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Evaluate the biomarkers of bone turnover in patients with in sickle cell anemia in Basra province

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***Abstract*---**Background: Sickle cell anemia (SCD) is one of many haemoglobinopathic disorders which is probably the most common known hereditary blood disorder. In this disease, the homozygous state in which the sickle gene is inherited from the father and the mother, and patients with sickle cell disorders often suffer from chronic hemolytic anemia, which causes bone marrow hyperplasia too. The present study was designed to evaluate the biomarkers which related with the bone turnover in sickle cell anemia 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. Aims: of this study to 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 no significant difference of both male and female patients (HbSS, HbSC and sickle- β-Thalassemia) in compared to control group. Conclusion: The study showed all the biomarkers that were significantly different in their concentration in both sex were higher in female patients than the males except FER which was higher in male.

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

Introduction

Sickle cell anemia (SCA) is the most widely form of SCD, SCA represents important health problem because of it is characterized by a high rate of morbidity and mortality, recurrence of crises , organ systems affected and no ideal long-term treatment (Romero , 2013; Ravikanth et al., 2017). Bone is the one of the most public clinical appearances of SCA and patients' bone skeletons are a main targets of the disease's repercussions (Fakunle *et al.*, 2012; Eaton 2020). 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). Complications of sickle cell disease include bone marrow necrosis, bone infarcts, osteomyelitis, stress fractures, vertebral collapse (Ballas, 2001; Leonard *et al.*, 2021), And fat embolism syndrome (FES) is another severs complication that occurs after lengthy bone fractures and surgery is characterized by respiratory failure, skin rashes, and thrombocytopenia (Ataga *et al.*, 2012). It is due to extensive bone marrow necrosis, and is associated with high mortality rates and severe neurological sequelae. (Tsitsikas *et al.*, 2020)

Various biomarkers of bone turnover remodeling which include bone formation such as bone-specific alkaline phosphatase, osteocalcin, and others whose roles are to stimulate the deposition of new bone and bone resorption markers are involved in bone remodeling N-terminal propeptide of type 1 procollagen (P1NP), cross-linked N-terminal propeptide of type 1 procollagen. They provide clinical evidence useful of the normal and pathological processes that reflect bone cell activities in the skeleton. 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 comprises of 180 Sickle cell anemia include 48 patients with HbSS , 22 with HbSC,50 patients with Sickle cell-β- thalassemia and 60 healthy individuals as control group who same sex and age range with patients. Intravenous blood was collected and centrifuged at 3000 rpm for separation of serum to perform biomarkers**.** Serum BCTx, BSAP, CRP, DPD, IGF-1, OT, TRACP-5b, , IGFBPT, Intact (iPTH), PINP, and Ostase were assessed using immunologic ELISA methods, while serum calcium and FER were measured using chemistry immunoassay technique by a full automatic (American Hipro device).

Statistical Analysis

Data are stated as means ± standard deviation (SD). Differences between groups means were tested by t-test, chi-square test. Correlations between variables were also determined. All statistical analyses were performed using SPSS for Windows

(version 23, USA), Non-parametric Kruskal-Wallis test was done. *P*-value ≤0.05 was considered statistically significant.

Result and Discussion

Table 1
Distribution of cases groups (HbSS, HbSCand Hb sickle- β-thalassemia) compared to control group according to age

Age group (years)	Category				Total No. (%)	*P-value*
	Control No. (%)	HbSS No. (%)	HbSC No. (%)	Hb sickle- β-Thalassemia No. (%)		
5-10	16 (26.7%)	12 (25.0%)	7 (31.8%)	14 (28.0%)	49 (27.2%)	0.99
11-20	12 (20.0%)	10(20.8%)	5 (22.7%)	8 (16.0%)	35 (19.4%)	
21-30	12 (20.0%)	11 (22.9%)	5 (22.7%)	8 (16.0%)	36 (20.0%)	
31-40	10 (16.7%)	8 (16.7%)	3 (13.6%)	11 (22.0%)	32 (17.8%)	
More than 40	10 (16.7%)	7 (14.6%)	2 (9.1%)	9 (18.0%)	28 (15.6%)	
Total	60(100.0%)	48 (100.0%)	22(100.0%)	50(100.0%)	180(100.0%)	

