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Vitamin E and Selenium Status as Antioxidants Biomarkers in Patients with Sickle Cell Anemia

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Abstract

Background: Sickle cell disease (SCD) is characterized by elevation of oxidative stress (OS) and measuring the antioxidants biomarkers like vitamin E (VE), lipid profiles, uric acid (UA), selenium (Se), and superoxide dismutase (SOD) may help to improve patients' outcomes and disease progression and disease progression. Objective: The current study aims to detect the role of OS in SCD and the protection effect of antioxidants. Methods: A case-control study was included three study groups; sickle cell anemia (SCA), sickle cell trait (SCT), and healthy controls (HC). Colorimetric, atomic absorption spectrophotometric, and enzyme-linked immune-sorbent assay (ELISA) techniques were performed to assess the levels of VE, Se, SOD, UA, and lipid profile in the blood (serum and plasma). Results: Sickle cell disease and SCT subjects had statistically reduced SOD, VE, and Se levels than the HC, with no significant differences between SCD and SCT patients. When comparing SCD and SCT groups to HC, lipid profiles reflected higher concentrations of triglycerides (TG), total cholesterol (TC), and very low-density lipoprotein (VLDL). The antioxidant biomarkers (VE, Se, and SOD) and lipid profiles illustrate significant correlational inter-relationships among the study groups. Conclusions: When compared to HC, subjects with SCD and SCT had considerably lower levels of VE, Se, and SOD, speculating a higher OS. On the contrary, these patients had higher concentrations of TC, TG, and VLDL and lower concentrations of high-density lipoprotein (HDL). The results verified that OS and dyslipidemia are common in SCD and SCT, emphasizing the utility need for lipid-lowering and antioxidant treatment determinants.

Key Words: SCD, vitamin E, selenium, SOD, oxidative, stress.

1. Introduction

The disease of SCA is an autosomal recessive hereditary pattern disease caused by the *sickle* gene which is characterized by the substitution of valine with glutamic acid in the beta-chain of Hb, which changes the structure of hemoglobin (Hb) [1]. Strong OS is linked to SCD, and to decrease continuous oxidative tissue damage, antioxidant levels must be kept at their optimal. Sickled red blood cells (RBCs) produce about twice as much superoxide, hydroxyl free radicals, and hydrogen peroxide [2]. Uric acid, the end product of purine metabolism, is considered a potential marker for OS due to its role as a significant antioxidant in humans. Elevated levels of OS have been reported in the SCA, and the cells can be protected from oxidative damage by UA due to its role in the ROS scavenged. Uric acid may play a protective compensatory role due to its antioxidant function [3]. Monitoring the crucial function of UA in SCA subjects is critical for treatment strategies designation that target the OS [4]. Therefore, UA determination may provide important information about potential interventions and oxidative balance to decrease patient outcomes.

Metabolic and cardiovascular functions are changed by lipid profile concentration. Sickle cell anemia is characterized by dyslipidemia that occurs due to inflammation and OS which are correlated with elevated lipids levels [5]. Detection of SCA patient’s lipid profiles is crucial for OS treatment designation and decreasing disease progression. Healthcare professionals can learn a great deal about the inflammatory and oxidative status of SCA subjects by measuring their lipid concentration. This information can then be used to potentially guide antioxidant and cholesterol-decreasing therapy to alleviate SCA-related problems.

The VE is a potent antioxidant that supports a healthy immune system and protects human cells from OS. It directly affects the development of a baby's immunological, respiratory, and cognitive systems. [6]. It was hypothesized that using it in SCA patients would stop oxidized RBCs from adhering and being phagocytosed and hemolysis. This may help by lowering anemia, preventing clinical issues, and enhancing the patient’s quality of life [7].

One of the key trace elements required for regular human cell activity is Se. The body absorbs Se from a plant- or animal-based diet. Optimizing the population's Se intake to prevent illnesses associated with excess or insufficiency represents one of the most urgent problems in contemporary medicine [8]. Patients with SCD have an inefficient antioxidant protection system as a result of little synthesis and/or high consumption of antioxidants such as trace elements [9].

Cellular defense, by using enzymatic and non-enzymatic antioxidant mechanisms, plays an essential role in protecting the body systems from ROS when the body is in a controlled state. Superoxide dismutase are enzymes that function in the body to fight free radicals [10]. Given its crucial function in mitigating OS, SOD has been studied as a potential biomarker for antioxidant status in SCA patients. Adequate activity of SOD is essential for maintaining RBCs integrity and preventing hemolysis. Numerous investigations have reported the role that SOD plays in lowering OS and enhancing the body's defense against free radicals in individuals with SCA [11]. Detection of SCA patients' VE, Se, and SOD levels could give important information about their oxidative state and direct treatment strategies meant to strengthen antioxidant

defenses. Comprehending the function of VE, Se, and SOD in reducing OS can aid in creating efficient methods for handling SCA and improving patient outcomes. The current study aims to detect the role of OS in SCD and the protection effect of antioxidants. It presents essential biomarkers for evaluating OS and antioxidant status in SCD patients, including UA, lipid profiles, VE, Se, and SOD. Monitoring these biomarkers may guide therapeutic interventions to improve patient outcomes.

2. Materials and Methods

2.1 Subjects and Study Design :This case-control paper was conducted at the Thi-Qar Center for Hereditary Diseases, Thi-Qar (Iraq), in the period between July 2023 and January 2024. Included three study categories: There were 31 SCA patients in the first group with HbSS (12 females and 19 males) with an age between 5 and 61 years. The second category included 34 SCT subjects HbAS (14 females and 20 males), their ages were 11–60 years, and the third category as control included a total of fifty healthy subjects HbAA (the control group) (20 females and 30 males) with a range of ages between 2 and 63 years.

