red blood cell, HGB: Hemoglobin, MCV: Mean cellular volume, MCH: Mean cellular hemoglobin, MCHC: Mean cellular hemoglobin concentration, RDW: Red cell distribution width, ESR: Erythrocyte sedimentation rate, SOD: Superoxide dismutase.

All the biochemical tests were classified into low, normal or high as compared with the reference normal levels (Table 3). All patients and patients of both thalassemia groups had a very high ferritin level and very low RBC count and HGB. Patients of all groups had significantly (p= 0.0001) deficient hepcidin and a high level of SOD compared to the normal values of 464.61–533.67 ng/ml and 0.65 ng/ml [18], respectively.

In the intermedia group, patients having low MCH (54.8%) were more than (p< 0.05) those having a normal MCH level (41.9%), in contrast to the major form group (31% vs. 69%). In the major group, patients having normal MCHC (86.2%) were significantly more than those having low concentration (10.3%), and only one patient had a high concentration. RDW was significantly high in 87.1% of patients with intermedia, 86.2% of those with major form, and 86.7% of the whole sample compared to those who had low RDW (12.9, 13.8, and 13.3%, respectively). Most patients of intermedia (67.7%), major (72.4%) and the whole (70%) samples had high levels of ESR.

Table 3: Biochemical assessment based on thalassemia form

Test[#]	Frequency n (%)								
	Intermedia (n= 31)			Major (n= 29)			Total (n= 60)		
	Low	Normal	High	Low	Normal	High	Low	Normal	High
RBC count	31 (100)	0	0	29 (100)	0	0	60 (100)	0	0
HGB	31 (100)	0	0	29 (100)	0	0	60 (100)	0	0
MCV	16 (51.6) [a]	15 (48.4) [a]	0	12 (41.4) [a]	17 (58.6) [a]	0	28 (46.7) [a]	32 (53.3) [a]	0
MCH	17 (54.8) [a]	13 (41.9) [b]	1 (3.2) [c]	9 (31) [b]	20 (69) [a]	0	26 (43.3) [a]	33 (55) [a]	1 (1.7) [b]
MCHC	11 (35.5) [a]*	19 (61.3) [a]	1 (3.2) [b]	3 (10.3) [b]*	25 (86.2) [a]	1 (3.4) [b]	14 (23.3) [b]	44 (73.3) [a]	2 (3.3) [c]
RDW	0	4 (12.9) [b]	27 (87.1) [a]	0	4 (13.8) [b]	25 (86.2) [a]	0	8 (13.3) [b]	52 (86.7) [a]
ESR	1 (3.2) [c]	9 (29) [b]	21 (67.7) [a]	0	8 (27.6) [b]	21 (72.4) [a]	1 (1.7) [c]	17 (28.3) [b]	42 (70) [a]
Ferritin	0	0	31 (100)	0	0	29 (100)	0	0	60 (100)
Hepcidin	31 (100)	0	0	29 (100)	0	0	60 (100)	0	0
SOD	0	0	31 (100)	0	0	29 (100)	0	0	60 (100)

Data are presented as frequencies and percentages of the number of patients with intermedia and major forms, and the total. Values in rows within each test in intermedia, major and total with different superscripts are significantly different (p<0.05).
*Indicates significant differences between intermedia and major (p<0.05).
[#]Assessment is based on the reference ranges for each test from the kits' reference method sheet: low < normal range, normal= normal range, high > normal range.
RBC: Red blood cell, HGB: Hemoglobin, MCV: Mean cellular volume, MCH: Mean cellular hemoglobin, MCHC: Mean cellular hemoglobin concentration, RDW: Red cell distribution width, ESR: Erythrocyte sedimentation rate, SOD: Superoxide dismutase.

The correlation coefficients of hepcidin and SOD with other biochemical markers classified by thalassemia form are as shown in Table 4, and by age group in Table 5. SOD was positively correlated with RBC count (r= 0.272, p= 0.044) and HGB (r= 0.262, p= 0.048) in patients with thalassemia major. It was negatively but insignificantly correlated with RBC count and HGB in the thalassemia intermedia patients. Hepcidin was negatively but not significantly correlated with RBC count and HGB in the intermedia group, whereas it was positively correlated with these markers in the major group. In child patients (Table 5), hepcidin was positively related to SOD (r= 0.848, p= 0.033) and SOD was correlated with MCV (r= 0.904, p= 0.013). Hepcidin tended to be significantly correlated with ferritin (r=- 0.257, p= 0.06) in the adolescent group. In male patients, SOD was positively linked to HGB (r= 0.496, p= 0.009), MCV (r= 0.502, p= 0.008), MCH (r= 0.499, p= 0.008), and RDW (r= -0.437, p= 0.023).

