Original Article

Study Serum Visfatin Level in Steady State of Sickle Cell Patients and Association with Frequency of Vaso-Occlusive Crises in Both Adults and Children

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Abstract

Background: Sickle cell anemia (SCA) is a known hematological disorder that arises from a single point mutation in codon 6 of the β-globin gene that results in a glutamic acid to valine substitution. **Objective:** This study aimed to (i) investigate the role of visfatin, as a marker of inflammation in SCA patients, (ii) evaluate the association between serum visfatin level in SCA patients and the frequency of vaso-occlusive crises, and (iii) to find the possible usage of these markers as a predictive index for vaso-occlusive crises occurrence. Study correlations between visfatin with different hemolysis and inflammation. **Materials and Methods:** The total number of participants are 100 including 35 patients with painful crisis, 35 patients with steady state, and 30 healthy control group; all of the patients and healthy control group were men, in addition, their ages ranged from 3 to 55 years were evaluated with respect to serum visfatin levels by means of enzyme linked immune sorbent assay. **Results:** The results showed there have been significant differences in concentrations of Ferritin, visfatin, and lactate dehydrogenase, in SCA patients (painful crisis, steady state) in comparison with the control group for both adults and children. No significant differences in body mass index were observed between SCA patients and the control group for both adults and children. **Conclusion:** SCA patients with the painful crisis have significant correlation between the combination of visfatin and LDH and that of visfatin and ferritin.

Keywords: Sickle cell disease, vaso-occlusive crises, visfatin

Introduction

Sickle cell anemia (SCA) is a known hematological disorder that arises from a single point mutation in codon 6 of the β-globin gene that results in a glutamic acid to valine substitution.[1] This mutation leads to the formation of abnormal hemoglobin called hemoglobin S (HbS).[2] When HbS is deoxygenated, it becomes less soluble and precipitates due to intraerythrocytic polymerization of deoxyhemoglobin S, and displays morphological change to a crescent shape, hemolytic anemia, and complications are brought on by these rigid sickle cells.[3] Homozygous hemoglobin S (HbSS disease) is the most common form of sickle cell disease (SCD).[4] In addition to homozygous SCD (HbSS), other forms such as HbSC and HbSB-thalassemia also exist.[4]

The pathophysiology of SCA arises from hemolytic anemia and acute vaso occlusion; organ damage develops from recurrent erythrocyte sickling, chronic hemolysis, and progressive endothelial vasculopathy.[5] Most of the time, many children with sickle cell anemia are in relatively good health. This is otherwise known as the steady state. This is interrupted occasionally by crises, especially the vaso occlusive type, which is the hallmark of SCA. Crises are characterized by episodes of acute illnesses that cause

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sickle cell anemia's clinical signs, such as pain, anemia, or jaundice.[6]

Hemoglobin electrophoresis is the definitive diagnostic for SCA. Nonetheless, a rapid screening test known as the "sickled test" can detect levels of HbS of more than 10% in emergency conditions, but it cannot distinguish between SCD and sickle cell trait.[7] SCA is the most common hemoglobinopathy globally, recent estimates suggest that globally, more than 300,000 children are born with SCA every year,[8] and its incidence is expected to increase to 400,000 neonates born per year by 2050.[9] In Iraq, sickle cell disorders are less uniformly distributed with carrier rates varying from 0% to 16.0%, and they cluster in the extreme north and south of the country.[10] The prevalence of sickle cell disease hemoglobin was low 0.56%, which is not representing the true figure of sickle cell carriers in the Karbala governorate because there is no definitive cure for these genetic conditions.[11]

Visfatin is a protein mediator produced by fat cells (having high levels of expression in visceral fat cells).[12] It appears that other organs and cells, like skeletal muscle, the liver, immune cells, cardiomyocytes, and the brain, are also involved in its production.[13] Visfatin is a product of the growth factor gene for early B cells, identified in 2005 in visceral adipose tissue at present. Visfatin is widely known to be produce by hepatocytes, myocytes, and lymphocytes. Visfatin has several important roles, including promoting preadipocyte differentiation into adipocytes and promoting cytokine production such as tumor necrosis factor-alpha, interleukin-1 beta, and interleukin.[14] Visfatin has been suggested as a marker of endothelial dysfunction, it can directly promote vascular inflammation by activating different cell types including endothelial cells and vascular smooth muscle cells,[15] in sickle cell disease patients.[16]

Its broad spectrum of effects is mirrored by its potential involvement in a variety of diseases, such as human immuno-deficiency virus infection, septicemia, myocardial failure, atherosclerosis, metabolic disorders, inflammatory diseases, malignancies, neurodegenerative disorders, and aging.[13]

Materials and Methods

Patients

The case-control study has been conducted during 3 months between November 2022 and January 2023 in the hereditary blood disease center/Karbala Health Directorate. Hematopathologist doctor diagnosed 70 patients, both new and old cases, with sickle cell anemia, according to data that patients provided to the center. Additionally, the patients' ages ranged from three to 55, and they were all men. The total number of participants are 100 including 35 patients with painful crisis (adults 19, children 16), 35 patients with steady state (adults 9, children 26), and (30) healthy control group (adults 20, children 10). The patients and healthy subjects were divided into six age groups, less than 10 years, (11-20), (21-30), (31-40), (51-60), and (41-50).

