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Evaluate the severity of bone impairment in different types of sickle cell anemia in Basrah province- Iraq

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Abstract---Sickle cell anemia (SCD) is one of the important health problems in Basrah,southern Iraq. which is probably the most common known hereditary blood disorder .patients with sickle cell disorders often suffer from chronic hemolytic anemia, which causes bone marrow hyperplasia too. The present study aimed to evaluate the severity of bone impairment in different types of sickle cell anemia in patients. Patients with SCA (n=120) 60 males and 60 females were on follow up in the Basra center for Hereditary Blood Disease, who were included in this study and age and sex matched healthy persons (n=60) as controls. biomarkers had important role in many biological processes. measure serum levels of different biomarkers such as; BCTx, BSAP, CRP, DPD, IGF-1, OT, TRACP-5b, FER, IGFBPT, Intact (iPTH), Ostase, PINP and serum Ca+. Using Kruskal-Wallis test as statistical test, markers in sickle cell anemia and control groups. The results showed were statistical significant differences among the groups of patients included in this study and control group for all the biomarkers that were measured (P=0.0001). Conclusion: The compound heterozygous (Hb SC Hb S/β-thal,) are less severe than the homozygous genotype (HbSS).

Keywords---sickle cell anemia, biomarkers, bone.

Introduction

SCA refers to all genotypes that contain at least one sickle gene, and HbS represents at least half of the present hemoglobin, A homozygous mutation for the S globin is homozygote hemoglobin of sickle (HbSS) when both globin genes are abnormal with insoluble and crystallizes at low oxygen partial pressure , causing RBCs to resemble sickles (Piel *et al.*, 2019; Hoffbrand *et al.*, 2019). And anotheris a heterozygous mutation linked to abnormal hemoglobin double heterozygote for hemoglobin such as hemoglobin C (Hb SC) and hemoglobin sickle- thalassemia (HbS-thal) including sickle β-thalassemia (Hb S/β-thal) , it is produced by sickle cell trait and β thalassemia trait There is no consensus about the classification of Hb S/ βThal, but it is usually classified in two types: Hb S/ β0Thal and Hb S/β+Thal. Hb S/ β0 Thal, which β globin production is zero, is often clinically indistinguishable from sickle cell anemia(SCA), Sickle cell β+ thalassemia, where β globin production is less than normal and the milder form is designated as Sickle cell/β+ thal (Zou *et al.,* 2021).

Bone is one of the most public clinical appearances of SCA and patients' bone skeletons are a main target of the disease's repercussions (Fakunle *et al.*, 2012; De Luna *et al.*, 2018). In excess of 30% of patients with sickle cell issues end up with complications of avascular necrosis of the bone (David *et al.*, 1993; Ballas *et al.*, 2012). Biomarkers of bone are very helpful in monitor osteoporosis treatment, which is a potential feature in evaluation of remodeling can be used to document the effects of therapeutic agents in some patients with sickle bone diseases represents a chronic and invalidating complication of SCD (Vanderhave *et al.*, 2018). More studies looking at early detection of osteoporosis are needed in patients with sickle cell disease, as well as targeted therapy to reduce bone complications and Improve disease outcome (Benenson and Porter, 2018).

Materials and Methods

This study was conducted during the period from January To February 2021. 180 Sickle cell anemia include 48 patients with HbSS, 22 with HbSC,50 patients with Sickle cell-β- thalassemia and registered at the center for genetic blood diseases at Basrah, their ages ranged from (5- More than 40 years), All of them were attending the CGBD for follow up, there were 60 healthy individuals as control group who were same sex and age range with patients. Five ml of venous blood were drawn from each patient in to jel tube and centrifuged at 3000 rpm for 15 minute to obtain serum, which was then transferred into Eppendorf tubes for biomarkers analysis. Serum BCTx, BSAP, CRP, DPD, IGF-1, OT, TRACP-5b, , IGFBPT, Intact (iPTH), PINP, and Ostase were assessed by enzyme immunoassay (ELISA) methods, while serum calcium and FER were measured using a chemistry immunoassay technique by a full automatic (American Hipro device). A consent was obtained from patients and parents before enrollment in the study. The work was also approved by the Collage of science , university .

