SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY

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

SHARBA, Intisar Razzaq[1*]; AL-DUJAILI, Arshad Noori;

[1] University of Kufa, Faculty of Science, Department of Biology, Iraq.

** Correspondence author*
e-mail: intisar.sharba@uokufa.edu.iq

Received 26 May 2021; received in revised form 11 September 2021; accepted 27 november 2021

ABSTRACT

Background: β-thalassemia is a blood disorder in which the body does not make hemoglobin normally. **Aim**: 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 (βT) patients (54 βT major and 15 βT 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 records by the questioner. **Results**: A significantly increased serum sclerostin level (mean 26.80±0.91) pg/ml was showed in βT 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 βT patients. **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.

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

1. INTRODUCTION

Beta-thalassemia (βT) is a hereditary hemopathy characterized by a defect in the production of the beta-globin chain resulting from a genetic defect on chromosome 11 (Galanello and Origa, 2010). 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). Affecting bone metabolism in βT and instability of bone mineral turnover with amplified resorptive average and inhibition of osteoblast activity leads to diminished bone mineral density (BMD) (Voskaridou *et al.,* 2012).

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). Also, it was found that osteoclast precursors can secrete sclerostin in bone marrow and reductions in the development of their maturation (Delgado-Calle *et al.,* 2017). Additionally, studies have demonstrated that sclerostin can be assessed in several clinical states, such as inflammatory (Wehmeyer *et al*., 2016). Sclerostosis, fracture risk spinal injury (Morse *et al.,* 2012), multiple myeloma (Terpos *et al.*, 2012), ankylosing chronic kidney disease,

type-II diabetes, and another disease (Kim *et al*., 2013; Pelletier *et al.,* 2013). 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

According to the approval of the ethical committee regulations of Kufa University, faculty of science at protocol number 2014. Sixty-nine females with β-thalassemia (54 major βT and 15 intermedia βT), the aged range from (8–40) year. Patients were enrolled at The Thalassemia Center in Al–Zahraa Educational 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; the patient and controls. This study was carried out from July 2014 to March 2015. 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 year), Group 2 (19 –29 year), and Group 3 (30 – 40 year), Splenic situation divided into Splenectomized 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/m^2 for (>20) years of age. (WHO, 2006).

2.2. Methods

An electronic balance and height unit carried out the BMI measurement to measure weight (kg) divided on the height square (m^2), according to Equation 1. (WHO, 2006). 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 (Wasmuth, 2010). The serum samples for ferritin, iron, and sclerostin were prepared through cool-centrifugation and freezing at (−80 °C) after the collection and stored until the time of the analysis. Estimated serum ferritin using the enzyme-linked immunosorbent assay (ELISA) immunoenzymatic 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) that depended on the technique of the quantitative sandwich enzyme immunoassay. Serum iron concentration was measured by iron (using the chromogen ferrozine method) kit (bt 35i, Turkey). The principal transferrin-bound iron is released at acid pH and reduced from ferric Fe^{3+} to ferrous ions Fe^{2+} iron. These ions react with ferrozine to form a violet-colored complex, which is measured spectrophotometrically at 560 nm. This absorbance is proportional to serum iron concentration in the sample (Persijn *et al*., 1971).

BMI= weight (kg) / height square (m^2) (Eq. 1)

2.3. Statistical analysis

The data were statistically analyzed by the computer software of packed SPSS V. 25 (Inc., Chicago, IL, USA). Data with normal distribution were defined as mean ± standard error (SE) and compared between two groups by independent t-test. While not normally distributed data as the median and interquartile range (IQR) and Mann-Whitney U tests. Nominal data is defined as frequency and percent. Pearson or Spearman rank correlation coefficients for finding related between serum sclerostin and study parameters. A receiver operator characteristic curve (ROC) with an area under the curve (AUC) was then applied to identify the predictive value of sclerostin for patients among the healthy group. statistically

significant at $p < 0.05$.