Data collection and blood sampling

Three mL of venous blood was drawn in an aseptic condition using a plastic syringe and the standard safety procedures used during venipunctures. Blood samples were taken in a gel tube and left for 30 min to clot, and then centrifugations were carried out for 20 min at 1500×*g*, and serum was separated for analyzing parameters.

Serum visfatin level was detected using the ELISA kits which were characterized according to the manufacturer's recommendations. LDH was measured automatically by DIRUI (Auto Chemistry Analyzer CS180). Ferritin was measured automatically by MINI Vidas Equipment.

Exclusion and inclusion criteria

Patients were excluded from the study if they had any of the following: acute illness or fever, malignancy, hepatic or renal dysfunction, autoimmune disease or cardiopulmonary disease, and surgery or trauma within a month before the study. The inclusion criteria included SCA patients who served as case group, healthy subject, who served as control group.

Body mass index

Calculated from the following equation:

$$\text{BMI}=\frac{\text{Weight (kg)}}{\text{Height}(\text{m}^2)}.$$

Statistical analysis

The version 24 of the computer program, SPSS (IBM Company, Chicago, IL, USA), analysis of variance table – one way. *R*-test and the results have been considered to have statistical significance at $P \leq 0.05$.

Ethical approvals

This study was approved by Ethical Committee at College of Applied Medical Science/ University of Karbala. All subjects involved in this work were informed and agreement was obtained verbally from each one before the collection of samples. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the document number 3 (including the number and the date in November 1, 2022) to get this approval.

Results

Visfatin

According to the findings of Table 1, SCA patients had higher concentrations of Visfatin than controls. In Table 1, it is also indicated significant differences

Table 1: Concentration of visfatin in patients with SCA and compare with control group

Categories	*P* value	Mean ± SD			LSD
		Patients (70)		Healthy (30)	
		Steady (35)	Painful (35)		
Children	0.0000 *	1.36 ± 0.25 [ab] (N=26)	2.28 ± 0.31 [a] (N=16)	0.29 ± 0.17 [b] (N=10)	1.398
Adults	0.0000 *	1.81 ± 0.38 [b] (N=9)	2.85 ± 0.15 [a] (N=19)	0.97 ± 0.36 [c] (N=20)	0.458
Total		1.48 ± 0.34	2.59 ± 0.37	0.82 ± 0.43	

NS: no significance

* Means significant differences at $P \leq 0.05$

Table 2: Concentration of LDH in patients with SCA and compared with control group

Categories	Mean ± SD			*P* value	LSD
	Patients (70)		Healthy (30)		
	Steady (35)	Painful (35)			
Children	929.34 ± 173.03[b] (*N* = 26)	1964.93 ± 546.1[a] (*N* = 16)	633.16 ± 91.92 [c] (*N* = 10)	0.0000 *	7.982
Adults	569.33 ± 69.08 [b] (*N* = 9)	959.57 ± 93.40 [a] (*N* = 19)	282.80 ± 72.72 [c] (*N* = 20)	0.0000 *	0.596
Total	836.77 ± 220.51	1419.17 ± 551	363.65 ± 168.44		

NS: no significance

* Means significant differences at $P \leq 0.05$

Table 3: Concentration of Ferritin in patients with SCA and compare with control group

Categories	Mean ± SD			*P* value	LSD
	Patients (70)		Healthy (30)		
	Steady (35)	Painful (35)			
Children	730.76 ± 252.1 [b] (*N* = 26)	1178.37 ± 493.1 [a] (*N* = 16)	181.0 ± 314.1 [c] (*N* = 10)	0.0000 *	5.313
Adults	998.0 ± 162.3 [b] (*N* = 9)	2625.6 ± 687.6 [a] (*N* = 19)	404.05±575.8 [c] (*N* = 20)	0.0000 *	2.880
Total	799.48 ± 258.82	1964.05 ± 944.85	352.57 ± 530.0		

NS: no significance

* Means significant differences at $P \leq 0.05$

($P \leq 0.05$) between adults, children in both patient groups compared with control.