* Chi^2 Test

The results of the our study was showed no statistically significant difference. P-vale (0.99) in age groups of patients (HbSS, HbSC and Hb- β- sickle- β-thalassemia), in comparison to control group table (1). HbSS, HbSC, and Hb sickle- β-thalassemia are three genotypes found in SCD patients which were detected by electrophoresis (Da Guarda *et al.,* 2020), firstly, when compare the various genotypes of SCD such as "HbSS, HbSC, and Hb sickle- β-thalassemia" distribution between total group cases and the control of this study from different age groups starting from 5 years old to 40 years old and more with 10 years intervals, using Chi^2 statistical test, it showed that there is no statistical significant difference in all age groups of patients (P value = 0.99; statistical significance when P value ≤0.05) in comparison to control group, is an indication that the blood cell disorders are evenly distributed and age has no influenced role in the results in the studied population ,This finding supports the concept of statistical conformity, so this study will be free of difference in results due to age and sex (Schleicher *et al.*, 2020;). 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
Distribution of cases groups (HbSS, HbSc and Hb sickle - β-thalassemia) compared to control group according to Age group and male sex

Gender			Category				Total No. (%)	*P-value
			Control No. (%)	HbSS No. (%)	HbSc No. (%)	Hb sickle - β-Thalassemia No. (%)		
Male	Age grup	5-10	8 (26.7%)	7 (25.0%)	4 (33.3%)	9 (45.0%)	28 (31.1%)	0.99
		11-20	6 (20.0%)	6 (21.4%)	3 (25.0%)	4 (20.0%)	19 (21.1%)	
		21-30	6 (20.0%)	7 (25.0%)	3 (25.0%)	3 (15.0%)	19 (21.1%)	
		31-40	5 (16.7%)	4 (14.3%)	1 (8.3%)	2 (10.0%)	12 (13.3%)	
		> 40	5 (16.7%)	4 (14.3%)	1 (8.3%)	2 (10.0%)	12 (13.3%)	
	Total		30 (100.0%)	28 (100.0%)	12 (100.0%)	20 (100.0%)	90 (100.0%)	

* Chi^2 Test

With regard to age and sex the data of the present study showed no significant difference of both male and female patients p-value (0.99). (Hbss, Hbsc and sickle- **β-**Thalassemia) in compared to control group (table 2, 3). When merge the male sex with age group and compare the results of presence these genotypes in case and control groups, shall find that data of the present study showed no statistically significant difference of male patients p-value (0.99) (Hbss, Hbsc and sickle-Thalassemia) when compared to control group to reveal that male sex has no additive effect on the genotype prevalence. The results of this study were agree with previous studies (Schleicher *et al.*, 2020; Broucek, 2021).

Table 3
Distribution of cases groups (HbSS, HbSc and Hb sickle - β-thalassemia) compared to control group according to age and female sex

Sex			Category				Total No. (%)	*P-value
			Control No. (%)	HbSS No. (%)	HbSc No. (%)	Hb sickle- β-Thalassemia No. (%)		
female	Age group	5-10	8 (26.7%)	5 (25.0%)	3 (30.0%)	5 (16.7%)	28 (31.1%)	0.99
		11-20	6 (20.0%)	4 (20.0%)	2 (20.0%)	4 (13.5%)	19 (21.1%)	
		21-30	6 (20.0%)	4 (20.0%)	2 (20.0%)	5 (16.7%)	19 (21.1%)	
		31-40	5 (16.7%)	4 (20.0%)	2 (20.0%)	9 (30.0%)	12 (13.3%)	
		> 40	5 (16.7%)	3 (15.0%)	1 (10.0%)	7 (23.3%)	12 (13.3%)	
	Total		30 (100.0%)	20 (100.0%)	10 (100.0%)	30 (100.0%)	90 (100.0%)	

* Chi^2 Test

Also, data of the present study showed no statistical significant difference of female patients p-value (0.99) (HbSS, HbSC and sickle-- **β-** Thalassemia) when compared to control group to reveal that female sex has no any additive effect on the genotype prevalence, which is in agreement with the theory which said that the incidence of SCD is not strictly gender-related as it is transmitted as an autosomal recessive disorder and all preliminary observations point to the need for further studies into gender differences in pain crisis in patients with SCD

(Udezue and Girshab 2004).But this data were in contrast to (Ceglie *et al.,* 2019) study who studied on total of 89 cases (29 in females, 60 in males) who found groups (p = 0.04). Also , found that sex hormones were recognized as responsible for gender differences in adult patients with SCD, but in the prepuberty setting of childhood their role could be less relevant in the pathogenesis of gender differences in the pediatric population.