3. RESULTS AND DISCUSSION:

3.1. The general characteristics of the studied groups

The general characteristics of the studied groups are shown in Table 1. In this table, 69 β-thalassemia patients (major βT 54 (78.2%), and intermedia βT 15 (21.8%), splenectomized 29 (42%) and unsplenectomized 40 (58%) indicated no significant difference ($p>0.05$) in the mean age of the β-thalassemia patients (16.62± 0.96) years when compared with the healthy controls (20.75±1.86) year, p=0.612. The results showed that β-thalassemia patients a significant difference ($p<0.05$), decreased levels of Hb (7.7±0.14) g/dl, RBC (3.13±0.07) 10^6/ml, and P.C.V (23±0.41) %, to compared with the healthy controls (12.01±0.19) g/dl, p=0.027, (4.36±0.07) 10^6/ml, p=0.038 and (36.34±0.52) %, p=0.036 respectively. But significantly increase in values of WBC (11.05±0.26) 10^3/ml and PLT (361.86±13.92) 10^3/ml in β-thalassemia patients, as compared to the healthy controls (7.59±0.33)10^3/ml, p=0.036, (244±14.23) 10^3/ml, p=0.034. The current results agree with Arshad *et al*. (2014), who reported that Thalassemia patients might have abnormalities associated with lower Hb levels due to the decrease of erythrocyte numbers and decreased values of RBC indexes (MCV, MCH, MCHC, HCT). Thus, these patients suffer from anemia resulting in less oxygen content in the blood. Some studies concluded that decreased Hb, PCV, and RBC count levels, observed in β-thalassemia patients, are due to their early degradation and continuous breakdown of erythrocytes because of the abnormal globin molecule leading to erythrocyte rupture before maturation (Shanthi *et al.,* 2013). Some studies referred to RBC mass, WBC, and PLT count a significant difference in β-thalassemia patients. These were attributed that might be due to continuing severe anemia accompanied by hyper-cellular (thrombocytosis and leukocytosis) resulting in stimulation of erythropoietin hormone, which acts on the bone marrow to increase proliferation of blood cells or resulting in the immune system activation by receiving blood from diverse donors (Yassin *et al*., 2013; Arshad *et al*., 2014).

BMI was showed a significant decrease ($p<0.05$) in β thalassemia patients in healthy inverse controls (17.59±0.43 vs. 22.15±0.36 kg/m^2, p=0.001). Iron and ferritin were reported high significant in β-thalassemia patients (172.2 ± 4.4) µg/ml and (5156.5±438.7) ng/ml, more than to the healthy controls (29.86±2.32) µg/ml, and (99.15±8.95) ng/ml, p=0.0001, respectively. These results correspond with Eissa and El-Gamal (2014), finding the relationship of growth differentiation factor (GDF15) (reflecting ineffective erythropoiesis), BMI, and iron overload, resulting in this who suggested that ferritin as markers of low BMI of β-thalassemia patients. Many researchers have postulated that the pathogenesis of growth failure β-thalassemia is multifactorial. The key contributing factors to stunt growth and delay in onset of puberty in β-thalassemia major patients may include chronic anemia, transfusions iron overload, hypersplenism, chelation toxicity, and developed endocrinopathies secondary to iron overload that include hypothyroidism, hypogonadism, GH deficiency/insufficiency. In addition to other contributing factors such as deficiency of essential nutritional elements, chronic liver disease, which are major contributing factors in producing underweight patients (Skordis, 2011; Salih and Al-Mosawy, 2013; Manali *et al*., 2015). In such cases, blood transfusions are frequently needed in β-thalassemia, which causes increased free iron overload, which leads to the development of abnormalities in the body. Moreover, the purpose of transfusion includes improvement of anemia, suppression of erythropoiesis, and inhibition of increased gastrointestinal iron absorption. As no excretory mechanism exists, excess iron gets deposited as hemosiderin and ferritin in the liver, spleen, and endocardium (Pasricha *et al*., 2013)**.**

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

ROC curve analysis and AUC of cutoff thresholds estimating serum sclerostin levels identified between beta-thalassemia patients and healthy controls group were shown in Figure 1. The results of ROC curve analysis showed that sclerostin (pg/ml) was a highly positive cutoff ratio of > 12.83 (AUC= 0.95; 95%CI= 0.919-0.996, sensitivity = 0.91; and specificity = 0.10, p=0.0001).

A significant increase ($p<0.05$) in sclerostin level in patients (26.80±0.91) pg/ml compared with the healthy controls (10.03±0.68) pg/ml (Figure 2A). A significant increase in Sclerostin level in β-thalassemia intermedia (32.28±3.20) pg/ml more than in β-thalassemia major (25.28±0.64) pg/ml. (Figure 2B). However, these results mentioned a

significant ($p<0.05$) decrease in the sclerostin level of age group (8-18) about (23.83±0.49) pg/ml as compared with that age group (19-29) and (30-40) about (29.45±1.55) pg/ml and (46.7±1.25) pg/ml respectively. It is also noticeable that the same significant decrease in age groups (19-29) and (30-40) was compared. (Figure 2C). Additionally, Sclerostin level indicated a significant increase ($p<0.05$) in the splenectomized group (28.95±1.49) pg/ml to compare with the unsplenectomized group (25.23±1.09) pg/ml (Figure 2D). The results in (Figure 2E) show that serum sclerostin levels significantly decrease ($p<0.05$) in underweight (24.44 ±0.66) pg/ml less than in the normal weight groups (31.51±2.09) pg/ml.