Statistical Analysis

Data are stated as means ± standard deviation (SD). Differences between groups means were tested by t-test, chi-square statistical test. Correlations between

variables were also determined. All statistical analyses were performed using SPSS for Windows (version 23, USA), Kruskal-Wallis test used for difference between the group non-parametric data, was done . *P*-value ≤0.05 was considered statistically significant.

Result and Discussion

Table 1
Comparison of mean values of biomarkers among control group and patients groups (HbSS, HbSCand Hb sickle - β-Thalassemia)

	Category				* *P value*
	Control	HbSS	HbSc	Hb sickle - β-Thalassemia	
		Mean± SD Median(Min.-Max.)	Mean± SD Median(Min.-Max.)	Mean± SD Median(Min.-Max.)	
BCTx pg /ml.	52.44±2.43 52.60(44.90-58.60)	77.95±0.89 77.95(75.60-79.88)	79.04±1.25 78.81(76.80-81.88)	78.44±1.29 78.62(74.52-81.00)	0.0001
BSAP ng/ml.	10.20±0.15 10.22 (9.86-10.52)	4.80±0.35 4.87(4.31-5.29)	4.97±0.19 4.89(4.63-5.37)	4.92±0.27 4.88(4.23-5.41)	0.0001
C-RP ng/ml.	15.98±0.45 16.05(14.90-16.90)	42.40±1.28 42.30(40.50-45.60)	40.57±0.92 40.65(38.99-42.12)	41.07±0.81 41.15(38.90-42.30)	0.0001
DPD ng/ml.	5.51±0.61 5.4(5.0-9.9)	10.34±0.84 10.4(5.1-11.5)	9.95±0.56 9.99(9.10-11.02)	9.98±0.50 9.99(8.1-10.8)	0.0001
IGf-1 ng/ml.	324.89±2.9 324.3(320.8-331.5)	151.77±1.99 151.2(148.6-156.8)	152.14±1.5 151.95(149.7-155.1)	152.51±1.77 152.35(147.9-156.1)	0.0001
OT pg /ml.	21.71±0.51 21.60 (20.8-22.6)	14.76±1.02 15.0(12.2-16.8)	14.72±0.82 14.6(13.2-16.5)	13.33±0.8 13.5(11.9-15.2)	0.0001
(TRACP-5b) mIU/ml.	2.36±0.08 2.39 (2.19-2.51)	3.32±1.10 2.51(2.31-4.79)	4.98±0.16 5.00(4.52-5.21)	4.99±0.10 4.98(4.65-5.14)	0.0001
FER ng/ml.	95.64±0.78 95.60(93.6-97.3)	247.85±4.82 245.60(241.5-256.3)	246.43±5.51 245.20(238.6-255.9)	247.39±4.04 245.55(242.6-256.6)	0.0001
IGFBPT ng/ml.	3.47±0.14 3.49 (3.23-3.67)	1.52±0.03 1.52(1.42-1.56)	1.50±0.02 1.51(1.48-1.53)	1.51±0.03 1.51(1.43-1.57)	0.0001
Intact (iPTH) pg /ml.	4.44±0.18 4.50(4.11-4.85)	1.84±0.03 1.84(1.75-1.89)	1.81±0.03 1.81(1.74-1.85)	1.85±0.03 1.85(1.78-1.92)	0.0001
Ostase mg/L	12.07±0.24 12.11(11.23-12.56)	4.73±0.32 4.73(4.22-5.21)	5.07±0.12 5.08(4.88-5.31)	5.09±0.19 5.06(4.63-5.80)	0.0001
PINP pg /ml.	121.90±1.02 121.70(119.8-124.1)	192.09±25.47 195.55(19.6-200.1)	190.29±1.47 190.15(187.9-193.1)	191.53±1.62 191.20(187.6-195.6)	0.0001
Serum Ca^{+} mg/dL	2.53±0.38 2.46 (2.26-5.41)	1.87±0.05 1.87(1.69-1.96)	1.86±0.04 1.86(1.77-1.92)	1.88±0.03 1.88(1.82-1.94)	0.0001