Table 4
Distribution of cases groups (HbSS, HbSc and Hb sickle-- β-thalassemia) compared to control group according to sex

Sex	Category				Total No. (%)	*P-value.
	Control No. (%)	HbSS No. (%)	HbSc No. (%)	Hb sickle- β-Thalassemia No. (%)		
Male	30 (50.0%)	28 (58.3%)	12 (54.5%)	20 (40.0%)	90 (50.0%)	0.32
Female	30 (50.0%)	20 (41.7%)	10 (45.5%)	30 (60.0%)	90 (50.0%)	
Total	60(100.0%)	48(100.0%)	22 (100.0%)	50(100.0%)	180(100.0%)	

* Chi^2 Test

While the results of this study as shows in table (4), no significant difference (P value= 0.32). of cases groups (HbSS, HbSC and Hb sickle-Thalassemia) in compared to control group. When neglect the age group impaction, will find that no significant difference of sex effect (with P value= 0.32) of all case groups (HbSS, HbSC and sickle-**- β-** Thalassemia) when compared to control group which is also discussed giving the same results in (Ceglie *et al.,* 2019) study who found that incidence of SCD is not strictly sex-related. In particular, the sex-related differences in pediatric SCD are not well-characterized. To address this state, they analyzed the clinical records of 39 pediatric patients retrospectively with a SCD diagnosis (hemoglobin SS genotype) focusing on sex differences analyzing many aspects of the disorder and comprising both acute symptoms and late complications.

There was no significant difference in the prevalence of the haemoglobin disorders in both male and females. This suggests that the distribution of the haemoglobin disorder is not sex-influenced. It has been reported that incidence of any haemoglobin related disorder is neither sex-linked nor sex related because the transmission of the trait is of autosomal origin being a recessive autosomal disorder. Nevertheless, some researchers have reported sex influenced differences in sickle cell diseases related deaths as well as morbidity. Narkhova (2021) reported a higher death rate among adult males compared to the females and this has been attributed to the presence of more episodes of crises in males than in females. This is however in contrast to the observations in this study as there was no significant difference in the distribution of the hemoglobin disorder among the adults of both sex.

Table 5
Comparison of mean values of the studied biomarkers according to sex.

Biomarkers	Sex		*P value*
	Male	Female	
	Mean± SD Median(Min.-Max.)	Mean± SD Median(Min.-Max.)	
BCTx pg /ml.	69.59±12.33 77.71(48.60-79.88)	69.84±12.47 77.52 (44.90-81.88)	0.273
BSAP ng/ml.	6.59±2.57 4.97(4.23-10.49)	6.72±2.49 5.25 (4.39-10.52)	0.035
C-RP ng/ml.	33.13±12.30 40.31(14.90-45.60)	32.88±11.99 40.80 (15.10-42.50)	0.503
DPD ng/ml.	8.48±2.25 9.60(5.10-10.90)	8.70±2.32 10.10 (5.00-11.50)	0.228
IGf-1 ng/ml.	209.18±82.24 152.35(147.90-331.50)	210.28±81.59 154.25 (150.20-330.60)	0.006
OT pg /ml.	16.84±3.53 14.90(12.50-22.60)	16.51±3.87 15.20 (11.90-22.60)	0.185
(TRACP-5b) mIU/ml.	3.31±1.26 2.43 (2.20-5.17)	4.02±1.19 4.63 (2.19-5.21)	0.015
FER ng/ml.	198.83±73.52 245.55 (93.80-256.60)	194.79±70.47 243.20 (93.60-248.10)	0.0001
IGFBPT ng/ml.	2.17±0.93 245.55 (93.80-256.60)	2.16±0.93 1.52 (1.43-3.66)	0.530
Intact (iPTH) pg /ml.	2.71±1.25 1.86 (1.78-4.85)	2.69±1.23 1.87 (1.74-4.68)	0.960
Ostase mg/L	7.19±3.47 4.99 (4.22-12.34)	7.44±3.30 5.23 (4.69-12.56)	0.0001
PINP pg /ml.	167.08±37.04 190.20 (19.60-198.20)	169.55±33.91 191.10 (120.20-200.10)	0.304
Serum Ca^{+} mg/dL	2.10±0.45 1.89 (1.69-5.41)	2.10±0.45 1.91 (1.78-2.74)	0.337

