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ASSESSMENT OF SERUM SCLEROSTIN LEVEL AS A BIOMARKER ASSOCIATED WITH BONE DISORDERS IN β -THALASSEMIA PATIENTS IN AL- NAJAF CITY, IRAQ

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ABSTRACT

Aim of the study: To assess serum sclerostin in female patients with beta-thalassemia and compare with the healthy controls and to predict its complication associated with the bone pathophysiology, for designed improvement the lifestyle goodliness for these patients. Material and methods: Sixty-nine female beta-thalassemia (β T) patients (54 β T major and 15 β T Intermedia), aged 8-40 years who dependent on transfused blood, and 20 healthy controls were evaluated serum sclerostin, and was examined the relationship with hematological parameters RBC, Hb, PCV, WBC, PLT, BMI, splenic status, iron, and ferritin levels. The information of beta-thalassemia patients was collected and recorded by the questioner. Results: A significantly increased serum sclerostin level (mean 26.80 ± 0.91) pg/ml was shown in β T patients compared with the healthy controls (10.03 ± 0.68 , $p < 0.001$) pg/ml. Furthermore, a significant decrease ($p < 0.05$) of the sclerostin level was observed in β -thalassemia major compared to intermedia β -thalassemia patients. Serum sclerostin level revealed a significant increase in progress age; it is highest in the age group (30-40) year as compared with age group (8-18) and (19-29) year respectively. Sclerostin showed no associations with the RBC, Hb, PCV, and significantly positively correlated ($p < 0.05$) with serum iron, ferritin levels, WBC, and PLT count. Significantly higher sclerostin levels in splenectomized and underweight groups were observed compared to unsplenectomized and normal-weight groups ($p < 0.05$) of β T patients. Conclusion: Sclerostin plays an important role in beta-thalassemia patients and can serve as a biomarker associated with the bone pathophysiology and indicator to prevent the continuation of such serious diseases caused by iron overload in these patients.

Keywords: Sclerostin, β -thalassemia, BMD, iron overload, ferritin, and biochemical analysis of blood.

1. INTRODUCTION

Beta-thalassemia (β T) is a hereditary hemopathy, and significant clinical symptoms of these patients are ineffective erythropoiesis, with extramedullary involvement, hemolysis, and severe anemia (Almousawi, and Sharba, 2019; Thein, 2013). Bone abnormalities play an important role in β -thalassemia patients, which arises from the hematological disorder and its complications with hypertransfusion, iron overload, treatment and iron chelation, nutritional deficits, and sedentarism (Sapunarova *et al.*, 2020).

Sclerostin is a member of the Wnt signaling pathway inhibitor. It is a glycoprotein expressed predominantly by mature osteocytes under physiological and pathological conditions released

into circulation and binding with the cell membrane receptors of osteoblasts (Drake *et al.*, 2010; Wijenayaka *et al.*, 2011). The major role of Sclerostin in β Thalassemia patients is related to the reduced bone mineral density and the development of osteoporosis (Voskaridou *et al.*, 2012). However, the research about the role of sclerostin in female patients with beta-thalassemia is still rather inadequate, and it required further investigations; for this causes, the current study aimed to assess serum sclerostin in female patients with beta-thalassemia and comparison with the healthy controls, the relationship between sclerostin levels with the hematological parameters, age, BMI and splenic status, to predict of its complication that associated with bone pathophysiology such as osteoporosis, for designed improvement the lifestyle goodliness for these patients.

2. MATERIALS AND METHODS

2.1. Study design

Sixty-nine females with β -thalassemia (54 major β T and 15 intermedia β T), the aged range from (8–40) year. Patients were enrolled at The Thalassemia Center in Al-Zahraa Teaching Hospital in Al-Najaf province, Iraq. They were frequently blood transfused and treated every two or three weeks to maintain a Hb level of more than 10 g/dl and regulate iron-chelating therapy. Twenty participate of healthy females with matched age served as a control group; β -thalassemia patients were classified into many divisions according to types of β -thalassemia into two groups Major and Intermedia β -Thalassemia, three aged groups Group 1 (8 –18 years), Group 2 (19–29 year), and Group 3 (30–40 year), Splenic situation divided into Splenectomized and unsplenectomized patients (Sharba and Al-Dujaili, 2020). Also, according to Body Mass Index (BMI), patients were divided into two groups: normal weight and underweight the normal range (5th – 85th) % for (≤ 20) years of age, and (18.5 – 25) kg/m² for (>20) years of age.