* Kruskal-Wallis Test

As show in the (table 1) the data of our current study appeared that there were no statistically significant difference among the groups of patients included in this study and control group for all the biomarkers that were measured (BCTx, BSAP, CRP, DPD, IGF-1, OT, TRACP-5b, FER, IGFBPT, Intact (iPTH)), Ostease, PINP and serum Ca+) (P=0.0001). Different biomarkers are used and assessed to get the relation between their elevation and SCD such as; BCTx, BSAP, CRP, DPD, IGF-1, OT, TRACP-5b, FER, IGFBPT, Intact (iPTH), Ostase, PINP, and serum Ca+. Using Kruskal-Wallis test as statistical test, the data of our current study it appeared

that there was a statistically significant difference among the groups of patients included in this study and control group for all the biomarkers that were measured with P value =0.0001.It was observed in this present study that serum Ca^{+}, Ostease, iPTH, IGFBPT, OT, IGf-1 and BSAP were significantly lower in the patients group compared to the control group whereas, the values of PINP, BCTx, CRP, DPD, TRACP-5b and FER were significantly higher in the patients group compared to the control group. The non-significant difference in the proportion of the patients with the SCA to the control may be attributed to better health care facilities available for the management of the diseases and early detection of the conditions (Zijlstra *et al.*, 2021b). Bone specific alkaline phosphatase (BSAP) is one of the predictors of early bone fractures, especially in children .The significant low level of BSAP in the patients with SCA is indicative of possible complications of bone health in the patients. According to Manoj & Patro (2021), a lowered level of BSAP is associated with a higher incidence of fracture in the children with juvenile osteoporosis.

The lower level of Ca+ observed in the serum of the patients group compared with the control , Vitamin D deficiency and low level of parathyroid hormone are the main causes of the low level of Ca^{2+} , this study was agree with (Sultana & Akhter, 2018; Lahhob *etal.*, 2021). Also, (Adewoye *et al.,* 2008) who found impaired levels calcium and vitamin D in patients with SCD makes bone disease with osteoporosis and osteomalacia are common in sickle cell disease patients, so treatment of adult SCD with vitamin D and calcium can restore 25(OH)D levels to normal and improve bone disorders, Additionally liver and kidney disease related to SCD may negatively contribute to bone homeostasis, vitamin D protecting agent reduced the complication of liver fibrosis (Ali *et al.,* 2021), vitamin D deficiency has emerged as a public health concern due to its skeletal and extra-skeletal effects (Giustina *et al.,* 2020)**.** When clacium is low in the blood of patients group, they experience bone (Ferrè *et al.*, 2020). but in contrast the report of Halo Jr *et al.* (2020) who reported that serum electrolyte levels are usually significantly higher in SCA patients than the values of the normal individuals. Also, previous study by Mehta & Gupta (2016) concluded that there is increased level of calcium in SCA patients when compared to controls. differences between our study and previous studies may be explained by different locality, different number of studied patients, ages of studied patients, severity of disease and degree of iron overload (Rabab &Khalid.,2019).

The low level of PTH observed in the patients group in this study It is a general knowledge that metabolic activities of the bone as well as regulation of blood mineral levels are both regulated by the PTH along with the kidney and intestines. PTH has been linked to regulation of the ionized calcium in the serum thus, the hyper production of PTH leads to high concentration of the calcium in the blood and vice versa. Therefore, the low level of the iPTH in the patients under investigation compared with their control group may be responsible for the low level of minerals in their blood (Cao *et al.*, 2021a). According to Evrensel & Tarhan (2020), parathyroid hormone plays a critical role in mineral balancing in the blood especially in the calcium and phosphorus homeostasis, which is carried out by their effect on special target organs including bone, intestine and kidney, which is a precursor of normal bone formation and mineralization, as well as normal physiological concentrations of plasma calcium and phosphorus.

