Biochemical Status of Beta-Thalassemia Major Patients in Erbil City: Case Control Study

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Background and objectives: β -thalassemia major patient is one of the hereditary hemolytic diseases, which can cause many hematological and biochemical changes in the affected patient. And these changes can happen even when the patient is treated adequately. The objective was to study biochemical changes in the level of serum hepcidin, osteocalcin, calcium, ferritin, iron, PTH and IL-6 in patients with β- thalassemia major and to compare it with control subjects.

Patients and method: In this research 40 patients with beta thalassemia major, 20 Female and 20 Male (age ranged from 10 to 38 years), and 40 control subjects 20 Female and 20 Male (age ranged from 9 to 33 years) were studied. Measurement of serum hepcidin, osteocalcin, calcium, ferritin, iron, PTH and IL-6 were done by the researcher for both cases and control groups.

Results: Serum Ferritin and Iron were higher significantly in all thalassemic patients (P< 0.001), this increment was proportional with increasing number of units of blood transfusion and aging. Mean serum Hepcidin, PTH, Osteocalcin and IL6 were significantly lower in thalassemic patients in contrast to the control subjects (P< 0.001). Reduction in S-PTH was proportional to increasing number of blood transfusion and aging. But there was no significant difference in the level of serum Calcium in the majority of patients, only 9 patients had low serum calcium.

Conclusion: Our study demonstrates that in B-thalassemia major patients Serum Ferritin and Iron were increased proportionally with increasing age and number of units of blood transfusion. Mean serum PTH, osteocalcin, hepcidin, and IL-6 were reduced, but mean serum calcium was remained normal.

Keywords: β- thalassemia, Serum.

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Introduction

Homozygotes thalassemia major either are unable to synthesize hemoglobin A or, at best, produce very little; after the first 4–6 months of life, they develop profound hypochromic anemia in thalassemia major there is many biochemical changes in the level of hepcidin, Osteocalcin , calcium, ferritin ,iron ,PTH and IL6[1].

Hepcidin is a small peptide hormone secreted by hepatocytes to regulate plasma iron concentration and distribution in different tissues.[2,3] Hepcidin dysregulation causes a majority of iron related disorders. Chronic excess of hepcidin causes iron deficiency anemia,[3] while the hepcidin deficiency leads to iron overload with iron deposition in the liver parenchyma[4]. Increased plasma and stored iron stimulate hepcidin production,

which in turn blocks dietary iron absorption and consequently reduce iron loading. Conversely, hepcidin is suppressed in iron deficiency[5]. Hepcidin is mainly regulated by hypoxia, anemia and iron stores.[6-10] Despite using iron chelation, patients with Thalassemia major have iron overload as one of the main mortality factor. In the future Hepcidin targeted therapy may help better management of iron overload in patients with Thalassemia.[11]

Osteocalcin which is produced by osteoblasts, also known as bone gamma-carboxyglutamic acid-containing protein, is a non-collagenous protein hormone found in bone and dentin. osteocalcin is secreted solely by osteoblasts and thought to play a role in the body's metabolic regulation and is pro-osteoblastic, or bone-building. It is also implicated in bone mineralization and calcium ion homeostasis.[12-14]

Repeated blood transfusion results in citrate toxicity and lead to iron deposition in the parathyroid gland, which in turn may cause hypoparathyroidism. A few studies have reported that some of the thalassemic patients who are on regular packed red blood cell transfusion, can develop hypoparathyroidism, especially after 10 years of age.[15-16]

Normal body iron stores are 3– 4 g; an excess of iron of 20 g or more can lead to organ damage.[17] Iron overload is a universal complication of transfusion-dependent thalassemia.[17,18] Increased intestinal absorption of iron occurs in response to ineffective erythropoiesis and chronic anemia. Each unit of transfused red blood cells contains 200 –250 mg of iron, and because the body has no mechanism for excreting excess iron, iron overload readily occurs in patients after 10 to 20 transfusions[19]. Excessive body iron can lead to increased free iron, which is highly toxic to cells.[17]

Interleukin-6, an inflammatory cytokine, is characterized by pleiotropy and redundancy of action. Apart from its hematologic, immune, and hepatic effects, it has many endocrine and metabolic actions. Specifically, it is a potent stimulator of the hypothalamic-pituitary-adrenal axis. It acutely stimulates the secretion of growth hormone, inhibits thyroid-stimulating hormone secretion, and decreases serum lipid concentrations.[20,21] The objective of this study to determine and compare some biochemical changes in the level of serum hepcidin, osteocalcin, calcium, ferritin, iron, PTH and IL-6 in patients of β-thalassemia major with controls.