In the present study, Table 2 and Figure 1 show a significant increase in the serum Sclerostin level in female patients with β-thalassemia, compared with the control group, figure 1A. These results coincided with Voskaridou *et al.* (2012), suggesting that high sclerostin is an important marker for increased osteocyte activity in thalassemia patients. Especially, Sclerostin expression by osteocytes and the major function is regulating the formation of bone. Therefore, suppression of sclerostin may suppose the increase of the osteoblastic bone formation. Moreover, it plays a role important in osteoporosis (Lewiecki, 2011). A previous study suggested that the increased serum Sclerostin level in β-thalassemia is probable of many causes. One of the driving forces of drugs, the most commonly used iron chelator, is suppression of DNA synthesis, which was enhanced formation and proliferation of elements of the bone (osteoblast, fibroblast, collagen, then differentiation of osteoblast precursor), in contrast to these agents causes osteoblast apoptosis (Haidar *et al*., 2011). In addition, the study of Chen *et al*. (2013) concluded that when the use of iron chelator of such as desferrioxamine, a hypoxia-inducible factor (HIF-1α) catalyzes, thereby causes active sclerostin expression, as a chief manager of hypoxia. (HIF-1α) acts as regularly combined angiogenesis to osteogenesis. However, these hypoxias and that HIF-1α inhibited osteoblast growth through inhibiting the Wnt pathway. Ultimately, Wnt act as antagonist Sclerostin was up-regulated in osteoblasts.

Recent studies reported that most thalassemia patients suffer from many severe complications such as osteoporosis, low (BMD), and poor bone quality, resulting in decreased bone matrix and increased threat of fractures. These findings concluded that circulating sclerostin is elevated in thalassemia patients with osteoporosis and correlated with their BMD (Lapauw *et al*., 2013; Hashemieh *et al*., 2014). Baldini *et al*. (2010) explained that the imperative of the blood cell manufacture and over activation of the hematopoietic system then stimulates osteoclasts and osteoblasts, all of these risk factors for enhancing bone turnover and osteoporosis cooperates with increase sclerostin expression. Figure 1B found that circulating Sclerostin level significantly decreased in β-thalassemia major compared with thalassemia intermedia. The reason, but it is possible that the study subjects had a wide range age of about (19) in patients with β- thalassemia major but the range of age patients with thalassemia intermedia about to (33), which leads to affect the relationship between sclerostin and age. In this study, age-related to sclerostin levels in females showed a significant increase in serum sclerostin associated with progressed age in β-thalassemia patients figure 1C. These were consistent with Mödder *et al*. (2011), who revealed that lower serum sclerostin in young women under 30 years resulted in skeletal immaturity, lower osteocytes activity, or both. In addition, Wnt-related protein expression by osteoblastic was regulated by aging. The researchers reported that it related to age and increased sclerostin levels. It seems that aging increases active osteocytes for sclerostin production; however, physiological changes that companion with age, such as disorder in some hormones, possibly will contribute to the rise of sclerostin (Ardaw *et al*., 2011).

The study showed a significant decrease in the serum sclerostin level in underweight as compared with normal weight of female patients with β-thalassemia Figure 1D. These are founding agreement with the results of Klangjareonchai *et al*. (2014), which concluded that sclerostin, an osteocyte-specific protein, is found related to adiposity and glucose metabolism. Many studies have observed sclerostin found positively related to BMI or other measures of adiposity in studies of non-diabetic subjects (Urano *et al*., 2012; Sheng *et al*., 2012; Amrein *et al*., 2012). Study each of Yeo Pei *et al*. (2014), and Hashemieh *et al*. (2014) revealed that low bone mineral density (BMD), growth failure, osteopenia, and osteoporosis in later life are significant problems in children with β-thalassemia. In addition, a confident relationship between serum sclerostin and BMD or bone mineral content is also found in healthy adults (Mödder *et al*., 2011; Garnero *et al*., 2013). One interpretation is that bones with higher mass contain more osteocytes, which secrete more sclerostin than the fewer osteocytes in a skeleton

of the lower mass (Richards *et al*., 2012).

All these problems are an effect of decreasing BMI in patients with β-thalassemia, and the present study hypothesis that serum sclerostin level decrease in underweight groups of these patients, consequently increased BMI followed by elevating of BMD, which attendant to increase of sclerostin level. Figure 1E observed a significant increase in serum Sclerostin level in the splenectomized group compared with the non-splenectomized group of female patients with β-thalassemia.

The reasons for this are unclear, but a probable increase in sclerostin levels might reflect the increased average age of splenectomized, which showed in the results of the current study (19.83±1.49) are higher than in non-splenectomized patients (14.3±1.14) and increased sclerostin level with progress age-previous discussed. On the other hand, the non-splenectomized group in the present study revealed low BMI (15.69±0.57) kg/m2 in comparison of those in the splenectomized group (17.82 ± 0.61) kg/m^2, these compatible with Salih and Al-Mosawy (2013), which explained decreasing underweight frequency in splenectomized patients. Since the sclerostin level decrease in the underweight group that was previously discussed in the present study led to increasing the sclerostin level of the splenectomized group, the results may discuss as the fluctuations in serum sclerostin related to other biological changeability such as nutritional, physical activity, and other existence factors for individuals.