Changes in PTH level usually leads to disturbance in the calcium homeostasis, which may result in metabolic bone disorders which explains the inherent physiological changes of the skeletal system in patients suffering from SCA, This result agrees with (Hirama & Sugimoto., 2018),They found that serum calcium and parathyroid hormone were lower level in SCP . The observed higher level of FER in the blood of patients group compared with the control group is indicative of iron overload in the patients . The level of Serum FER in the blood changes in patients with endocrine disease especially hypothyroidism, and Thus the effect is reversed on HB (Khairallah *et al.*, 2022).). Previous reports indicated that an elevated iron level (iron overload) occurs when a patient receives a blood transfusion. An elevated iron level may also be due to high level of hemolysis, iron supplementation or chelating agents although, many factors such as hepatitis and inflammation in sickle cell patients are likely to contribute to an elevated iron level. This position is supported by previous research reports (Evrensel & Tarhan, 2020 ; Zhou *et al.*, 2021).

According to Shi *et al.* (2020), a high level of C-reactive protein (CRP) has been described as a major protein (marker) used to detect inflammation both acute and chronic. In sickle cell disease patients, earlier reports suggest CRP levels are usually elevated in sickle cell disease patients. These support the finding obtained in this study. The higher level of the CRP in the sickle cell patients may be linked with chronic inflammation or hypothalamus-pituitary-adrenal axis dysfunction . Mehta & Gupta (2019) concluded that hs-CRP was the most significant correlate of hospitalizations for painful episodes in SCD patients across different age groups. Our study showed higher levels of tartrate-resistant acid phosphatase 5b (TRACP 5b) than control group. Furthermore, high TRACP 5b levels were associated with severe VOC (Nouraie *et al.,* 2011) This agree with other study conducted by (Faienza *et al* (2015) ,TRACP 5b levels were significantly higher in SCD adult patients than controls, regardless of age, sex, body mass index, and disease severity, and they strongly correlated with alkaline phosphatase. , iron overload may cause osteoporosis by decreasing osteoblast function and increasing the TRACP 5b gene (Liberati *et al.,* 2009). The result show that DPD levels were higher in patients with SCA when compared to control group. This agreement with other studies conducted by (Bolarin *et al.* 2010; Nolan *et al.,* 2015). Also this study was incompatible with Fung *et al.*(2008) who reported higher levels of OT and BSAP, and lower levels of DPD in SCA children. There was no significant difference in mean OT in both the control and patient groups. The value of OT as biomarker of bone matrix formation is unclear because fluctuations in circulating may only reflect changes in the equilibrium between bone matrix and bone. (Duggan,2001).

The current study has shown that there was a decrease in the levels of IGf-1 and IGFBPT in patients with SCA compared to control group. This result agrees with Luporini *et al.* (2001); Bennett. (2011); Mandese *et al.* (2019) and Al-Hejaj *et al.* (2021) they found that serum levels of IGf-1 and IGFBPT are lowered in patients with SCA might be due to hydroxic vascular insults to their hypothalamic pituitary during sickling episodes. Also, the severity of the disease microcirculation disorder the affection of GH-IGF-1 , IGFBPT(Bennett , 2011). The same results were reported by (Fanestil and Van Siclen 2015) who found that there is no difference between the case results of hemoglobin electrophoresis

results in patients with HbSS and Hb sickle- β-thalassemia “75% to 90%” when compared to results of control group.

Table 2
Comparison meam values of biomarkers between Case groups (HbSS + HbSC)