Patients and method

Patients. In this research 40 patients, 20 Female and 20 Male (age ranged from 10 to 38 years) with beta thalassemia major, and 40 control subjects 20 Female and 20 Male (age ranged from 9 to 33years) were studied. The study period started from February 1st 2017 to July 1st 2017. Patients with beta thalassemia major were seen in the Erbil thalassemic center, which is the only health center specific for thalassemic patient. All patients are seen on regular visits each month for the clinical, hematological and biochemical assessment and the need for blood transfusion. Hematological assessment includes CBC and Blood film morphology. Assessment of some biochemical tests like the level of serum hepcidin, Osteocalcin, calcium, ferritin, iron, PTH and IL6 were done by the researcher for both cases and control groups. In this study we compared thalassemic patients with control subjects for some biochemical status in relation to the age, number of blood transfusion.

Method. The blood samples were collected under sterile conditions, and injected into a gel tube that is free from any chemical substance except a small amount of gel. The function of the gel is only to separate the blood cells from the serum. Then this process is done after the blood in the tube is clotted, using a centrifuge at the speed of 6000 rpm. Then the serum which is the top yellow fluid is used to

perform the tests. After turning on the analyzer (Cobas Integra 400 plus), and after the automatic checks, the analyzer goes to standby status. By clicking the Order section, a window opens that shows some editable fields and the available tests to order. The fields are to enter the details of the patient ID, and to specify the location of the specimen on a suitable rack. Multiple tests can be selected from the screen as long as the reagent is on board and a suitable calibration has performed. After inputting the specimen's info, it's position on the rack, and selecting the required tests, save button will save the entered data and will start the analysis process. Finally, in the Results screen, the result of all selected tests can be found with all necessary details such as units and time of pipetting and time of the result output.

Statistical analysis. All the data was entered and analyzed by SPSS version 22. Data was summarized as mean and standard deviation for numerical data number and percentages were used to express categorical data, and the results were analyzed using the independent t-test to compare the mean of the parameters where necessary and p value of less than 0.05 was considered significant.

Results

In Table 1, showing the difference between serum level of different biochemical among thalassemic patients and control subjects. Ferritin and iron were higher significantly in thalassemic patients. Hepcidin, PTH, Osteocalcin and IL6 were significantly lower in thalassemic patients in contrast to the control subjects. But serum calcium was not different in both groups.

In Table 2 shows the minimum and maximum level of some biochemical materials in thalassemic patients. In which the serum ferritin and iron level are markedly higher than normal range. But the serum level of hepcidin, PTH, Osteocalcin and IL6 are lower than the normal ranges, in which their maximum level does not reach the level of upper normal value. serum calcium was below normal only in 9 patients, and in the rest 31 patients were normal. (as shown in the Table 3).

In Table 4, mean serum ferritin and iron are higher in all age groups than control and increasing the level of mean serum ferritin and iron with increasing the age. mean serum hepcidin is lower than control in all age groups.

In Table 5, serum calcium in all age groups are with in normal range. Serum PTH in less than 20 years old patients are normal, while in those patients 21 years old and more, serum PTH is low. Mean serum Osteocalcin is lower than normal in all age groups.

In Table 6, serum IL6 is with in normal limit in age group of 10 years and less. But above 10 years of age, serum IL6 is lower than normal.

In Table 7, serum ferritin is increasing with increasing number of blood transfusion in 18 patients who received an average 100-150 units of blood, serum ferritin was 1858.6ng/ml. In 11 patients who received an average 151-200 units of blood, serum ferritin was 3233ng/ml. In 3 patients who received an average 201-250 units of blood, serum ferritin was 3529.6ng/ml. And among 8 patients who received more than 250 units of blood, serum ferritin was 4066ng/ml. serum ferritin is proportional to the increasing the number of blood transfusion. Regarding serum iron, it is increasing with increasing number of blood transfusion. in those who received between 100-150 units of blood (18 patients), S. iron in was 208 μg/dl. and those who received between 151-200 units of blood (11patients), serum iron was 266. and those who received between 201-250 units of blood (3 patients), serum iron was 301.3. and finally in those who received more than 250 units of blood. (8 patients), serum iron was 317.3. Serum hepcidin is reduced in all thalassemic patients who received blood transfusion (mean 1.1 ng/ml). while in control group, range is 1.563-100 ng/ml.