The eligibility criteria of the first and second groups were: the patient is diagnosed with SCD or SCT, no severe jaundice, obesity, metabolic syndrome, cancer, hypertension, peripheral vascular disease, chronic or autoimmune diseases, not taking hydroxyurea or receiving blood transfusions within the last three months, no pregnancy or breastfeeding no corticosteroid treatment for the previous four weeks, not receiving biological agent therapy, and not had surgery within the last 6 months.

For the HC group, any individual with the following criteria was enrolled in the present research; a person without any family history of any type of hemoglobinopathies, especially SCD, there is no endogamy history or smoking, non-pregnant women, there is no inflammation or infection, no antibiotics or biological agents are taken currently, no current chronic or autoimmune diseases, no recent blood transfusions, and no recent surgery.

2.2 Sample Collection :A puncture from the vein was used to collect 2–5 milliliters (ml) of whole blood from each subject. Then the 2-3 ml of collected blood was placed in a gel vacuum tube and left to clot for a while at room temperature, then centrifuged for 10 minutes at 3600 xg to obtain the serum. The separated serum was stored at -80 degrees Celsius for further analysis. There were 1-2 ml of whole blood placed in an ethylene diaminetetraacetic acid tube for plasma separation for lipid profile tests.

2.3 Biochemical Assays : Serum VE was detected in picograms (pg)/ml by using the human VE ELISA kit (Sun Long, China). The findings were interpreted and categorized into three categories; below-normal (<2 pg per ml), normal (2–5 pg per ml), and above-normal (>5 pg per ml) based on HC results, whereas serum SOD was detected in units of nanograms (ng)/ml) using a human SOD ELISA kit (Sun Long, China). The results were interpreted and scaled into; below-normal (<1 ng per ml), normal (1-3 ng per ml), and above-normal (>3 ng per ml), based on their levels in the HC group.

Serum Se was detected by the atomic absorption spectrophotometer technology using the Atomic Absorption Spectrophotometer Device (PG, England) in micrograms per litter (µg/L).

The findings were interpreted and categorized into three scales; below-normal (<70 µg per L), normal (70–120 µg per L), and above-normal (>120 µg per L) based on HC results. Furthermore, serum UA level was detected and titrated in milligram (mg)/deciliter (dl) with using UA uricase–PAP test kit (Bio Research, Germany) which based on colorimetric methods. Colorimetric methods were used to measure the lipid profile biomarkers (TC, TG, and HDL), which were detected in mg/dl using a cholesterol kit (Bio Research, Germany), TG test Kit (Bio Research, Germany), and ultra HDL assay kit (Abbott Laboratories, Germany), respectively. The levels of VLDL and low density lipoprotein (LDL) were calculated in mg/dl by using the formulas numbered 1, and 2, respectively.

VLDL (mg/dl)= TG/5------(1) [12]

LDL (mg/dl)= TC-(HDL+ VLDL)--------2 [13]

2.4 Statistical Analyses : The data are tabulated and statistically analyzed by using statistical package for the social sciences" (version 31). Means, or relative frequencies, had been obtained using descriptive methods. The relationships among parameters are detected by using the chi-square statistical test. The p-value<0.05 was regarded as statistically valuable [14].

3. Results :The study was carried out at the Thi-Qar Center for Hereditary Diseases, Thi-Qar (Iraq), in the period between July 2023 and January 2024. Included three study categories; there were 31 SCA patients in the first group with HbSS (12 females and 19 males) with an age between 5 and 61 years. The second category included 34 SCT subjects HbAS (14 females and 20 males 20), their ages were 11–60 years, and the third category as control included a total of fifty healthy subjects HbAA (20 females and 30 males) with a range of ages between 2 and 63 years.

Figure 1-A shows that most SCD and SCT subjects (71% and 70.6%, respectively) had an increase of below-normal VE compared to the HC subjects (32%) with statistically valuable ($p<0.05$) differences. There are no statistical differences ($p>0.05$) among subjects of SCD and SCT for VE frequency percent. The titer of the mean for serum VE was statistically ($p<0.05$) decreased within SCD and SCT subjects (2.42 pg/ml and 1.87 pg/ml, respectively) compared to the HC subjects (3.85 pg/ml). There is no valuable statistical difference ($p>0.05$) was detected for the VE mean value between SCD and SCT groups (Figure 1-B).

Figure 2-A shows that most SCD, and SCT, subjects had elevated below-normal Se levels (64.5%, and 82%, respectively) than the HC subjects (0%) with statistically valuable ($p<0.05$) differences. There are no statistical differences ($p>0.05$) in the frequency percent of serum Se between patients of SCD and SCT groups. The mean value of serum Se was statistically ($p<0.05$) low in SCD patients and SCT individuals (63.22 µg/l, and 61.17 µg/l respectively) compared to the HC (122.54 µg/l). No valuable statistical differences ($p>0.05$) were detected in serum Se mean value between SCD and SCT groups (Figure 2-B).

Figure 3-A illustrates that the SCD and SCT patients had increased the below-normal SOD frequency (45.2% and 58.8%, respectively) when compared to the HC group (38%) with statistically valuable differences ($p<0.05$). The mean titer of serum SOD was statistically ($p<0.05$) decreased in the SCD patients (0.92 ng/ml) and SCT individuals (1.11 ng/ml) when

compared to the HC subjects (2.38 ng/ml) (Figure 3-B). No valuable differences (p>0.05) in frequency percent and mean titer of serum SOD were reported between SCD and SCT subjects. The findings revealed statistically (p<0.05) elevated mean values of TC, TG, and VLDL in SCD (166.9±67 mg/dl, 141.2±69.43 mg/dl, and 28.2 ±13.88 mg/dl, respectively) and SCT (182.1±74.93 mg/dl, 146.5±62.29 mg/dl and 29.31±12.45 mg/dl, respectively) than HC group (144.5±29.97 mg/dl, 90.48 ±27.53 mg/dl and 17.2±5.55 mg/dl, respectively). Furthermore, LDL mean titer was significantly (p<0.05) higher in SCT (108.4±76.4 mg/dl) compared to the HC (76.52±31.45 mg/dl), whereas the HDL was statistically (p<0.05) elevated in the HC (48.2±5.35 mg/dl) than SCA (44.29±9.21 mg/dl) and SCT (44.4±9.45 mg/dl). The UA level revealed no statistical differences (p>0.05) among the study groups (Table 1).