Biomarkers	Category		* *P value*
	HbSS	HbSC	
	Mean± SD Median(Min.-Max.)	Mean± SD Median(Min.-Max.)	
BCTx pg /ml.	77.95±0.89 77.95(75.60-79.88)	79.04±1.25 78.81(76.80-81.88)	0.001
BSAP ng/ml.	4.80±0.35 4.87(4.31-5.29)	4.97±0.19 4.89(4.63-5.37)	0.135
C-RP ng/ml.	42.40±1.28 42.30(40.50-45.60)	40.57±0.92 40.65(38.99-42.12)	0.0001
DPD ng/ml.	10.34±0.84 10.4(5.1-11.5)	9.95±0.56 9.99(9.10-11.02)	0.001
IGf-1 ng/ml.	151.77±1.99 151.2(148.6-156.8)	152.14±1.5 151.95(149.7-155.1)	0.158
OT pg /ml.	14.76±1.02 15.0(12.2-16.8)	14.72±0.82 14.6(13.2-16.5)	0.436
(TRACP-5b) mIU/ml.	3.32±1.10 2.51(2.31-4.79)	4.98±0.16 5.00(4.52-5.21)	0.0001
FER ng/ml.	247.85±4.82 245.60(241.5-256.3)	246.43±5.51 245.20(238.6-255.9)	0.293
IGFBPT ng/ml.	1.52±0.03 1.52(1.42-1.56)	1.50±0.02 1.51(1.48-1.53)	0.020
Intact (iPTH) pg /ml.	1.84±0.03 1.84(1.75-1.89)	1.81±0.03 1.81(1.74-1.85)	0.001
Ostase mg/L	4.73±0.32 4.73(4.22-5.21)	5.07±0.12 5.08(4.88-5.31)	0.0001
PINP pg /ml.	192.09±25.47 195.55(19.6-200.1)	190.29±1.47 190.15(187.9-193.1)	0.0001
Serum Ca^{+} mg/dL	1.87±0.05 1.87(1.69-1.96)	1.86±0.04 1.86(1.77-1.92)	0.558

* Kruskal-Wallis Test

However, The results of this study as in the table (2) showed a significant difference between patients groups (HbSS and HbSC) p-value (0.001,0.001, 0.001, 0.0001, 0.0001, 0.02, 0.001 and 0.0001) an biomarkers (BCTx, CRP, DPD, TRACP-5b, IGFBT, Intact (iPTH), Ostase and PINP) respectively, while the study did not show any significant Statistical difference between the two groups above with regard of biomarkers (BASAP, IGF-1, OT, FER and Serum Ca+), P-value 0.135, 00436, 0.293 and 0.558) respectively. Belonging to these biomarkers, the main two genotypes are being compared, however, the results of this study showed a significant difference between patients’ groups (HbSS and HbSC) p-value (0.001,0.001, 0.001, 0.0001, 0.0001, 0.02, 0.001 and 0.0001) for biomarkers (BCTx, CRP, DPD, TRACP-5b, IGFBT, Intact (iPTH), Ostase and PINP) respectively, while the study did not show any significant statistical difference between the two groups above with regard of biomarkers (BSAP, IGF-1, OT, FER

and Serum Ca+) P-value 0.135, 00436, 0.293 and 0.558) respectively. The non-significant differences in the level of the biomarkers assessed in this study among the various sickle cell disorder patients strongly suggests that the production of these biomarkers are uniform in the sickle cell disease condition irrespective of the type of sickle disorder. Moreover, the biomarkers which are non-significantly different in the disease groups may be major common denominators in the early detection and management of the disease. However, those which are significantly different in the patient may be due to individual response to various environmental conditions as well as treatment regimens they may have been exposed to. That was in agreement with (Gualandro *et al.,* 2015) who found that HbSC disease are driven by different aspects of blood abnormalities and variations in different studied biomarkers.

Table 3
Comparison mean values of biomarkers between Case groups (HbSS + Hb sickle - β-Thalassemia)