Table 1: Serum level of some biochemical among cases and control subjects.

Parameters	Case (n=40) Mean±SD	Control (n=40) Mean±SD	P-value
S-Ferritin	2803.42 ± 1555.74	128.80 ± 57.19	P< 0.001
S-Iron	250.87 ± 60.33	94.35 ± 43.07	P< 0.001
hepcidin	1.11 ± 0.47	27.48 ± 32.68	P< 0.001
S-Ca	9.19 ± 0.62	9.28 ± 0.38	P> 0.05
PTH	20.01 ± 12.15	28.38 ± 11.93	P< 0.001
S-Osteocalcin	0.70 ± 0.29	2.83 ± 2.11	P< 0.001
IL6	0.046 ± 0.05	0.137 ± 0.01	P< 0.001

Table 2: Showing minimum and maximum levels of some biochemical materials in thalassemia.

Parameters	Normal Ranges	Minimum	Maximum
S-Ferritin	F=9.3-159 M=68-434 ng/ml	797	7225
S-Iron	33-193 µg/dl	124	381
hepcidin	1.563-100 ng/ml	0.0040	1.9470
S-Ca	8.8-10.8 mg/dl	7.5	10.6
PTH	15-65pg/ml	4.50	49.94
S-Osteocalcin	0.938-60 ng/ml	0.109	1.321
IL6	0.06-0.2 ng/ml	0.0010	0.1470

Table 3: Serum calcium in 40 thalassemic patients.

Calcium	Frequency	%
< 8.8 mg/dl	9	22.5
8.8-10.8 mg/dl	31	77,5
Total	40	100

Table 4: Case and control comparison of serum ferritin, iron, and hepcidin in different age groups.

Parameters	Age	No.		Mean	
		case	control	case	Control
S-Ferritin	<=10	4	10	1131.5	95.3
	11-20	28	23	2681.4	134
	>=21	8	7	4066.2	159.7
	Total	40	40	2803.4	128.8
S-Iron	<=10	4	10	168.5	93.2
	11-20	28	23	248.2	97.5
	>=21	8	7	301.3	85.7
	Total	40	40	250.8	94.4
S-hepcidin	<=10	4	10	1.57	4.5
	11-20	28	23	1.03	29.7
	>=21	8	7	1.17	52.8
	Total	40	40	1.11	27.5

Table 5: Case and control comparison of serum calcium, PTH, and Osteocalcin in different age groups.

Parameter	Age	No.		Mean	
		case	control	case	control
S-Ca	<=10	4	10	8.88	9.1
	11-20	28	23	9.37	9.3
	>=21	8	7	8.71	9.3
	Total	40	40	9.19	9.3
PTH	<=10	4	10	27.68	18.3
	11-20	28	23	20.58	32.0
	>=21	8	7	14.19	30.7
	Total	40	40	20.01	28.4
S-Osteocalcin	<=10	4	10	0.9	2.8
	11-20	28	23	0.67	3.1
	>=21	8	7	0.73	1.9
	Total	40	40	0.7	2.8

Table 6: Case and control comparison of serum IL6 in different age groups.

Parameter	Age	No.		Mean	
		case	control	case	Control
IL6	<=10	4	10	0.11	0.1
	11-20	28	23	0.03	0.1
	>=21	8	7	0.05	0.1
	Total	40	40	0.05	0.1

Table 7: Relation of T.N.O.BT with serum ferritin, serum iron and Hepcidin.

Parameters	T.N.O.BT	N	Mean	SD
S-Ferritin	100-150	18	1858.6	853.2
	151-200	11	3233	2061.6
	201-250	3	3529.6	242.6
	>250	8	4066.2	996.9
	Total	40	2803.4	1555.7
S-Iron	100-150	18	208	54.5
	151-200	11	266.1	35.4
	201-250	3	301.3	24.1
	>250	8	317.3	34.6
	Total	40	250.8	60.3
S-Hepcidin	100-150	18	1.2	.457
	151-200	11	0.9	.461
	201-250	3	0.9	.200
	>250	8	1.1	.515
	Total	40	1.1	.466

Table 8: Relation of T.N.O.BT with serum calcium, PTH and Osteocalcin.