Lactate dehydrogenase

According to Table 2 findings, there are significant differences ($P \leq 0.05$) between adult and children in both patient groups compared with control. In the same table revealed LDH level higher in all patient groups (painful crisis and steady state) compared to control.

Ferritin

Results of Table 3 demonstrated that there were significant changes ($P \leq 0.05$) in the ferritin concentration in both children and adults with SCA when compared to the control group. The ferritin concentration increased in patients with SCA (painful crisis, steady state) when compared to the control group.

Visfatin

According to Table 4, all age groups less than 10 years, (11-20), (21-30), (31-40), (51-60), and (41-50), showed significant differences ($P \leq 0.05$). These results agreed with other studies that found the value of serum visfatin is increased in SCD patients compared with that in healthy children from (1-20) years and is associated with the frequency of vaso-occlusive crises (VOC) (17).

Lactate dehydrogenase

All age groups less than 10 years, (11-20), (21-30), (31-40), (51-60), and (41-50) showed significant differences ($P \leq 0.05$) in the results shown in Table 5.

Ferritin

The age groups (<10), (11-20), (21-30), (31-40), and (51-60) showed significant differences ($P \leq 0.05$), according to the data in Table 6. There were no significant differences ($P > 0.05$) in age group (41-50) only.

According to the results in Table 7 there was no significant correlation between Visfatin and LDH in steady patients group in both children and adults ($R^2 = 0.163$). Which indicates that the rise in LDH is not due to the rise in

Table 4: Visfatin of study sample

Age Categories (year)	Mean ± SD			*P* value	LSD
	Patients (70)		Healthy (30)		
	Steady (35)	Painful (35)			
≤10	1.30 ± 0.24 [b]	2.18 ± 0.29 [a]	0.28 ± 0.15 [c]	0.000 *	0.288
11-20	1.44 ± 0.24 [b]	2.34 ± 0.32 [a]	0.31 ± 0.29 [c]	0.000 *	0.502
21-30	1.64 ± 0.41 [b]	2.84 ± 0.15 [a]	0.76 ± 0.34 [c]	0.000 *	0.831
31-40	2.01 ± 0.21 [b]	2.88 ± 0.18 [a]	1.23 ± 0.09 [c]	0.000 *	0.582
41-50	1.79 ± 0.00 [b]	2.88 ± 0.13 [a]	1.15 ± 0.27 [c]	0.002 *	0.613
51-60	2.26 ± 0.00 [a]	2.76 ± 0.00 [a]	1.37 ± 0.06 [b]	0.000 *	0.712

NS: no significance

* Means significant differences at $P \leq 0.05$

Table 5: LDH of study sample

Age categories (year)	Mean ± SD			*P* value	LSD
	Patients (70)		Healthy (30)		
	Steady (35)	Painful (35)			
≤10	1020.3 ± 152.9 [b]	2414.6 ± 459.8 [a]	659.5 ± 98.7 [c]	0.000 *	8.979
11-20	805.27 ± 112.2 [b]	1695.1 ± 404.7 [a]	580.5 ± 68.5 [b]	0.000 *	432.8
21-30	563.0 ± 67.76 [b]	988.4 ± 94.77 [a]	313.6 ± 79.4 [c]	0.000 *	205.0
31-40	573.0 ± 117.3 [b]	920.2 ± 50.2 [a]	253.2 ± 16.9 [c]	0.000 *	12.53
41-50	634.0 ± 0.00 [b]	850.5 ± 28.9 [a]	271.0 ± 48.5 [c]	0.000 *	4.722
51-60	529.0 ± 0.00 [b]	1422.3 ± 0.00 [a]	190.0 ±0.00 [c]	0.000 *	18.562

NS: no significance

* Means significant differences at $P \leq 0.05$

Table 6: Ferritin of study sample

Age categories (year)	Mean ± SD			*P* value	LSD
	Patients (70)		Healthy (30)		
	Steady (35)	Painful (35)			
≤10	697.2 ± 277.3[a]	696.3 ± 270.2[a]	255.0 ± 376.7[b]	0.036 *	3.220
11-20	776.5 ± 217.5 [b]	1467.6±341.8[a]	33.00 ± 42.42[c]	0.000 *	2.465
21-30	963.6 ± 163.0 [b]	2351.0 ± 527.5[a]	250.2 ± 260.9[c]	0.000 *	96.66
31-40	933.0 ± 210.7 [a]	3327.2 ± 793 [b]	445.7 ± 495.6[b]	0.001 *	507.8
41-50	1148.0 ± 0.00	3007.5 ± 202.9	963.0 ± 1352.1	0.159	NS
51-60	1150.0 ± 0.00 [a]	2978.2 ± 0.00 [c]	328.0 ± 98.9[b]	0.001 *	32.41