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

The correlation coefficient of sclerostin with other variables in beta-thalassemia patients showed in Table 2. Serum level of Sclerostin no correlated with RBC (r=0.094), PCV (r=0.031), and, Hb (R =0.054), respectively. While it is correlated positively significantly ($p<0.05$) with WBC (r=0.770) and PLT and (r=0.260), respectively. A positive significance ($p<0.05$) correlated between Sclerostin level with Serum Iron (r =0.513) and Ferritin levels (r =0.522). The current study results have found serum concentration of Sclerostin nonexistence of a relationship with RBC, PCV, and HB. These results presented a significantly positive correlation to WBC and PLT, iron, and ferritin level. Elevated sclerostin level in thalassemia patients is related to osteoporosis, which refers to an imbalance between bone cells, active osteoblasts, osteoclasts, and osteocytes (Voskaridou *et al.,* 2012). Inflammation influence of bone cells activity, the result of the activation of leukocytes is in patients with β-thalassemia stimulation of proinflammatory cytokines, thereby inducing osteocytes to release sclerostin (McLean, 2009). A recent study showed the development of osteoporosis and bone marrow density in β-thalassemia major directly correlated with iron overload, ferritin level, and liver iron concentration (Rossi *et al*., 2014). As the sclerostin is an important elemental in the progress of osteoporosis in these patients (Voskaridou *et al.,* 2012), the present study is educated that elevated serum sclerostin level is associated with a high level of iron. A previous study demonstrated that oxidative stress result from iron overload might be a necessary mechanism of the negative effects of aging on bone mass and strength (Manolagas, 2010). An increase in sclerostin level is possible due to ROS rise, which greatly impacts the generation and survival of bone cells, including osteoclasts, osteoblasts, and osteocytes, by high effects of oxidative stress on apoptosis osteoblast. Iron level indirectly affected the sclerostin level. Endocrinopathies are frequent in thalassemia patients due to the oxidative stress that causes the damage of these endocrine organs (Ardawi *et al.,* 2012; Garnero *et al*., 2013). Likely, iron overload reduced the synthesis and release of their hormones such as parathyroid and estrogen hormones by iron overload. These hormones act as sclerostin production antagonists, increasing sclerostin expression.

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.

5. DECLARATIONS

5.1. Study Limitations

No limitations were known at the time of the study.

5.2. Acknowledgements

The authors would like to express their gratitude to all participants of thalassemic patients

in the Thalassemia Center of Al–Zahraa Educational Hospital in Al–Najaf province, and the Department of Biology of the University Kufa for their assistance.

5.3. Funding source

The authors funded this research.

5.4. Competing Interests

The authors have declared that no competing interests exist.

5.5. Open Access

6. HUMAN AND ANIMAL-RELATED STUDIES

The work involves the use of the database of the thalassemia patients.

6.1. Ethical Approval

The Ethics Committee approved this study protocol of Faculty of Science, Kufa University at approval number 2014, and conducted in accordance with the Declaration of Helsinki.

6.2. Informed Consent

Informed consent was verbally obtained from the participants as well as from the parents of the minor participants.

7. REFERENCES:

1. **Galanello,** R., and Origa, R. (2010). Beta-thalassemia. Orphanet journal of rare diseases, 5(1), 1-15.
2. **Almousawi**, A. S., and Sharba, I. R. (2019). Erythroferrone Hormone, a Novel Biomarker, is associated with Anemia and Iron Overload in Beta-Thalassemia Patients. Journal of Physics: Conference Series, 1294, 062045.
3. **Delgado-Calle**, J., Sato, A. Y., and Bellido, T. (2017). Role and mechanism of action of sclerostin in bone. Bone, 96, 29-37.
4. **Sapunarova,** K., Goranova-Marinova, V., Georgiev, P., Deneva, T., Tsvetkova, S., & Grudeva-Popova, Z. (2020). Associations of serum sclerostin with bone mineral density, markers of bone metabolism, and thalassemia characteristics in adult patients with transfusion-dependent beta-thalassemia. Annals of medicine, 52(3-4), 94–108.
5. **Wehmeyer**, C., Frank, S., Beckmann, D., Böttcher, M., Cromme, C., König, U., Fennen, M; Held, A; Paruze, P; Hartmann, C; Stratis, A; and Dankbar, B. (2016). Sclerostin inhibition promotes TNF-dependent inflammatory joint destruction. Science translational medicine, 8(330), 330ra35-330ra35.
6. **Amrein** K, Amrein S, Drexler C, Dimai HP, Dobnig H, Pfeifer K, Tomaschitz A, Pieber TR, and Fahrleitner-Pammer A. (2012).Sclerostin and its association with physical activity, age, gender, body composition, and bone mineral content in healthy adults. J Clin Endocrinol Metab. 97(1):148–154.
7. **Ardawi** M. S., Rouzi A. A., Al-Sibiani S. A., Al-Senani N. S., Qari M. H., and Mousa SA. (2012). High serum sclerostin predicts the occurrence of osteoporotic fractures in postmenopausal women: The Center of Excellence for Osteoporosis Research Study. J Bone Miner Res. 27(12):2592–2602.
8. **Arshad** B, Iqbal T, Jami A and Hasan IJ. (2014). Hematological and Biochemical Changes in Male Patients with Thalassemia Major. Pak. j. life soc. Sci. 12(1): 31-35.
9. **Baldini M,** Forti S, Marcon A, Ulivieri FM, Orsatti A, Tampieri B, Airaghi L, Zanaboni L, and Cappellini MD**.** (2010). Endocrine and bone disease in appropriately treated adult patients with beta-thalassemia major. Annals of Hematology.89(12):1207-1213.
10. **Bellido** T, Saini V, and Pajevic PD. (2013). Effects of PTH on osteocyte

function. Bone 54 (2): 250–7.