Biomarkers	Category		*P value**
	HbSS	Hb sickle - β-Thalassemia	
	Mean± SD Median(Min.-Max.)	Mean± SD Median(Min.-Max.)	
BCTx pg /ml.	77.95±0.89 77.95(75.60-79.88)	78.44±1.29 78.62(74.52-81.00)	0.002
BSAP ng/ml.	4.80±0.35 4.87(4.31-5.29)	4.92±0.27 4.88(4.23-5.41)	0.080
C-RP ng/ml.	42.40±1.28 42.30(40.50-45.60)	41.07±0.81 41.15(38.90-42.30)	0.0001
DPD ng/ml.	10.34±0.84 10.4(5.1-11.5)	9.98±0.50 9.99(8.1-10.8)	0.0001
IGf-1 ng/ml.	151.77±1.99 151.2(148.6-156.8)	152.51±1.77 152.35(147.9-156.1)	0.015
OT pg /ml.	14.76±1.02 15.0(12.2-16.8)	13.33±0.8 13.5(11.9-15.2)	0.436
(TRACP-5b) mIU/ml.	3.32±1.10 2.51(2.31-4.79)	4.99±0.10 4.98(4.65-5.14)	0.0001
FER ng/ml.	247.85±4.82 245.60(241.5-256.3)	247.39±4.04 245.55(242.6-256.6)	0.534
IGFBPT ng/ml.	1.52±0.03 1.52(1.42-1.56)	1.51±0.03 1.51(1.43-1.57)	0.283
Intact (iPTH) pg /ml.	1.84±0.03 1.84(1.75-1.89)	1.85±0.03 1.85(1.78-1.92)	0.055
Ostase mg/L	4.73±0.32 4.73(4.22-5.21)	5.09±0.19 5.06(4.63-5.80)	0.0001
PINP pg /ml.	192.09±25.47 195.55(19.6-200.1)	191.53±1.62 191.20(187.6-195.6)	0.0001
Serum Ca^{+} mg/dL	1.87±0.05 1.87(1.69-1.96)	1.88±0.03 1.88(1.82-1.94)	0.162

* Kruskal-Wallis Test

Regarding, comparison between patient's case groups (HbSS and sickle-- **β-** Thalassemia), Our results in this study showed significant statistically difference p-value (0.002, 0.0001, 0.0001, 0.015, 0.0001, 0.0001, 0.001 and 0.0001) on

biomarkers (BCTx, CRP, DPD, IGF-1, TRACP-5b, Ostase, and PINP) respectively. While weakly significant difference between above groups regarding Intact (iPTH) (P=0.055) conversely, this study was illustrate non-significant difference with biomarkers (BSAP, FER, IGFBPT and serum Ca+, p-value (0.080, 0.53, , 0.283, 0.055 and 0.162) respectively. When add Hb sickle - β-Thalassemia genotype in the comparison, the results in this study showed significant statistically difference P-value (0.002, 0.0001, 0.0001, 0.015, 0.0001, 0.0001, 0.001 and 0.0001) on biomarkers (BCTx, CRP, DPD, IGF-1, TRACP-5b, Ostase, and PINP) respectively.

While weakly significant difference between above groups regarding Intact (iPTH) (P=0.055) conversely, this study was illustrating non-significant difference with biomarkers (BSAP, FER, IGFBPT and serum Ca+, P-value (0.080, 0.53, 0.283, 0.055 and 0.162) respectively This could be because Hb sickle - β-Thalassemia is a disorder caused by the co-existence of a sickle cell and a beta-thalassemia gene. It's the most important and severe type lead to severe transfusion dependent anemia if untreated properly (Adnan *etal*,2016), The clinical phenotype is determined by the type of beta-thalassemia gene that is inherited (beta (+) or beta (o). Typically, a definite diagnosis is essential for early supportive treatment and determining the later clinical course in patients with homozygous sickle cell disease (SS illness). Additionally, thalassemia is often identified through family history, complete blood count, and testing of hemoglobin A2 and hemoglobin F levels. Clinical manifestations of Hb sickle - β-Thalassemia are quite diverse, ranging from totally asymptomatic condition to a severe disorder similar to homozygous sickle cell disease, depending on how each gene is inherited.

This heterogeneity is most likely owing to the presence of multiple - thalassemia alleles or interaction with genetic factors that modulate the disease. The results agree with (Mathias *et al.* 2010) the found C-reactive protein, and fibrinogen were increased in patients with the SS genotype, (VOC) due to microvascular occlusion, which leads to increased inflammation and tissue ischemic damage (Ballas *etal.* 2010). FER level showed non-significant statically behavior can be due to the fact that all Patients with SCA suffer from a chronic hemolytic state and the iron produced by the destruction of red blood cells is usually stored in the body. The results matches with the previous studies (Odunlade *etal.*, 2019; Lahhob *etal.*, 2020b,). Shi *et al.* (2020) reported that children with phenotype, HbSS and HbS-β-Thal have significantly higher hs-CRP levels than those with HbSC disease