* Kruskal-Wallis Test

The results of the current study as shown in the (table 5), according to sex was appeared strong significant difference (p=0.0001) regarding both of (FER and Ostease). Moreover, the results showed statistically significant variation (p=0.035, p=0.006, p=0.015) between male and female of the following biomarkers (BSAP, IGF-1 and TRACP-5b) respectively. While there are non-significant difference (p=0.273, p=0.503, p=0.0228, p=0.185, p=0.530, p=0.960, p=0.304 and p=0.327)

of the (BCTx, CRP, DPD, OT, GFBPT, Intact (iPTH), PINP and serum Ca+) respectively. Different biomarkers are used and assessed to get the relation between their elevation and SCA 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, All the biomarkers that were significantly different in their concentration in both sex were higher in female patients than the males except FER which was higher in males. This is an indication that they may be possibly applied in female patients than that of male. Blood ferritin level in relation to sickle cell disease patients has been analysed by different researchers and there have been no report of significant difference in the level found in both sex. However, more iron have been found in the serum of younger children with the sickle cell disease who have undergone at least a transfusion or may have been treated with chelating drugs or with iron supplement.

When take sex in consideration, the study results appeared strong significant difference (p=0.0001) regarding both of (FER and Ostase). Moreover, the results showed statistically significant variation (p=0.035, p=0.006, p=0.015) between male and female of the following biomarkers (BSAP, IGF-1 and TRACP-5b) respectively. While there is no significant difference (p=0.273, p=0.503, p=0.0228, p=0.185, p=0.530, p=0.960, p=0.304 and p=0.327) of the (BCTx, CRP, DPD, OT, GFBPT, Intact (iPTH), PINP and serum Ca+) respectively. It may give a little bit impact in this study as shown in (Kalpatthi and Novelli 2018) study male sex and markers of hemolysis were associated with increased risk of near-term death.

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Conclusion

Although specific biomarkers related to these different events needs to understand for assessment of pathogenesis, the ones we have studied can be useful to spike in the sickle cell anemia patients may be harnessed in detecting the disease at early stage or monitoring the progress of the disease for appropriate medical interventions.

References

Ataga KI, Brittain JE, Desai P, May R, Jones S, Delaney J, et al. (2012) Association of coagulation activation with clinical complications in sicklecell disease. PLoS ONE.;7(1):e29786:1-7.

Ballas, S. K (2001). Sickle cell disease: current clinical management. Seminars in Hematology, 2001. Elsevier, 307-314.

Benenson, I. an-d Porter, S. (2018) _Sickle Cell Disease: Bone, Joint, Muscle, and Motor Complications‘, *Orthopaedic Nursing*, 37(4), pp. 221–227. doi: 10.1097/NOR.0000000000000464.

Broucek, J. (2021). Action of High Temperatures on the Biochemical Parameters of Cows. *Zivocisna Vyroba*

Ceglie G, Di Mauro M, Tarissi De Jacobis I, *et al.* (2019). Gender-Related Differences in Sickle Cell Disease in a Pediatric Cohort: A Single-Center Retrospective Study. front Mol Biosci. 6:140.

Da Guarda CC, Yahouédéhou S.(2020), Santiago RP, *et al.* Sickle cell disease: A distinction of two most frequent genotypes (HbSS and HbSC). PLoS One.15(1). 0228399 .

DAVID, H., BRIDGMAN, S., SC DAVIES, A. H. & EMERY, R.(1993). The shoulder in sickle-cell disease. *The Journal of Bone and Joint Surgery* 75-B, No. 4.

Fakunle E, Eteng,KI, Shokunbi,WA.(2012) .D- dimer levels in patients with sickle cell disease during bone pain crises and in the steady state. Pathology and aboratory Medicine International;4:21-25.