Table 4: Correlations between serum levels of hepcidin and SOD and other biochemical markers in thalassemia intermedia and major forms

Biomarker	Intermedia (n= 31)		Major (n= 29)		Total (n= 60)	
	Hepcidin	SOD	Hepcidin	SOD	Hepcidin	SOD
RBC count (10^{12}/L)	-0.216	-0.325	0.225	0.272*	0.007	0.008
HGB (g/dl)	-0.060	-0.075	0.018	0.262*	-0.037	0.088
MCV (fl)	0.125	0.189	-0.123	-0.012	0.010-	0.051
MCH (pg)	0.146	0.236	-0.204	-0.002	0.033	0.051
MCHC (g/dl)	0.134	0.209	-0.259	-0.114	-0.040	0.007
RDW (%)	-0.166	-0.237	0.160	0.070	0.023	-0.030
ESR (mm/hr)	0.119	0.235	-0.021	-0.116	0.045	0.047
Ferritin (ng/ml)	-0.236	0.099	-0.004	0.102	-0.109	0.124
Hepcidin (ng/ml)	1	0.035	1	0.187	1	0.091
SOD (ng/ml)	0.035	1	0.187	1	0.091	1

Data are presented as correlation coefficients (r).
*Indicates significant correlations ($p<0.05$).
SOD: Superoxide dismutase, RBC: Red blood cell, HGB: Hemoglobin, MCV: Mean cellular volume, MCH: Mean cellular hemoglobin, MCHC: Mean cellular hemoglobin concentration, RDW: Red cell distribution width, ESR: Erythrocyte sedimentation rate.

Table 5: Correlations between serum levels of hepcidin and SOD and other biochemical markers by age and sex

Biomarker	Children (n= 6)		Adolescents (n= 27)		Adults (n= 27)		Females (n= 33)		Males (n= 27)	
	Hepcidin	SOD	Hepcidin	SOD	Hepcidin	SOD	Hepcidin	SOD	Hepcidin	SOD
RBC count (10^{12}/L)	-0.74	-0.515	0.193	-0.008	0.039	0.136	-0.182	-0.076	0.207	0.052
HGB (g/dl)	0.233	0.508	0.029	-0.030	-0.021	0.024	-0.18	-0.195	0.15	0.496*
MCV (fl)	0.791	0.904*	-0.149	-0.153	-0.051	0.216	0.044	-0.292	-0.127	0.502*
MCH (pg)	0.724	0.772	-0.226	0.004	-0.02	-0.095	0.031	-0.301	-0.157	0.499*
MCHC (g/dl)	0.551	0.497	-0.181	0.163	0.016	-0.292	0.012	-0.123	-0.158	0.203
RDW (%)	-0.588	-0.525	0.208	-0.006	-0.009	0.046	-0.018	0.264	0.139	-0.437*
ESR (mm/hr)	-0.3	-0.121	-0.307	-0.145	0.354	0.045	0.118	-0.076	0.015	0.233
Ferritin (ng/ml)	-0.029	0.371	-0.257#	-0.159	0.043	0.281	-0.160	-0.047	-0.011	0.25
Hepcidin (ng/ml)	1	0.848*	1	-0.225	1	0.266	1	-0.066	1	0.245
SOD (ng/ml)	0.848*	1	-0.225	1	0.185	1	0.1	1	0.245	1

Data are presented as correlation coefficients (r).
*Indicates significant correlations ($p<0.05$).
# Indicates significance tendency ($p = 0.06$)
SOD: Superoxide dismutase, RBC: Red blood cell, HGB: Hemoglobin, MCV: Mean cellular volume, MCH: Mean cellular hemoglobin, MCHC: Mean cellular hemoglobin concentration, RDW: Red cell distribution width, ESR: Erythrocyte sedimentation rate.