In the SCD group, significant differences were observed for SOD and TG. For SOD, the mean levels were 1.78±2.36 ng/ml for below-normal, 1.47±0.15 ng/ml for normal, and 8.52±6.64 ng/ml for above-normal VE levels (P<0.05). For TG, the mean levels were 132.63 ±61.42 mg/dl for below-normal, 127.67±65.95 mg/dl for normal, and 179.50±95.65 mg/dl for above-normal VE levels (P< 0.05). Furthermore, there are statistical differences in VLDL levels (P <0.05), with the highest mean VLDL in the above-normal VE category (35.90±19.13 mg/dl) than normal (25.53±13.19 mg/dl) or below-normal (26.52±12.28 mg/dl) VE category. In group SCT, significant differences were noted for Se and SOD. For Se, the mean levels were 60.68±9.11 µg/l for below-normal, 60.33±8.38 µg/l for normal, and 65±5.35 µg/l for above-normal VE levels (P<0.05). For SOD, the mean levels were 0.85±0.28 ng/ml for below-normal, 1.54±1.06 ng/ml for normal, and 1.99±2.27 ng/ml for above-normal VE levels (P<0.05). In a group of HC, significant differences were observed for LDL. The mean levels were 64.06±30.38 mg/dl for below-normal, 84.65±29.24 mg/dl for normal, and 88.64±21.63 mg/dl for above-normal VE levels (P<0.05). However, all other comparisons revealed non-statistical differences (p<0.05) (Table 2).

For SOD, significant differences were found in groups SCD and SCT. In SCD, the mean SOD levels were 3.10±2.58 ng/ml for below-normal and 1.67±1.32 ng/ml for normal Se levels (P<0.05). In SCT, the mean SOD levels were 1.21±1 ng/ml for below-normal and 0.75±0.28 ng/ml for normal Se levels (P<0.05). The HC group did not show significant differences. Regarding TC, the SCT group displayed an important difference. The mean TC levels were 190.76 ±79.44 mg/dl for below-normal and 143.23 ±27.89 mg/dl for normal Se levels (P<0.05). Sickle cell disease and HC groups did not significantly differ in TC levels. In the case of LDL, significant differences were observed in SCD and SCT groups. For SCD, the mean LDL levels were 102.04 ±74.59 mg/dl for below-normal and 80.66±64.63 mg/dl for normal Se levels (P<0.05). In SCT, the mean LDL levels were 116.55±81.53 mg/dl for below-normal and 75.03 ±31.80 mg/dl for normal Se levels (P<0.05). The HC group did not show significant differences in LDL levels. Furthermore, all other comparisons revealed non-significant differences (Table 3).

For TC, significant differences were found in the SCT group. The mean TC levels were 181.47±75.33 mg/dl for below-normal, 192.54±79.71 mg/dl for normal, and 126.3±6.64 mg/dl for above-normal SOD levels (P<0.05). The SCD and HC groups did not show significant

differences in TC levels. Regarding TG, the SCT group also displayed a significant difference. The mean TG levels were 162.25±59.28 mg/dl for below-normal, 121.91±65.13 mg/dl for normal, and 137.5±47.37 mg/dl for above-normal SOD levels (P< .05). The SCD and HC did not show significant differences in TG levels. In the case of HDL, significant differences were observed in both SCD and SCT groups. For SCD, the mean HDL levels were 43.5 ±10.24 mg/dl for below-normal, 40.12±9.15 mg/dl for normal, and 49.11±5.51 mg/dl for above-normal SOD levels (P< 0.05). In SCT, the mean HDL levels were 45.2±8.78 mg/dl for below-normal, 41.75±10.59 mg/dl for normal, and 53±2.82 mg/dl for above-normal SOD levels (P<0.05). The HC group did not show significant differences in HDL levels. For LDL, significant differences were found in both SCD and SCT groups. In SCD, the mean LDL levels were 108.17±72.08 mg/dl for below-normal, 82.57±64.59 mg/dl for normal, and 83.66±78.37 mg/dl for above-normal SOD levels (P<0.05). In SCT, the mean LDL levels were 103.82±75.76 mg/dl for below-normal, 126.40±80.38 mg/dl for normal, and 45.8±18.95 mg/dl for above-normal SOD levels (P<0.05). The HC group did not show significant differences in LDL levels. Furthermore, all other comparisons revealed non-significant differences (Table 4).