Table 4
Comparison mean values of biomarkers between Case groups (HbSC and Hb sickle- β- Thalassemia)

Biomarkers	Category		* P value
	HbSC	Hb sickle- β- Thalassemia	
	Mean± SD Median(Min.-Max.)	Mean± SD Median(Min.-Max.)	
BCTx pg /ml.	79.04±1.25 78.81(76.80-81.88)	78.44±1.29 78.62(74.52-81.00)	0.204
BSAP ng/ml.	4.97±0.19 4.89(4.63-5.37)	4.92±0.27 4.88(4.23-5.41)	0.437

C-RP ng/ml.	40.57±0.92 40.65(38.99-42.12)	41.07±0.81 41.15(38.90-42.30)	0.039
DPD ng/ml.	9.95±0.56 9.99(9.10-11.02)	9.98±0.50 9.99(8.1-10.8)	0.736
IGf-1 ng/ml.	152.14±1.5 151.95(149.7-155.1)	152.51±1.77 152.35(147.9-156.1)	0.426
OT pg /ml.	14.72±0.82 14.6(13.2-16.5)	13.33±0.8 13.5(11.9-15.2)	0.0001
(TRACP-5b) mIU/ml.	4.98±0.16 5.00(4.52-5.21)	4.99±0.10 4.98(4.65-5.14)	0.581
FER ng/ml.	246.43±5.51 245.20(238.6-255.9)	247.39±4.04 245.55(242.6-256.6)	0.250
IGFBPT ng/ml.	1.50±0.02 1.51(1.48-1.53)	1.51±0.03 1.51(1.43-1.57)	0.236
Intact (iPTH) pg /ml.	1.81±0.03 1.81(1.74-1.85)	1.85±0.03 1.85(1.78-1.92)	0.0001
Ostase mg/L	5.07±0.12 5.08(4.88-5.31)	5.09±0.19 5.06(4.63-5.80)	0.807
PINP pg /ml.	190.29±1.47 190.15(187.9-193.1)	191.53±1.62 191.20(187.6-195.6)	0.006
Serum Ca^{+} mg/dL	1.86±0.04 1.86(1.77-1.92)	1.88±0.03 1.88(1.82-1.94)	0.079

* Kruskal-Wallis Test

Regarding, comparison between patients case groups(HbSC and sickle- **β-** Thalassemia), our results in this study showed significant statistically difference p-value (0.039, 0.0001, 0.0001, 0.0001 and 0.006) on biomarkers (CRP, OT, Intact (iPTH)and PINP), while this study was illustrate non-significant statistically difference p-value (0.0204, 0.437, 0.730, 0.420, 0.581, 0.250, 0.236, 0.807 and 0.079) respectively. (BCTx, BSAP,DPD, IGF-1, TRAP, FER, IGFBT, Ostase, and serum Ca+) respectively. Previous study reported, These are common factors to both SC and and sickle- **β-** Thalassemia may explain why some biomarkers were found in similar proportions in the blood of the patients group (Udezue and Girshab 2004;Broucek, 2021) The non-significant difference in the proportion of the patients with the hemoglobin disorders to the control may be attributed to better health care facilities available for the management of the diseases and early detection of the conditions .

Conclusion

SCA is characterized by a broad variation of abnormal levels of biomarkers that have been identified and associated with different pathological conditions. It was concluded, The increase and decrease in the level of bone biomarkers lead to the imbalance of bone formation and resorption cells, as well as the low level of calcium and PTH, causing impairment and bone disease among patients group .In addition to following up many biomarkers as their elevation may have a great role in SCD deterioration, The genotypes HbSS, HbSC, and Hb sickle- β- Thalassemia are contributing to SBD progression according to multiple factor observed in this study as hemolysis iron overload ,ischemic damage, low calcium , PTH level and vit .D deficiency in which there was no significant difference in the proportion of

the patients with these genotypes. Finally ,the compound heterozygous(Hb SC Hb S/β-thal) are less severe than the homozygous genotype (HbSS) according to biomarkers, which showed a strong significant difference between the control and the patient groups with each other (BSAP, CRP, DPD, IGF-1 and Ostase).

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