DISCUSSION

Iron overload is the predominant contributor to serious health complications in β-thalassemia patients. Its accumulation is continually increasing despite the use of iron chelators [19] even in non-transfused thalassemia patients [20]. Therefore, there is another source of iron than blood transfusion and hemolysis in thalassemia patients. In this study, ferritin was shown to be very high (3036.9±309.7 ng/ml). Ferritin is very high in thalassemia patients despite regular chelation therapy due to the role of low hepcidin levels in iron overload [21]. Hepcidin binds to the iron exporter protein, ferroportin (FPN), on the surface of absorptive and storage cells, causing a

degraded ability to control dietary iron absorption and its release from stores [22]. A study found that hepcidin is deficient in thalassemia patients because of the active ineffective erythropoiesis, raising the blood iron concentration [23].

Hepcidin measurement has recently become possible with the development of assays for its levels in serum and urine. Hepcidin assessment in β-thalassemia began in the early twenty-first century in mice and was extended to humans, focusing on its mRNA expression, and serum and urine levels [23]. In this study, hepcidin was found to be very deficient (2.21±0.16 ng/ml) compared to the normal range (464.61–533.67 ng/ml) [18]. In a study conducted on adult thalassemia patients (35.2 ± 2.9 years), hepcidin was 0.95 ± 0.2 µg/L, indicating the ineffectiveness of chelator use on serum iron [24]. Another study by Smesam et al. [25] found a significantly (p= 0.005) decreased level of hepcidin in thalassemia major patients (36.06 ng/ml) compared to healthy controls (80.34 ng/ml). The sample of our study included three age groups and showed that hepcidin insignificantly decreased with age; children had 2.28±0.60 ng/ml, adolescents 2.25±0.22 ng/ml, and adults 2.16±0.25 ng/ml.

The total thalassemia intermedia patients in this study had an insignificantly lower level of serum hepcidin (2.15±0.21 ng/ml) than the patients who had thalassemia major (2.28±0.24 ng/ml). Huang et al. [26] found that transfusion-dependent thalassemia major patients had significantly higher hepcidin levels (31.6 ng/ml) than non-transfusion-dependent patients with thalassemia intermedia (14.6 ng/ml). In our study, the patients with the intermedia form were transfusion-dependent, which might be responsible for the insignificant difference in the hepcidin levels compared with patients of the major form. Moreover, hepcidin was found to be significantly (p= 0.034) higher in child patients with thalassemia major (1.9 ng/ml) than those with intermedia form (1.3 ng/ml) [27]. Assem et al. [28] also reported the same finding.

Most of the hepcidin studies in thalassemia patients investigated the relationship of its level in the body with iron biomarkers, particularly ferritin, and found variable correlations. We found hepcidin into be insignificantly negatively correlated to ferritin in all patients and both groups of thalassemia patients. It only tended to be significantly linked with ferritin (r= -0.257, p= 0.06) in the adolescent group. Hepcidin was also negatively but insignificantly correlated with RBC count and HGB in thalassemia intermedia patients, while it was positively correlated with these two blood markers in thalassemia major patients. These findings could be explained by the difference in the mutation degree of β-globin genes between the two forms of thalassemia. Both thalassemia forms are caused by a mutation in both hemoglobin genes, leading to reduced production of β-globin in thalassemia intermedia and a complete absence of β-globin in the major form [29]. As a result of the difference in mutation degree, a few healthy RBCs are expected to be produced in thalassemia intermedia patients as compared with complete impairment of RBCs in thalassemia major. Another possible reason is that thalassemia major patients have blood transfusion more frequently than patients with thalassemia intermedia. More blood transfusions for thalassemia major patients result in more RBCs and a rise in hepcidin [20], albeit it is still deficient.

Similar to our finding, hepcidin was not found correlated with the serum ferritin, hemoglobin, or serum free iron (p> 0.05) [21,30–32]. These results indicate that hepcidin may be affected more by erythropoiesis or iron chelation therapy than iron storage, whereas it was positively correlated with hemoglobin, ferritin and serum iron (p< 0.05) [20,26,27,33]. On the other hand, Hendy et al. [34] showed a positive relationship of serum hepcidin with hemoglobin (r= 0.97, p< 0.01) but it was negative with ferritin (r= - 0.72, p< 0.01).