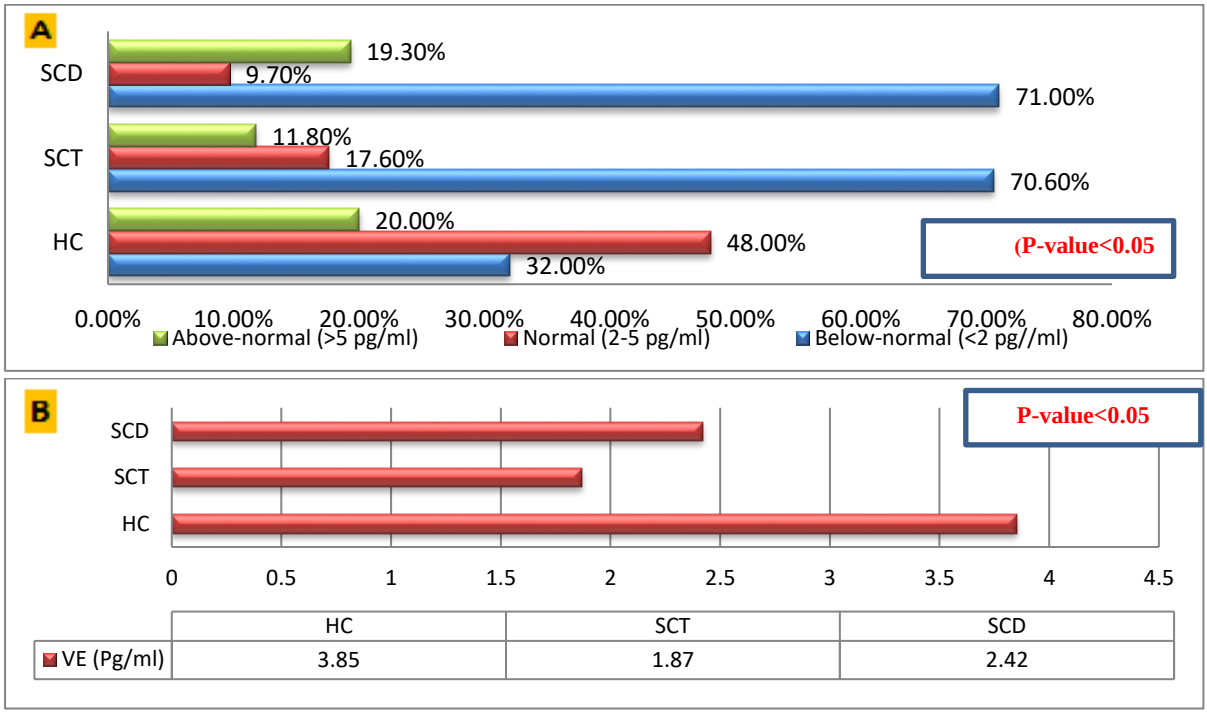

Figure (1): Result of vitamin E in our study groups, (A): frequency (%), and B): mean titer (SCD; sickle cell disease, SCT; sickle cell trait, HC; healthy control, VE; vitamin E, pg; picogram, ml; milliliter).

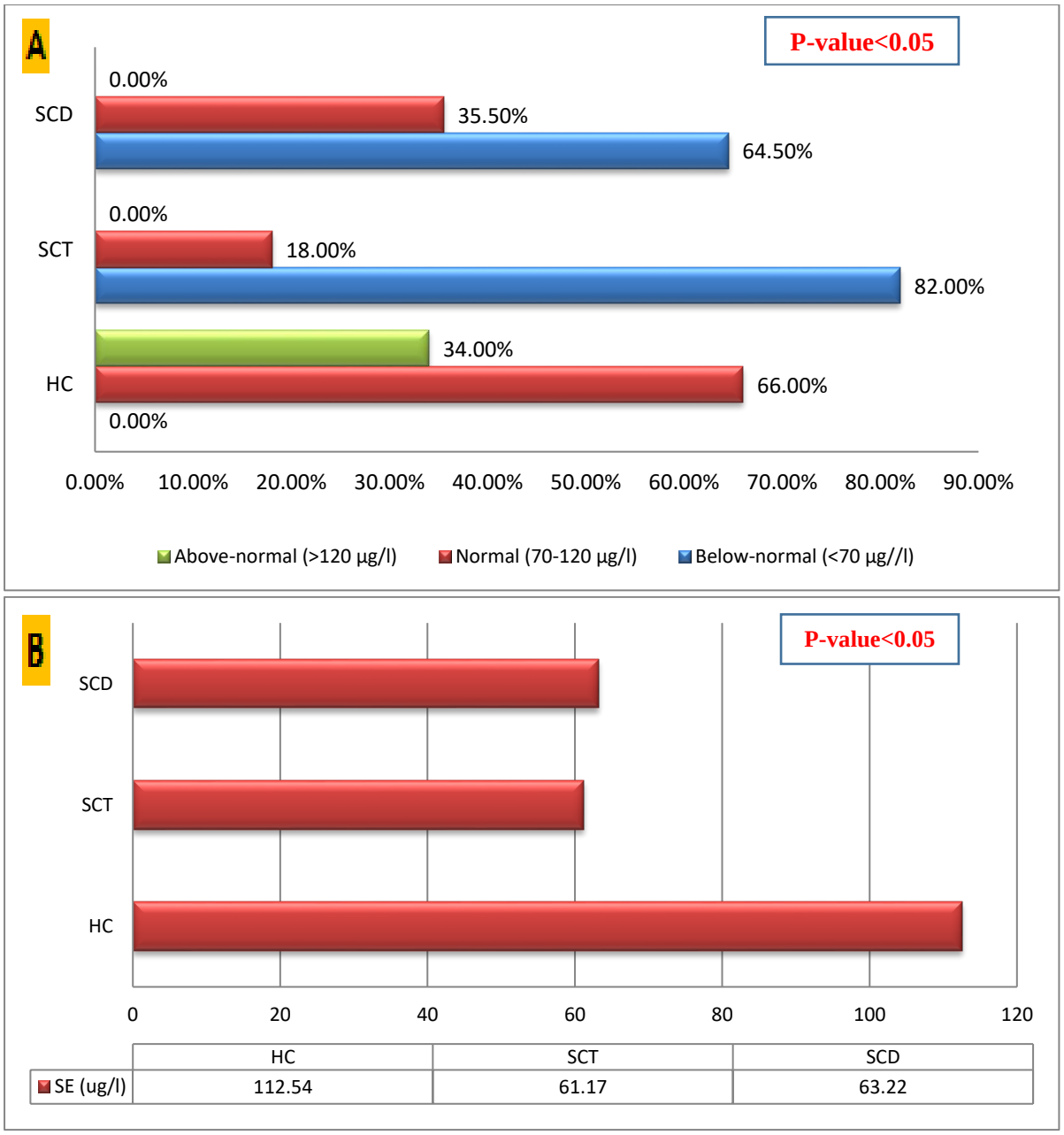

Figure (2): The result of selenium in our groups, (A): frequency (%), and (B): mean titer (SCD; sickle cell disease, SCT; sickle cell trait, HC; healthy control, SE; selenium, µg; microgram, l; Litter).