Parameter	T.N.O.BT	N	Mean	SD
S-Ca	100-150	18	9	.55
	151-200	11	9.5	.42
	201-250	3	9.9	.25
	>250	8	8.7	.59
	Total	40	9.1	.62
PTH	100-150	18	23.9	12
	151-200	11	17.7	11.7
	201-250	3	20.1	19.3
	>250	8	14.1	8.9
	Total	40	20	12.1
S-Osteocalcin	100-150	18	.68	.30
	151-200	11	.70	.32
	201-250	3	.72	.05
	>250	8	.73	.31
	Total	40	.70	.29

Table 9: Serum PTH in 40 thalassemic patients.

PTH	Frequency	%
<15pg/ml	20	50
15-65pg/ml	20	50
Total	40	100

Table 10: Serum IL6 and its relation to T.N.O.BT.

Parameter	T.N.O.BT	N	Mean	SD
IL6	100-150	18	.057	.060
	151-200	11	.025	.022
	201-250	3	.026	.017
	>250	8	.053	.057
	Total	40	.045	.050

In Table 8, Serum calcium is kept within normal range regardless of blood transfusion. PTH is not increasing with blood transfusion but in fact it is remained in the lower limit of normal range or reduced especially in those who received more 250 units of blood transfusion (In 50% of patients PTH is less than 15 pg/ml as shown in Table 9). Serum Osteocalcin is low in all thalassemic patients regardless of blood transfusion (mean 0.7 ng/ml).

In Table 10, Serum IL6 is low in all thalassemic patients regardless of blood transfusion (mean 0.046 ng/ml).

Discussion

Thalassemia major can cause many biochemical changes in addition to its hematological abnormalities. In our study serum ferritin and serum iron were high significantly in comparison to control subjects. This is related to regular blood transfusion, in transfused thalassemic patients, iron is preferentially distributed to the reticuloendothelial system, stimulating ferritin synthesis and its release to the circulation, resulting in high serum ferritin levels[22]. Our result is in agreement with the work of Hagag et al. who found serum ferritin and iron levels, in the thalassemic patient were significantly higher than those in the control group[23]. In our study serum hepcidin was lower than control subjects. Hepcidin concentration in patients with iron-loading anemia is decreased and consequently this leads to increased iron absorption. Our result of low hepcidin is similar to another study by Muhammad J etal and Pasricha SR.[9,24] In another study the serum hepcidin levels were similar in both, patients and controls, because these patients were on chelation therapy. This discrepancy between our results and the result of other studies, probably it is due to inadequate use of chelating therapy in our patients.[25]

In this study, mean PTH levels are significantly lower in patients compared to the control group. in our study, 50% of thalassemic patients had hypoparathyroidism. This is in agreement with the recent work of Bash et al.[26] Its explained by excess iron deposition in the parathyroid gland which causes its damage. This is particularly observed in cases of suboptimal chelation therapy. In another study hypoparathyroidism was detected in 12 out of 60 (20%) thalassemic patients.[27]

In our study, mean serum calcium was within normal range in 31 (77.5%) patients. but 9 patients (22.5%) had low s. calcium. In a study by Napoli et al, found no alteration in serum calcium levels in thalassemia patients.[28] While Saboor et al, in his study found serum calcium levels to be significantly lower in the cohort.[29] Hypocalcaemia seems to be related to hypoparathyroidism, as repeated blood transfusion results in iron deposition in the parathyroid gland which affects its normal functioning.[30]

In this study, number of blood transfusion had an influence on the level of serum calcium and PTH. With increasing number of blood transfusion lead to decrease in the level of

both s. calcium and PTH, which explained by parathyroid damage by excess iron deposition. This result is similar to the study done by Abdel-Hafez and colleagues conducted a prospective research evaluating endocrinal status in B-thalassemia children and they found a significant decrease in parathyroid hormone levels in the thalassemia group compared to the control group.[31]

In our study, in thalassemic patients mean IL-6 was low, The reason for the low serum levels of IL-6, is probably due to reduced activity of CD4+ lymphocyte.[32]

Serum Osteocalcin level was low in our study, while in study done by Zoga J etal, they found no significant change in the osteocalcin between patients and control.[33] In another study done by Ozturk O etal, osteocalcin and IL-6 were not changed with increasing number of blood transfusion, and this finding is comparable with our result.[34]

Conclusion

Thalassemia major is causing many biochemical changes even when managed properly. In our study serum ferritin and s-iron were increased proportionally with increasing age and increasing number of units of blood transfusion. while serum PTH was reduced in 50% 0f patients and this reduction in PTH was proportional to increasing number of blood transfusion and aging. serum osteocalcin, hepcidin, and IL-6 were reduced, but mean serum calcium was normal.

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