Poor correlations between hepcidin and ferritin or hemoglobin in this study may be explained by low hepcidin and the consequent hyper-absorption of dietary iron being a major cause of systemic iron overload, contributing less to the total iron load than transfusions. More simply, hepcidin deficiency could be a major cause of iron overload in thalassemia but it has a very weak correlation with iron biomarkers in the presence of blood transfusion. To recap, it seems that hepcidin in thalassemia loses its function in controlling FPN, and thereby the iron levels, contributing to iron overload [35].

All patients in this research had significantly high levels of SOD (3.13±0.14 ng/ml) compared to the normal value of 0.65 ng/ml (p= 0.0001). In β-thalassemia, the thalassemic red blood cells hemolyze and release heme, iron and unpaired α-globins, thus developing free radicals and a state of oxidation [36], which stimulates SOD synthesis [16,37]. SOD is involved in the detoxification processes that neutralize the harmful free radicals and protect the cells from oxidation and consequent damage [37, 38]. However, Choudhary et al. [10] showed an inverse correlation between the oxidative stress and the levels of SOD, indicating its role in the inactivation of superoxide anions in increased

oxidative stress. Impaired levels of antioxidant enzymes implicate their increased needs in thalassemic patients with repeated transfusions [39].

Mohammed and Abd-El Rasoul [40] found a significant increase in SOD activity in thalassemia children compared to healthy controls (91.49 U/ml vs. 56.63, p= 0.0001). Similarly, Abdalla et al. [19], who conducted the only study in Jordan to assess SOD, reported significantly increased activity in thalassemia children compared to healthy children (210±6.3 U/mL vs. 70 ± 3.1 U/mL,p< 0.05). In contrast to our finding, SOD was observed to be significantly lower in thalassemia patients compared to healthy controls [10,36,39,41]. However, most of the studies measured the SOD as activity, not a quantity as we did.

The activity of SOD was found to be decreased by more than 30% in the blood of β-thalassemia major patients compared to controls [42]. It was deficient in 50% of the studied thalassemia patients, indicating its role in oxidative stress [43]. The decreased SOD levels in thalassemia patients could be due to the reported deficiency of zinc and copper, which are essential components of the SOD enzyme [23]. However, its activity was observed to be higher (p< 0.0004) in thalassemia major patients (1478.4 U/ml) than controls (1261.7 U/ml) [44]. On the other hand, SOD intensity was significantly lower (162.41 U/ml) than the normal cutoff point (164–264 U/ml, p= 0.001) in Rujito et al. [45]. All these results indicate an oxidant-antioxidant uproar and enhanced oxidative stress in thalassemia [23].

SOD was positively correlated with RBC count and HGB in patients with thalassemia major; MCV in child patients; HGB, MCV, and MCH in male patients (p< 0.05). These correlations showed the protective role of SOD against RBC oxidation attributed to excess iron. However, it was found negatively but insignificantly correlated with RBC count and HGB in thalassemia intermedia patients. The converse relationships between SOD and RBC count and HGB among thalassemia intermedia and major patients may be attributed to the differences in gene mutation and blood transfusion frequency between them.

SOD was not correlated with ferritin levels in this study, which is in agreement with the findings of other researchers. Maskoen et al. [43] did not find a significant relation between SOD and serum ferritin (r= -0.073, p= 0.634). Moreover, the correlation was not significant (r= -0.239, p= 0.167) in Mohammed and Abd-El Rasoul [40]. On the contrary, it was detected that SOD is inversely related to ferritin levels in order to compensate for iron overload and enhanced oxidation [39, 41]. SOD activity was inversely linked with hemoglobin, indicating a positive association between SOD and anemia severity [44]. The inverse relationship indicates the increased demand for SOD that accompanies increased body iron. On the other hand, Abdalla et al. [19] showed a positive relation between ferritin and SOD levels (r= 0.32, p= 0.046) in response to iron overload.

Hepcidin was not significantly correlated with SOD levels except in child patients (r= 0.848, p= 0.033). Hepcidin levels in these patients were very low compared to the normal values. Children have high serum ferritin, although fewer blood transfusions than adolescents and adults, which decreases the causative effect of iron overload from the transfusion. Increased SOD in child patients, along with high ferritin and a positive relation to RBC health, can be attributed to the defensive role of SOD against iron accumulation. In brief, it seems that hepcidin deficiency in child patients increases iron absorption and release from stores, causing iron accumulation more than that caused by blood transfusion.