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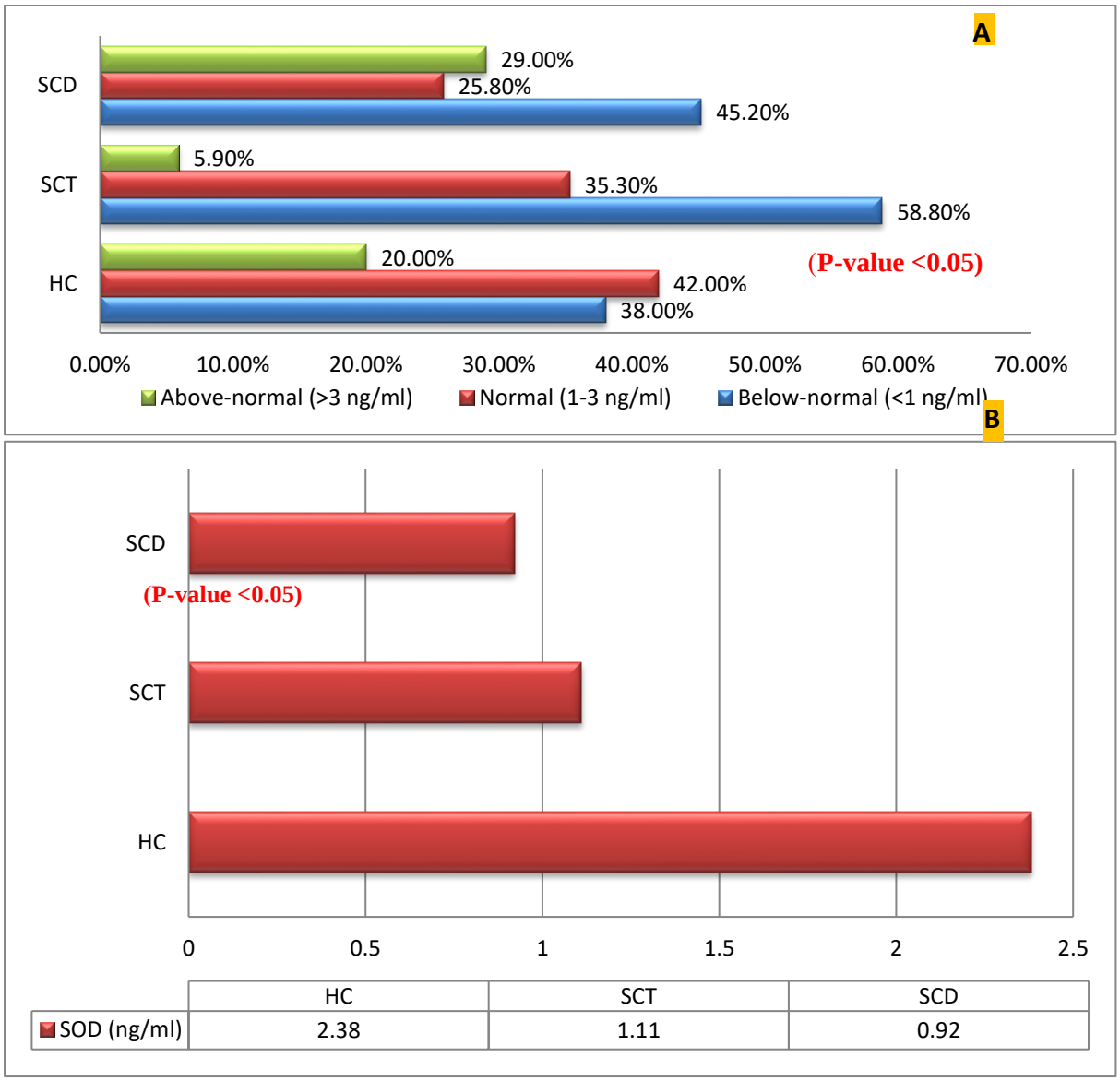

Figure (3): Result for superoxide dismutase activity in the study groups, (A): frequency (%), and (B): mean titer, (SCD; sickle cell disease, SCT; sickle cell trait, HC; healthy control, SOD; superoxide dismutase, ng; nanogram, ml; milliliter).

Table (1): The Results Of Uric Acid And Lipidprofile

Variables	G1 (N=31)	G2 (N=34)	G3 (N=50)	P-Value
	Mean ±SD	Mean ±SD	Mean ±SD	
Uric Acid (Mg/Dl)	4.7 ± 1.89	5.01 ±1.37	4.7 ±1.31	G1 × G3: >0.05 G2 × G3: >0.05 G1 × G2: >0.05
Total Cholesterol (Mg/Dl)	166.9 ± 67	182.1 ±74.93	144.5 ±29.97	G1 × G3: <0.05 G2 × G3: <0.05 G1 × G2: >0.05
Triglyceride (Mg/Dl)	141.2 ±69.43	146.5 ±62.29	90.48 ±27.53	G1 × G3: <0.05 G2 × G3: <0.05 G1 × G2: >0.05
High Density Lipoprotein (Mg/Dl)	44.29 ±9.21	44.4 ±9.45	48.2 ±5.35	G1 × G3: <0.05 G2 × G3: <0.05 G1 × G2: >0.05
Low Density Lipoprotein (Mg/Dl)	94.45 ±70.88	108.4 ±76.4	76.52 ±31.45	G1 × G3: >0.05 G2 × G3: <0.05 G1× G2: >0.05
VLDL (Mg/Dl)	28.2 ±13.88	29.31 ±12.45	17.2 ±5.55	G1 × G3:<0.05 G2 × G3: <0.05 G1 × G2: >0.05

Abbreviations: G1; Sickle Cell Disease, G2; Sickle Cell Trait, G3; Healthy Control, SD; Standard Deviation, VLDL; Very Low Density Lipoprotein, Mg; Milligram, Dl; Deciliter, N; Number,×; Indicate Comparison.