2.2. Methods

An electronic balance and height unit carried out the BMI measurement to measure weight (kg) divided on the height square (m²), according to Equation 1. Both patients and healthy were withdrawn 5 ml of venue blood for hematological criteria were conducted on EDTA anticoagulated blood by using a completely automated hematology analyzer Mythic 18 (RINGELSAN CO., Turkey) for estimated complete blood count (CBC), which included RBC, PCV, Hb, WBC, and PLT. Estimation of biomarkers by using ELISA technique using the bioelisa ELx 80000 reader (biokit, USA). According to the Manufacturing Firm, the Human Accu Bind Ferritin ELISA Kit (Monobind Inc., USA, code number 2825-300). (Anderson and Kelly, 1981). The assay Human Sclerostin (SOST) ELISA Kit was conducted according to the manufacturing company (CUSABIO BIOTECH Co., Ltd., PRC, code number CSB-E13146h). Serum iron concentration was measured by iron (using the chromogen ferrozine method) kit (bt 35i, Turkey).

BMI= weight (kg) / height square (m²) (Eq. 1)

3. RESULTS AND DISCUSSION:

3.1. The general characteristics of the studied groups

The β -thalassemia patients and healthy controls are shown in Table 1.

3.2. Sclerostin level effects by study categories (Thalassemia types, age, BMI, and splenectomy status)

Figure 1 describes the ROC curves of sclerostin for β -thalassemia patients, and Figure 2 summarizes several comparisons of Sclerostin levels in the studied groups of β T control groups.

3.3. Relationship of Sclerostin level with the hematological Parameters in beta-thalassemia patients

It is described in Table 2 the correlation coefficient of sclerostin with other variables in β -thalassemia patients

4. CONCLUSIONS:

Sclerostin plays an important role in beta-thalassemia patients and can serve as a biomarker associated with the bone pathophysiology and indicator to prevent the continuation of such serious diseases caused by iron overload in these patients.

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Table 1. Demographic of the clinical characteristics between female patients with β -thalassemia and the healthy controls

Clinical characteristics	Patients n= 69	Healthy Controls n= 20	P- Value
Age (year)	16.62 $\pm$ 0.96	20.75 $\pm$ 1.86	0.612
Group (8 – 18) n (%)	51(74%)		
Group (19 – 29) n (%)	12(17.4%)		
Group (30 – 40) n (%)	6(8.7%)		
Major β T n (%)	54(78.2%)	0	NA
Intermedia n (%)	15(21.8%)	0	
Hb (mg/dl)	7.7 $\pm$ 0.14 *	12.01 $\pm$ 0.19	0.027
RBC (10^6 /ml)	3.13 $\pm$ 0.07 *	4.36 $\pm$ 0.07	0.038
WBC (10^3 /ml)	11.05 $\pm$ 0.26 *	7.59 $\pm$ 0.33	0.036
PCV %	23 $\pm$ 0.41 *	36.34 $\pm$ 0.52	0.036
PLT (10^3 /ml)	361.86 $\pm$ 13.92 *	244 $\pm$ 14.23	0.034
BMI (kg/m ²)	17.59 $\pm$ 0.43 *	22.15 $\pm$ 0.36/	0.001
Underweight, n (%)	13.09 $\pm$ 0.52, 40(58%)	0	
Normal weight, n (%)	23.7 $\pm$ 2.04, 29(42%)	20(100%)	
Iron (μ g/ml)	172.2 $\pm$ 4.4 *	29.86 $\pm$ 2.32	0.0001
Ferritin (ng /ml)	5156.5 $\pm$ 438.7 *	99.15 $\pm$ 8.95	0.0001
Splenectomized n (%)	29 (42%)	0	NA
Unsplenectomized n (%)	40(58%)	0	NA

* Statistically significant at $p < 0.05$; NA: not application; RBC: red blood corpuscles; Hb: hemoglobin; PCV: pact corpuscular volume; WBC: White Blood Cells. BMI: body max index; PLT: platelets. Data represented as mean $\pm$ standard division.

Table 2: Correlation coefficient of sclerostin with other variables in β -thalassemia patients

Variables	Sclerostin (pg/ml)	
	P-value	R
RBC (106/ml)	0.440	0.094
PCV %	0.751	0.031
PLT (103/ml)	0.030	0.260 *
Hb (mg/dl)	0.661	0.054
WBC (103/ml)	0.001	0.770 **
Iron (μ g/ml)	0.0006	0.513 **
Ferritin (μ g/ml)	0.0005	0.522 **

Significant difference * p-value < 0.05 . ** p-value < 0.01

Figure 1. ROC curves of sclerostin for β -thalassemia patients. ROC: receiver operating characteristic; AUC: area under the curve

Figure 2. A: Comparison of Sclerostin levels in studied groups β T patients and healthy control groups. B: Effects of thalassemia types on Sclerostin levels in patients with β T major and β T intermedia. C: Effects of Age on Sclerostin levels among age groups. D: Effects of splenectomy status on Sclerostin levels in splenectomized and un splenectomized groups. E: Effects of BMI on Sclerostin levels in underweight and normal-weight groups. * and deferent litters significant $p < 0.05$. data represented as mean $\pm$ SD.