CONCLUSION

Compliance with chelation therapy is inadequate and iron overload remains a major cause of morbidity and mortality in thalassemia patients. SOD level was high in these patients, indicating enhanced oxidative stress. Therefore, in addition to considering hepcidin deficiency in β-thalassemia patients, hepcidin-targeted therapeutics may help improve the treatment of iron overload in β-thalassemia. Future preclinical and clinical studies are necessary to assess the side effects and benefits of hepcidin replacement therapy for β-thalassemia patients.

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وضع الهيبسيدين وإنزيم تدمير أيونات الأكسجين (SOD) في عينة من مرضى الثلاسيميا الأردنيين

دانه عدنان العمري[1]، حامد رباح التكروري[1]

[1] قسم التغذية والتصنيع الغذائي، كلية الزراعة، الجامعة الأردنية، عمان، الأردن

الملخص

المقدمة والهدف: لقد وُجِدَ أن مستوى هرمون الهيبسيدين يكون منخفضًا عند مرضى البيتا–ثلاسيميا مما يشير إلى دوره الحرج في تراكم الحديد. ولم يتم تقييم مستوى الهيبسيدين في مرضى الثلاسيميا الأردنيين، كما لم يتم البحث في علاقة استجابة إنزيم تدمير أيونات الأكسجين (SOD) مع التراكم الزائد والمتباين للحديد وربطه مع الهيبسيدين. لذا هدفت هذه الدراسة إلى تقييم مستويات مصل الدم من الهيبسيدين و SOD في المرضى الذين تم تشخيصهم بالثلاسيميا الوسطى والكبرى وبحث العلاقة بينهما في هؤلاء المرضى.

عينة ومنهجية البحث: تم في هذا البحث إشراك60 مريضًا مصابًا بالبيتا– ثلاسيميا تتراوح أعمارهم بين 2–25 سنة من وحدة الثلاسيميا في مستشفى الزرقاء الحكومي الجديد، وتمَّ تصنيفهم إلى نوعين من الثلاسيميا، كما تمَّ تقييم مستويات المصل من الهيبسيدين وSOD لديهم. وقد تم إجراء التحليل الإحصائي للتحقّق من الفروق بين مرضى نوعيّ الثلاسيميا، ومقارنة مستويات الهيبسيدين وSOD مع مستوياتهما المرجعية ، وحساب معاملات الارتباط بينها.

النتائج: اشتملت عينة الدراسة على 31 مريضًا بالثلاسيميا الوسطى و 29 مريضًا بالثلاسيميا الكبرى منهم 33 أنثى و 27 ذكراً. وجد نقص كبير لدى جميع المرضى في مستوى الهيبسيدين (2.21 ± 0.16 نانوغرام / مل) وارتفاع في مستوى SODفي مصل الدم (3.13 ± 0.14 نانوغرام / مل) مقارنة بالقيم الطبيعية. وقد كان مستوى الفيريتين مرتفعًا جدًا (3036.9 ± 309.7 نانوغرام / مل) في جميع المرضى وكان أعلى في المرضى البالغين والذين يعانون من الثلاسيميا الكبرى. ولم يتم العثور على ارتباط معنوي بين الهيبسيدين أوSOD والفيريتين ($P>0.05$)، بينما وُجدت علاقة معنوية فقط في مرضى الأطفال بين مستويات الهيبسيدين وSOD ($r = 0.848$ $P= 0.033$).

الاستنتاجات: يعاني مرضى الثلاسيميا الأردنيين من نقص الهيبسيدين مما يساهم في تراكم الحديد. وقد وُجدَ أنَّ لإنزيمSOD دوراً وقائياً ضد الأكسدة في مرضى البيتا–ثلاسيميا. كما تبيَّن أنَّ استخدام علاج طارد الحديد غير كافٍ لمعالجة فرط تراكمه، والذي لا يزال المسبب الرئيسي للمضاعفات الصحية لدى مرضى الثلاسيميا. ولذا فقد يكون استخدام أدوية منشطة للهيبسيدين مفيدًا لعلاج تراكم الحديد الزائد.

الكلمات الدالة: البيتا– ثلاسيميا، الثلاسيميا الوسطى، الثلاسيميا الكبرى، هبسيدين، إنزيم تدمير أيونات الأكسجين