Table (2): The Correlation Between Vitamin E And Some Study Biomarkers					
Variables/ Groups		Vitamin E (Pg/Ml)			P-Value
		Below-Normal (<2)	Normal (2-5)	Above-Normal (>5)	
Selenium (µg/L) Mean±SD	G1	62.90±10.86	67.33±17.67	62.33±12.11	>0.05
	G2	60.68±9.11	60.33±8.38	65±5.35	<0.05
	G3	110.81±19.02	114.54±14.79	110.5±15.52	>0.05
SOD (Ng/Ml) Mean±SD	G1	1.78±2.36	1.47± 0.15	8.52± 6.64	<0.05
	G2	0.85±0.28	1.54±1.06	1.99±2.27	<0.05
	G3	2.65±4.26	1.89±2.40	2.41±1.90	>0.05
Uric Acid (Mg/Dl) Mean±SD	G1	4.93± 2.03	3.97± 0.65	4.30±1.77	>0.05
	G2	5.05±1.29	4.95±1.72	4.87±1.69	>0.05
	G3	5.01±1.68	4.54±1.15	4.61±1.08	>0.05
TC (Mg/Dl) Mean±SD	G1	169.48±65.85	133.54± 41.35	174.56±85.29	>0.05
	G2	186.66±78.81	157.80±69.99	191.48±67.19	>0.05
	G3	134.14±31.12	150.57±29.04	152.88±22.57	>0.05
TG (Mg/Dl) Mean±SD	G1	132.63±61.42	127.67± 65.95	179.50±95.65	<0.05
	G2	146.88±67.29	165.50±43.00	116.25±54.29	>0.05
	G3	95.44±34.07	93.48±24.67	74.00±14.95	>0.05
HDL (Mg/Dl) Mean±SD	G1	44.45± 9.88	43.33± 3.51	44.17±9.66	>0.05
	G2	44.13±8.85	43.67±13.61	47.50±7.72	>0.05
	G3	51.00±5.76	46.38±4.75	46.60±4.62	>0.05
LDL (Mg/Dl) Mean±SD	G1	98.50± 70.10	64.68± 49.05	94.49±89.07	>0.05
	G2	113.16±78.31	81.03±71.03	120.74±66.17	>0.05
	G3	64.06±30.38	84.65±29.24	88.64±21.63	<0.05
VLDL (Mg/Dl) Mean±SD	G1	26.52± 12.28	25.53± 13.19	35.90±19.13	<0.05
	G2	29.38±13.46	33.10±8.60	23.25±10.85	>0.05
	G3	18.06±6.90	17.64±4.99	14.39±3.11	>0.05
Abbreviations	Pg; Picogram, Ml; Milliliter, G1; Sickle Cell Disease, G2; Sickle Cell Trait, G3; Healthy Control, µg/L; Microgram Per Litter, SD; Standard Deviation, SOD; Superoxide Dismutase, Ng; Nanogram, Mg; Milligram, Dl; Deciliter, TC; Total Cholesterol, TG; Triglyceride, HDL; High Density Lipoprotein, LDL; Low Density Lipoprotein, VLDL; Very Low Density Lipoprotein.				

Table (3): The Correlation Between Selenium And Some Study Biomarkers					
Variables/ Groups		Selenium (µg/L)			P-Value
		Below-Normal (<70)	Normal (70-120)	Above-Normal (>120)	
SOD (Ng/Ml) Mean±SD	G1	3.10± 2.58	1.67±1.32	⏚	<0.05
	G2	1.21±1	0.75±0.28	⏚	<0.05
	G3	⏚	2.14±1.91	2.85±2.75	>0.05
Uric Acid (Mg/Dl) Mean±SD	G1	4.7± 2.10	4.75± 1.53	⏚	>0.05
	G2	5.18±1.42	4.23±0.7	⏚	>0.05
	G3	⏚	4.57±1.45	4.96±0.95	>0.05
TC (Mg/Dl) Mean±SD	G1	173.52± 72.38	155.11± 57.22	⏚	>0.05
	G2	190.76±79.44	143.23±27.89	⏚	<0.05
	G3	⏚	138.58±30.47	156.12±26.03	>0.05
TG (Mg/Dl) Mean±SD	G1	135.9± 78.83	150.91±49.96	⏚	>0.05
	G2	150.67±64.09	137.50±42.86	⏚	>0.05
	G3	⏚	88.88±25.11	93.59±32.31	>0.05
HDL (Mg/Dl) Mean±SD	G1	44.3± 9.73	44.27±8.64	⏚	>0.05
	G2	44.07±9.66	45.50±9.18	⏚	>0.05
	G3	⏚	49.24±5.08	46.18±5.44	>0.05
LDL (Mg/Dl) Mean±SD	G1	102.04± 74.59	80.66±64.63	⏚	<0.05
	G2	116.55±81.53	75.03±31.80	⏚	<0.05
	G3	⏚	69.96±34.63	89.25±19.22	>0.05
VLDL (Mg/Dl) Mean±SD	G1	27.18± 15.76	30.18±9.99	⏚	>0.05
	G2	30.13±12.81	22.70±8.57	⏚	>0.05
	G3	⏚	16.78±4.91	18.21±6.68	>0.05
Abbreviations	Ml; Milliliter, G1; Sickle Cell Disease, G2; Sickle Cell Trait, G3; Healthy Control, µg/L; Microgram Per Litter, SD; Standard Deviation, SOD; Superoxide Dismutase, Ng; Nanogram Mg; Milligram, Dl; Deciliter, TC; Total Cholesterol, TG; Triglyceride, HDL; High Density Lipoprotein, LDL; Low Density Lipoprotein, VLDL; Very Low Density Lipoprotein, ⏚; No Participants.				