HOFFBRAND, A. V., VYAS, P., CAMPO, E., HAFERLACH, T. & GOMEZ, K.(2019). *Color Atlas of Clinical Hematology: Molecular and Cellular Basis of Disease*, John Wiley & Sons. *Blackwell,* 1-485.

Kalpatthi R, Novelli EM. (2018) Measuring success: utility of biomarkers in sickle cell disease clinical trials and care. Hematology Am Soc Hematol Educ Program. (1):482-492.

Lahhob, Q. R., Mohammed, N. Y., & Abbas, H. J. (2021b). Study of Some Minerals and Trace Elements Levels in patients with Sickle Cell Anemia and Sickle Cell Anemiathalassemia in South of Iraq. Biochemical and Cellular Archives, 21(1), 1091–1095.

Leonard, A. *et al.* (2021) ‗Curative therapy for hemoglobinopathies: an International Society for Cell & Gene Therapy Stem Cell Engineering Committee review comparing outcomes, accessibility and cost of ex vivo stem cell gene therapy versus allogeneic hematopoietic stem cell transpl', *Cytotherapy*.

Narkhova, A. G. (2021). Biochemical parameters of canned mountain ASH. *AGRO-industrial technologies of the central RUSSIA*, *2*, 17–24.

PLATT, O. S., BRAMBILLA, D. J., ROSSE, W. F., MILNER, P. F., CASTRO, O., STEINBERG, M. H. & KLUG, P. P.(1994). Mortality in sickle cell disease--life expectancy and risk factors for early death. *New England Journal of Medicine,* 330, 1639-1644.

Ravikanth , R. , Abraham , M. J. , & Alapati , A. (2017). Musculoskeletal manifestations in sickle cell anemia *Medical Journal of Dr. DY Patil Vidyapeeth* , *10* (5), 453 .

Romero Z, Urbinati F, Geiger S, Cooper AR, Wherley J, Kaufman ML, et al. (2013). Beta- globin gene transfer to human bone marrow for sickle cell disease. *The Journal of clinical investigation,* 123, 3317-3330.

Saud WH, Hassan MK, Al- Salait SK.(2017). Coagulation activation in patients with sickle cell disease in Basra, Iraq. J Appl Hematol;8:54-60.

Schleicher, J. *et al.* (2020) ‗Statistical matching for conservation science', *Conservation Biology*, 34(3), pp. 538–549. doi: 10.1111/cobi.13448.

SHI, X., LI, C., LIANG, B., HE, K. & LI, X.(2014). Weak cation magnetic separation technology and MALDI-TOF-MS in screening serum protein markers in primary type I osteoporosis. *Genet Mol Res,* 14, 15285-15294.

Tsitsikas, D.A.; Vize, J.; Abukar, J.(2020) Fat Embolism Syndrome in Sickle Cell Disease. J. Clin. Med., 9, 3601.

Udezue E, Girshab AM. (2004). Differences between males and females in adult sickle cell pain crisis in eastern Saudi Arabia. Ann Saudi Med. 24(3):179-182. FanestilVichaka , Van Siclen Carleen. (2015). Differentiation Between Sickle Cell Anemia and S/β^0 Thalassemia. *Laboratory Medicine*. 46(3). 79-81.

Eaton, W. A. (2020) ‗Hemoglobin S polymerization and sickle cell disease: A retrospective on the occasion of the 70th anniversary of Pauling's Science paper', *American Journal of Hematology*, 95(2), pp. 205–211Widana, I.K., Dewi, G.A.O.C., Suryasa, W. (2020). Ergonomics approach to improve student

concentration on learning process of professional ethics. *Journal of Advanced Research in Dynamical and Control Systems*, *12*(7), 429-445.

Widana, I.K., Sumetri, N.W., Sutapa, I.K., Suryasa, W. (2021). Anthropometric measures for better cardiovascular and musculoskeletal health. *Computer Applications in Engineering Education*, *29*(3), 550–561. https://doi.org/10.1002/cae.22202

Yuriah, S., Kartini, F., & Isnaeni, Y. (2022). Experiences of women with preeclampsia. International Journal of Health & Medical Sciences, 5(3), 201-210. https://doi.org/10.21744/ijhms.v5n3.1901