11. **Center** for Disease Control (CDC). (2014). Body Mass Index: BMI for Children and Teens.

12. **Chen** D, Li Y, Zhou Z, Wu C, Xing Y, Zou Y, and Tian W. (2013). HIF-1α Inhibits Wnt Signaling Pathway by Activating Sost Expression in Osteoblasts. PLoS ONE. 8(6): e65940.

13. **Drake** MT, Srinivasan B, Modder UI, Peterson JM, McCready LK, Riggs BL, Dwyer D, Stolina M, Kostenuik P, and Khosla S. (2010). Effects of parathyroid hormone treatment on circulating sclerostin levels in postmenopausal women. J Clin Endocrinol Metab. 95(11):5056-5062.

14. **Easa** ZO. (2009). Complications of High Serum Ferritin Level after Splenectomy in â Thalassemic Patients. Kufa Med. Journal. 12(1):243-250.

15. **Eissa** DS and El-Gamal RA. (2014). Iron overload in transfusion-dependent β-thalassemia patients: defining parameters of comorbidities. Egypt J Haematol. 39(3):164-70.

16. **Garnero** P, Sornay-Rendu E, Munoz F, Borel O, and Chapurlat RD. (2013). Association of serum sclerostin with bone mineral density, bone turnover, steroid, and parathyroid hormones, and fracture risk in postmenopausal women: the OFELY study. Osteoporos Int. 24(2):489–494

17. **Ginzburg** Y. and Rivella S. (2012). β-thalassemia: a model for elucidating the dynamic regulation of ineffective erythropoiesis and iron metabolism. Blood. 118 (16): 4321-4330.

18. **Haidar** RK, Musallam M, and Taher AT. (2011). Bone disease and skeletal complications in patients with β- thalassemia major. Bone. 48(3): 425–432.

19. **Hashemieh** M, Azarkeivan A, Radfar M, Saneifard H, Hosseini-Zijoud SM, Noghabaei G, and Yaseri M. (2014). Prevalence of Osteoporosis among Thalassemia Patients from Zafar Adult Thalassemia Clinic, Iran. IRANIAN JOURNAL OF BLOOD AND CANCER (IJBC). 6(3): 143-148

20. **Ibrahim** HA, Fouda MI, Yahya RS, Abousamra NK, Abd Elazim RA (2013b). Erythrocyte Phosphatidylserine Exposure in β-Thalassemia. J Blood Lymph 4: 115.

21. **Klangjareonchai** T, Nimitphong H, Saetung S, Bhirommuang N, Samittarucksa R, Chanprasertyothin S, Sudatip R, and Ongphiphadhanakul B. (2014). Circulating sclerostin and irisin are related and interact with gender to influence adiposity in adults with prediabetes. Int J Endocrinol. 2014:261545.

22. **Kim** MK, Lee JW, Baek KH, Song KH, Kown HS, Oh KW, Jang EH, Kang M, and Lee KW. (2013). Endocrinopathies in transfusion-associated iron overload. Clinical Endocrinology. 78(2):271–277.

23. **Lapauw B,** Vandewalle S, Taes Y, Goemaere S, Zmierczak H, Collette J, and Kaufman JM. (2013). Serum Sclerostin Levels in Men with Idiopathic Osteoporosis. Eur Jn Endocrinol. 168(4):615-620.

24. **Lewiecki** EM. (2011). Sclerostin: a novel target for intervention in the treatment of osteoporosis. Discov Med. 12(65):263–273.

25. Manali Arora, Nayeemuddin SM, Ghatak S, Singh B. (2015). Growth Impairment and Dental Caries in Thalassemia Major Patients. Indian Journal of Clinical Anatomy and Physiology (IJCAP). 1(1): 15-21.

26. **Manolagas** SC. (2010). From estrogen-centric to aging and oxidative stress: a revised perspective of the pathogenesis of osteoporosis. Endocr. Rev. 31(3): 266–300.

27. **McLean** RR. (2009). Proinflammatory cytokines and osteoporosis. Curr Osteoporos. Rep. 7(4):134-139.

28. **Mödder** UI , Hoey KA , Amin S , McCready L K , Achenbach SJ , Riggs BL , Melton LJ , an d Khosla S. (2011). Relation of age, gender, and bone mass to circulating sclerostin levels in women and men. J Bone Miner Res 26(11):373–379.