Table (4): The Correlation Between Superoxide Dismutase And Some Study Biomarkers					
Variables/ Groups		Superoxide Dismutase (Ng/Ml)			P-Value
		Below-Normal (<1)	Normal (1-3)	Above-Normal (>3)	
Uric Acid (Mg/Dl) Mean±SD	G1	5.11± 2.30	4.6± 1.55	4.2± 1.44	>0.05
	G2	4.77±1.31	5.39±1.36	5.15±2.33	>0.05
	G3	5±1.65	4.40±1.06	4.74±0.98	>0.05
TC (Mg/Dl) Mean±SD	G1	182.37± 64.11	145.20± 55.30	162.41± 81.04	>0.05
	G2	181.47±75.33	192.54±79.71	126.3±6.64	<0.05
	G3	130.25±34.80	146.40±22.91	167.8±16.16	>0.05
TG (Mg/Dl) Mean±SD	G1	153.14± 60.85	112.5± 54.67	148.22± 91.19	>0.05
	G2	162.25±59.28	121.91±65.13	137.5±47.37	<0.05
	G3	95.73±28.64	81.90±18.37	98.5±37.88	>0.05
HDL (Mg/Dl) Mean±SD	G1	43.57± 10.24	40.12± 9.15	49.11± 5.51	<0.05
	G2	45.2±8.78	41.75±10.59	53±2.82	<0.05
	G3	49.21±5.68	47±5	48.8±5.47	>0.05
LDL (Mg/Dl) Mean±SD	G1	108.17± 72.08	82.57± 64.59	83.66± 78.37	<0.05
	G2	103.82±75.76	126.40±80.38	45.8±18.95	<0.05
	G3	61.62±37.55	80.94±23.61	95.54±20.23	>0.05
VLDL (Mg/Dl) Mean±SD	G1	30.62± 12.17	22.5± 10.93	29.64± 18.23	>0.05
	G2	32.45±11.85	24.38±13.02	27.5±9.47	>0.05
	G3	18.38±5.73	15.29±3.64	19.26±7.51	>0.05
Abbreviations	Ml; Milliliter, G1; Sickle Cell Disease, G2; Sickle Cell Trait, G3; Healthy Control, SD; Standard Deviation, Ng; Nanogram Mg; Milligram, Dl; Deciliter, TC; Total Cholesterol, TG; Triglyceride, HDL; High Density Lipoprotein, LDL; Low Density Lipoprotein, VLDL; Very Low Density Lipoprotein.				

4. Discussion

The SCD is a defect of RBCs that occurs by a point mutation exchange of thymine by adenine at the sixth position of Hb protein [15]. The continuous production of ROS causes oxidative damage. That is felt by patients with SCA and can result in acute inflammation and endothelial dysfunction [16]. Vitamin E is a lipophilic antioxidant that have a vital role in maintaining cellular redox equilibrium [17]. It has been shown that both VE deficiency and supplementation impact the immune system and inflammation by playing various regulatory roles, like changing the cell cycle, modulating inflammatory mediators, and altering membrane integrity and signal transmission [18]. Consistent with these observations, the present research revealed a significantly lower concentration of VE in both SCD and SCT groups compared to the HC group (Figure 1). Patients with hereditary hemolytic anemia (HHA) have a higher chance of

forming erythroblasts and circulating erythrocytes' membrane lipid peroxidation due to prolonged OS, which reduces. A RBCs common characteristic of certain of these HHA is a VE deficiency [19]. Thus, a person may acquire anemia as a result of increased OS. Decreases in VE levels have been noted in chronic hemolytic anemia. In which the RBCs suffer from severe oxidative damage [20]. These observations revealed the crucial role of VE in the pathogenicity of SCA in which VE is a medicinal ingredient for the management of anemia.

The biological, chemical, and molecular functioning of cells depends largely on trace elements [21]. Therefore, monitoring trace element concentrations in SCA is essential for reducing morbidity linked to this disorder. Selenium is a main component of selenoproteins, which are involved in redox reactions, and structural/transport functions [22]. A previous research reported that SCA and SCT had decreased Se levels [15]. In line with these findings, the present study revealed the same findings (Figure 2). The best explanation for lower Se levels in both SCD and SCT is due to a continuous state of OS that eventually decreases all antioxidants [23]. The development of anemia may be caused by Se deficiency, which is of particular interest to our country.

The present research observations reveled decrease concentrations of SOD within the SCT and SCD groups when compared with the group of HC (Figure 3). In line with current study findings, a study by Khorshied *et al*. [24]. Illustrate a noteworthy negative association between the annual hospitalization level and SOD activity, which may be indicative of SOD's protective effect in SCD patients. Also, another study showed that the low peripheral blood level of SOD is related with more cardiomyopathy and hemolysis in patients with SCD [25]. Low SOD concentrations may indicate the need for antioxidant treatments. The antioxidant mechanism of the body against OS is helped by the SOD enzyme [26]. The SOD supplements can be used in a range of pathological conditions and can activate the body's natural antioxidant machinery to neutralize excess free radicals.

The present research showed that the individuals with SCD and SCT have statistically elevated mean values of TC, TG, and VLDL compared to HC. Additionally, LDL concentrations are statistically elevated in the SCT subjects, while HDL concentrations are considerably higher in the HC group. No significant differences were detected in UA levels within the groups (Table 1). Dantas *et al*,. [27] recorded increased TC, TG, and VLDL levels within SCD patients compared to HC, suggesting a potential risk for cardiovascular complications in these patients. Similarly, Yalcinkaya *et al*., [28] detected that SCD subjects had noticeably increased LDL and decreased HDL levels, indicating a disturbances in lipid profile that increases the risk of atherosclerosis index. The higher concentrations of TC, TG, and VLDL in the SCD and SCT groups could be attributed to the increased hemolysis and inflammation associated with SCD, which can fluctuate lipid metabolism. Furthermore, the lower HDL levels in the SCD and SCT groups in comparison to the HC group support the notion that these patients have dyslipidemia. In contrast to results of other research where higher UA concentration have been correlated with SCD, the lack of statistical significance in UA concentration in the present paper groups speculates that hyperuricemia may not be a prominent character in patients with SCD or SCT. Nonetheless, not all SCD populations revealed the same findings [29]. The amount of

hemolysis, and the incidence of concomitant diseases such as kidney impairment, and metabolic disorders may all influence the heterogeneity in UA concentration in subjects with SCD. Debnath *et al*., [30] recorded that some SCD subjects had elevated UA value, while others did not. This hypothesis suggests that parameters other than anemia, such as genetic predisposition and kidney function, influence UA value.