NS: no significance

* Means significant differences at $P \leq 0.05$

Table 7: Correlation between markers

Correlation between markers	R^2
Correlation between Visfatin and LDH in steady patients group	0.163
Correlation between Visfatin and LDH in painful patients group	0.553
Correlation between Visfatin and Ferritin in steady patients group	0.074
Correlation between Visfatin and Ferritin in painful patients group	0.450

VISFATIN, but rather for other reasons. As per the results in Table 7, there was a significant correlation between Visfatin and LDH in painful crisis patients group in both children and adults ($R^2 = 0.553$). This indicates that the rise in LDH is associated with the rise in visfatin in painful crisis whereas there was no significant correlation between visfatin and ferritin in steady patients group in both children and adults ($R^2 = 0.074$), which indicates

that the rise in Ferritin in steady patients group is not due to the rise in visfatin , but rather for other reasons.

According to the results shown in Table 7 there was a significant correlation between Visfatin and Ferritin in painful crisis patients group in both children and adults (R^2 = 0.450). These findings were in agreement with other previous published data in which authors reported that Serum visfatin level was positively correlated with serum ferritin level.[17-19]

Discussion

Depending on the findings of the current investigation in Table 1, patients' levels of visfatin elevated in response to the inflammatory process compared to control subjects. Numerous studies have addressed and supported the findings of this investigation, which demonstrates that the serum visfatin is increased in SCD patients compared with that in healthy children and is associated with the frequency of VOC; it can be used as a predictive index for VOC occurrence and follow-up in those patients.[17]

The concentration of LDH in Table 2 in SCA patients was significantly higher in both crisis and steady state compared to control groups in this work; hemolysis always present is supported scientifically with high values of Lactate Dehydrogenases, in some study that supports current results the Serum LDH is increased in SCA patients (crisis, steady) compared with that in healthy.[20] In this study also Lactate dehydrogenase levels were significantly increased in VOC state compared to steady-state in both children and adults.[21] The patients group with SCA patient group (painful crisis and steady state), according to the most recent findings in Table 3, had considerably higher ferritin concentrations than the control group. The rise in the serum ferritin in sickle cell anemia patients due to the excess free iron, due to the excess breakdown of Hb and the abnormal circulating mass of Hb in reticuloendothelial cells, exert a positive feedback on ferritin synthesis, ferritin was significantly higher in patients with SCA during both steady state and acute VOC compared to control.[22] According to Table 4 results, all age groups less than 10 years, (11-20), (21-30), (31-40), (51-60), and (41-50), showed significant differences ($P \leq 0.05$). These results agree with other studies that found the value of Serum visfatin is increased in SCD patients compared with that in healthy children from (1-20) years and is associated with the frequency of VOC.[17]

In the present study, there was a significant increase of serum LDH in Table 5 in patients with SCA compared to the control group. These results are in agreement with the other study that found a significant increase of LDH compared to a control group in the same study population at steady state found an elevation in serum LDH levels in patients with SCA in both steady state and in crises in age from 15 to 55 years.[23] Numerous studies have addressed and supported the findings of this investigation in Table 6, which demonstrates that the serum ferritin levels were marginally higher in children in acute crises or with acute disease complications compared with those in a steady state from age was 6 month to 15 years.[24] Also there were higher levels of ferritin in age group from (12-40) years.[25] Our results are in disagreement with this study in age from (40-46) because they found an increase in ferritin level in SCA patients (steady state and crisis).[26]

According to the results shown in Table 7 there was no significant correlation between Visfatin and LDH in steady patients group in both children and adults (R^2 = 0.163), which indicates that the rise in LDH is not due to the rise in Visfatin, but rather for other reasons, whereas in there was significant correlation between Visfatin and LDH in painful crisis patients group in both children and adults (R^2 = 0.553). This indicates that the rise in LDH is associated with the rise in visfatin in painful crisis.

The results in Table 7 there were no significant correlation between Visfatin and Ferritin in steady patients group in both children and adults (R^2 = 0.074). Which indicates that the rise in Ferritin in steady patients group is not due to the rise in VISFATIN, but rather for other reasons. The results also revealed that there was significant correlation between Visfatin and Ferritin in painful crisis patients group in both children and adults (R^2 = 0.450). These findings were compatible with other previous published data in which authors reported that serum visfatin level was positively correlated with serum ferritin level.[17]

Conclusion

The study concludes that serum visfatin level is significantly higher among patients with sickle cell anemia in both (steady state and painful crisis) compared to healthy individuals, with a positive correlation between serum visfatin level and the frequency of VOC, With positive correlation between serum visfatin level and LDH and ferritin in painful crisis.

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Conflicts of interest

There are no conflicts of interest.

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