29. **Moester** MJ, Papapoulos SE, Löwik CW, and van Bezooijen RL. (2010). Sclerostin: Current knowledge and future perspectives. Calcif Tissue Int 87(2): 99-107.

30. **Morse** LR, Sudhakar S, Danilack V, Tun C, Lazzari A, Gagnon DR, Garshick E, and

Battaglino RA. (2012). Association between sclerostin and bone density in chronic spinal cord injury. J Bone Miner Res. 27(2):352–359.

31. **Pasricha** SR, Frazer DM, Bowden DK, and Anderson GJ. (2013). Transfusion suppresses erythropoiesis and increases hepcidin in adult patients with β-thalassemia major: a longitudinal study. Blood. 122(1):124-133.

32. **Pelletier** S, Dubourg L, Carlier MC, Hadj-Aissa A, and Fouque D. (2013).The relation between renal function and serum sclerostin in adult patients with CKD. Clin J Am Soc Nephrol. 8(5):819–823.

33. **Persijn**, J.P., Clin. ACTA 35:91, (1971).phenotype of beta-thalassemia. Proc Natl Acad Sci USA105:1620–1625.

34. **Richards** WG, Ke HZ, Li X, and Ominsky MS. (2012). Sclerostin and Dickkopf-1 as therapeutic targets in bone diseases. Endocr Rev. 33(5):747–783.

35. **Rossi** F, Perrotta S, Bellini G, Luongo L, Tortora C, Siniscalco D, Francese M, Torella M, Nobili B, Di Marzo, and Maione S. (2014). Iron overload causes osteoporosis in thalassemia major patients through interaction with transient receptor potential vanilloid type1 TRPV1channels. Haematologica. 99(12):1876-84.

36. **Salih** KM and Al-Mosawy WF. (2013). Evaluation Some Consequences of Thalassemia Major in Splenectomized and Non-Splenectomized Iraqi Patients. Int J Pharm Pharm Sci, 5(4): 385-388.

37. **Shanthi** G, Balasubramanyam D, and Srinivasan R. (2013). Studies on the Haematological Aspects of Beta β–Thalassemia in Tamilnadu. RJPBCS. 4(3): 784-790.

38. **Sheng** Z, Tong D, Ou Y, Zhang H, Zhang Z, Li S, Zhou J, Zhang J, and Liao E. (2012). Serum sclerostin levels were positively correlated with fat mass and bone mineral density in Central South Chinese postmenopausal women. Clinical Endocrinology (Oxf). 76(6):797–801.

39. **Skordis** N. (2011). Endocrine investigation and follow-up in Thalassemia Time for specific guidelines. Thalassemia Reports. 1(s2):e22, 70-82.

40. **Taher** AT, Musallam KM, Cappellini MD, Weatherall DJ. (2011). Optimal management of beta-thalassemia intermedia. Br J Haematol. 152(5):512-23.

41. Taher AT, Porter JB, Viprakasit V, Kattamis A, Chuncharunee S, Sutcharitchan P, Siritanaratkul N, Galanello R, Karakas Z, Lawniczek T, Habr D, Ros J, Zhu Z, and Cappellini MD. (2012). Deferasirox continues to reduce iron overload in non-transfusion-dependent thalassemia: a one-year, open-label extension to a one-year, randomized, double-blind, placebo-controlled study. Blood. 120(5):970-977.

42. **Terpos** E, Christoulas D, Katodritou E, Bratengeier C, Gkotzamanidou M, Michalis E, Delimpasi S, Pouli1 A, Meletis J, Kastritis E, Zervas K and Dimopoulos M. (2012). Elevated circulating sclerostin correlates with advanced disease features and abnormal bone remodeling in symptomatic myeloma: reduction post-bortezomib monotherapy. Int J Cancer.131:1466–1471.

43. **Thein** SL. (2013). Molecular basis of β-thalassemia. Cold Spring Harb Perspect Med. 3(5):a011700.

44. **Urano** T, Shiraki M, Ouchi Y, and Inoue S. (2012). Association of circulating sclerostin levels with fat mass and metabolic disease-related markers in Japanese postmenopausal women. Journal of Clinical Endocrinology and Metabolism. 97(8): E1473–E1477.

45. **Voskaridou** E, Christoulas D, Plata E, Bratengeier C, Anastasilakis AD, Komninaka V, Kaliontzi D, Gkotzamanidou M, Polyzos SA, Dimopoulou M, and Terpos E. (2012). High circulating levels of sclerostin correlate with bone mineral density in patients with thalassemia and osteoporosis: the role of the Wnt signaling in the pathogenesis of bone loss in thalassemia. Horm Metab Res. 44(12):909-13.

46. **Wasmuth** AK. (2010). Evaluation of the Mythic 18, hematology analyzer for its use in dogs, cats, and horses. Inaugural-Dissertation. Faculty of Veterinary Medicine, University of Zurich.

47. **Wijenayaka** AR, Kogawa M, Lim HP, Bonewald LF, Findlay DM, and Atkins GJ. (2011). Sclerostin Stimulates Osteocyte Support of Osteoclast Activity by a RANKL-

Dependent Pathway. PLoS One 6:e25900.

48. **World** Health Organization (WHO). (2006). BMI Classification". Global Database on Body Mass Index.

49. **Yassin** M, Sirdah M, Al Haddad R, Lubbad AM, Al-Yazji M. (2013). Genotype-phenotype characteristics of β thalassemia children in the Gaza Strip, Palestine. J Genet Disor Genet. 2:2.

50. **Yeo** PS, Noor AM, Suzana S, and Roslee R. (2014). Bone Health Status among Thalassemia Children. International Journal of Public Health Research. 4(1):399-404.

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 ± 0.96	20.75 ± 1.86	
Group (8 – 18) n (%)	51(74%)		0.612
Group (19 – 29) n (%)	12(17.4%)		
Group (30 – 40) n (%)	6(8.7%)		
Major βT n (%)	54(78.2%)	0	NA
Intermedia n (%)	15(21.8%)	0	
Hb (mg/dl)	7.7 ± 0.14 *	12.01 ± 0.19	0.027
RBC (10^6/ml)	3.13 ± 0.07 *	4.36 ± 0.07	0.038
WBC (10^3/ml)	11.05 ± 0.26 *	7.59 ± 0.33	0.036
PCV %	23 ± 0.41 *	36.34± 0.52	0.036
PLT (10^3/ml)	361.86 ± 13.92 *	244 ± 14.23	0.034
BMI (kg/m^2)	17.59 ± 0.43 *	22.15 ± 0.36/	
Underweight, n (%)	13.09±0.52, 40(58%)	0	0.001
Normal weight, n (%)	23.7±2.04, 29(42%)	20(100%)	
Iron (µg/ml)	172.2 ± 4.4 *	29.86 ± 2.32	0.0001
Ferritin (ng /ml)	5156.5 ± 438.7 *	99.15 ± 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± standard division.

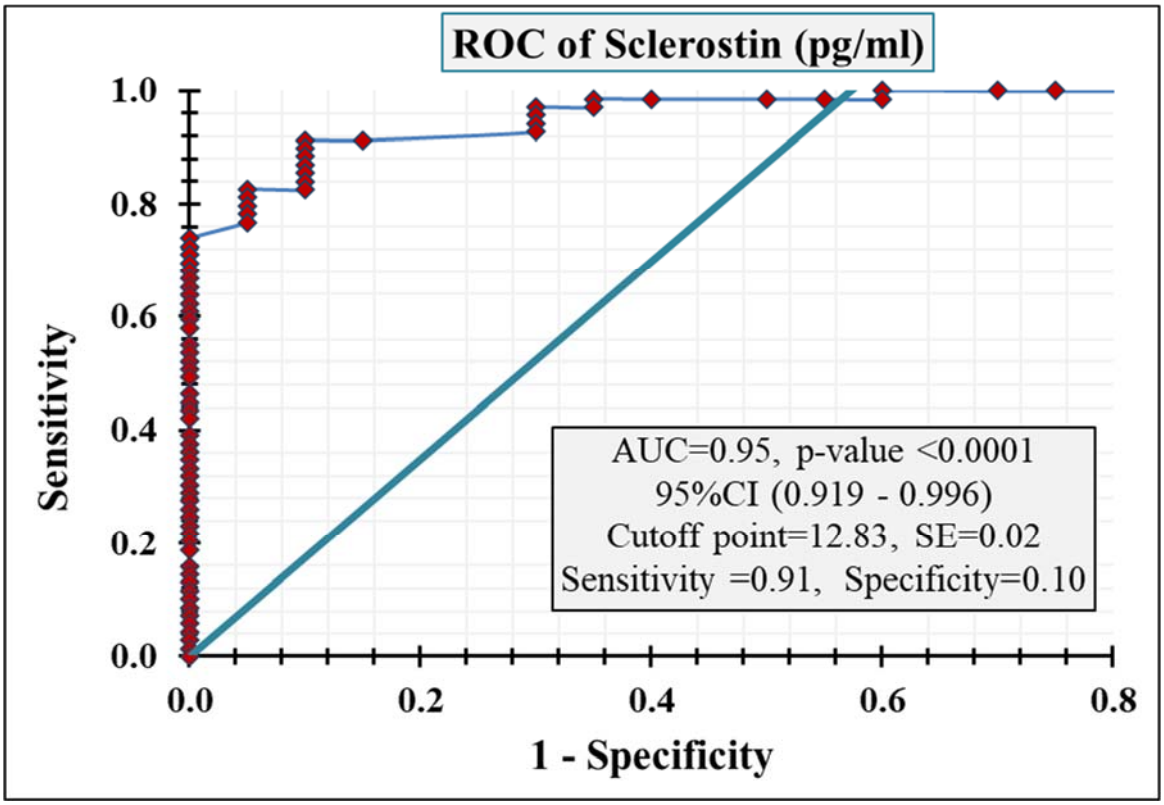

Figure 1. *ROC curves of sclerostin for beta-thalassemia patients. ROC: receiver operating characteristic; AUC: area under the curve*

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

.

Variables	Sclerostin (pg/ml)	
	P-value	R
RBC (10^6/ml)	0.440	0.094
PCV %	0.751	0.031
PLT (10^3/ml)	0.030	0.260 *
Hb (mg/dl)	0.661	0.054
WBC (10^3/ml)	0.001	0.770 **
Iron (µg/ml)	0.0006	0.513 **
Ferritin (µg/ml)	0.0005	0.522 **

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

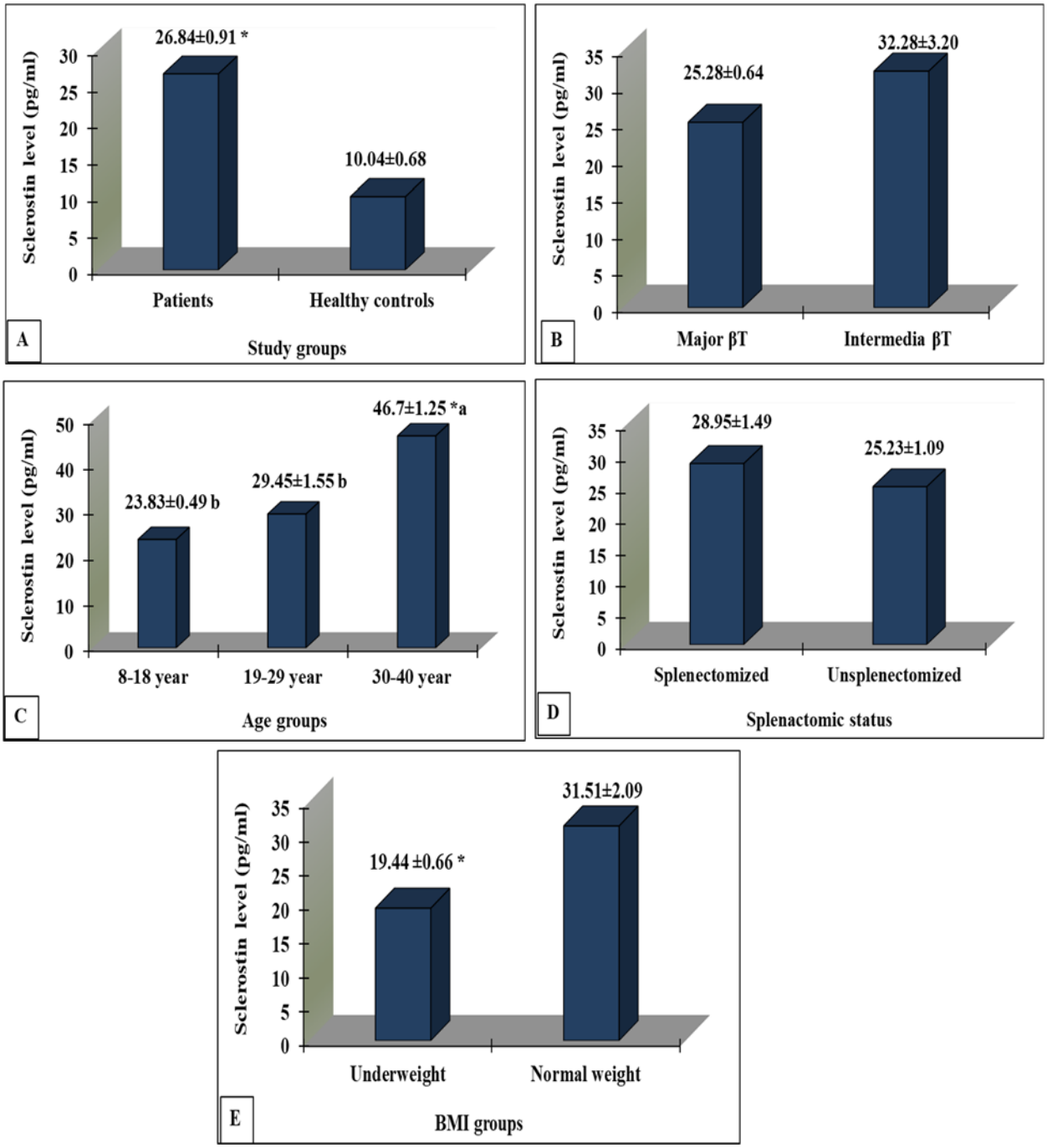

Figure 2. A: Comparison of Sclerostin levels in studied groups βT patients and healthy control groups. B: Effects of thalassemia types on Sclerostin levels in patients with βT major and βT intermedia. C: Effects of Age on Sclerostin levels among age groups. D: Effects of splenectomy status on Sclerostin 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 ±SD