The results showed that the SOD and TG concentrations in the SCD group, the Se and SOD concentrations in the SCT group, and the LDL concentrations in the HC group all differ statistically relative to the VE concentration. The SOD concentration in the SCD group varied statistically between VE categories, with the above-normal VE group having the higher concentration. This proposed that SOD is an essential factor in OS mitigation, and the highest VE concentration may be associated with elevated antioxidant capacity. Similarly, the above-normal VE group had higher concentrations of TG and VLDL, speculating a possible relationship between VE and lipid metabolism in SCA subjects (Table 2). This is in line with previous studies reporting that VE and other antioxidants can impact lipid profiles and OS in patients with SCD [31]. Selenium and SOD concentrations varied statistically within the SCT group. The Se concentration was higher in the above-normal VE category (Table 2), which was in line with data reporting that enhanced antioxidant status can strengthen enzymatic antioxidant defenses such as SOD, as evidenced by both Se and VE concentrations. This confirms Se's function as an antioxidant cofactor, essential for decreasing oxidative damage in SCT subjects [31]. Furthermore, the incidence of statistical variations in SOD concentration, with the higher concentration in the above-normal VE group, lends credence to the idea that appropriate antioxidant concentration can boost the body's defense against OS. Statistical differences in LDL concentration were reported in the HC group in relation to the VE groups, with the above-normal VE group having the higher LDL concentration (Table 2). This observation contrasts with a previous study, which reported no conclusive relationship between VE concentration and LDL in the healthy group [32], reporting that lipid concentration in the HC group may be influenced by other factors such as dietary consumption and metabolic changes. Given the intricate correlation between lipid metabolism, OS, and antioxidants, our results emphasize the importance of personalized interventions in treating SCD and SCT. More studies are needed to detect the precise fate and optimal antioxidant concentration required for protective effects in these groups.

The results indicate that SOD concentration differs statistically among patients with SCD and SCT concerning Se level. In SCD, higher SOD concentrations were linked with below-normal Se concentration as opposed to normal Se concentration. Similarly, below-normal Se concentration in SCT was correlated with elevated SOD concentration than normal Se concentration (Table 3). These results speculate that SOD level may be elevated as a compensatory response to decreased Se concentration. This could be due to an elevated OS environment that is common in SCD and SCT. Recent studies have corroborated these findings, noting increased OS markers and altered antioxidant enzyme activities in individuals with SCD [33]. Regarding TC, significant differences were reported in the SCT group, where below-normal Se levels were associated with higher TC levels compared to normal Se levels (Table

3). This suggests a possible link between Se deficiency and dyslipidemia in SCT individuals. This finding aligns with another study that reported an inverse relationship between Se status and cholesterol levels, suggesting Se's role in lipid metabolism [32]. In terms of LDL levels, both SCD and SCT groups exhibited significant differences based on Se status. In SCD, below-normal Se levels corresponded to higher LDL levels compared to normal Se levels. Similarly, in SCT, below-normal Se levels were associated with higher LDL levels versus normal Se levels (Table 3). This suggests a potential role of Se in regulating LDL levels, which could be crucial for managing cardiovascular risk in these populations. A previous study highlighted the beneficial effects of Se supplementation in reducing LDL levels, supporting our findings [34]. The HC group did not show significant differences in SOD, TC, or LDL levels based on Se status, which might indicate a more stable oxidative and lipid metabolic environment compared to SCD and SCT groups. This aligns with research suggesting that in healthy individuals, the impact of Se on these parameters might be less pronounced, with other factors such as diet and genetics playing more significant roles [33]. Overall, these findings underscore the importance of Se in modulating OS and lipid metabolism, particularly in individuals with SCD and SCT, and highlight the potential benefits of addressing SE deficiency as part of the management strategy for these conditions.

The current study findings reveal significant differences in various lipid parameters and antioxidant enzyme levels in individuals with SCD and SCT based on SOD levels. In the SCT group, TC levels varied significantly across different SOD levels. Specifically, the mean TC levels were highest in the normal/below-normal SOD categories and lowest in the above-normal SOD category (Table 4). This suggests a complex interaction between OS and cholesterol metabolism, as dyslipidemia in SCT may be more sensitive to variations in OS and antioxidant enzyme levels. The SCD and HC groups did not show significant differences in TC levels, aligning with research indicating distinct metabolic or compensatory mechanisms in these groups [33]. In the SCT patients, there were also notable variations in TG levels. The higher TG levels were correlated with below-normal SOD levels, whereas the lowest TG levels were correlated with normal/above-normal SOD levels (Table 4). This could reflect that at optimal SOD levels, lipid peroxidation is mitigated and TG levels are lowered. The lack of statistical variations in TG levels among the SCD and HC groups implies the involvement of distinct metabolic or compensatory processes [32]. Based on SOD status, there were notable disparities in HDL levels between the SCD and SCT groups. Higher HDL levels were linked to above-normal SOD levels in SCD subjects as opposed to below-normal and normal SOD levels. Likewise, in SCT, the highest HDL levels were correlated with above-normal SOD levels (Table 4). These results are the same as other research demonstrating that elevated antioxidant enzyme activity can raise HDL levels by guarding against lipoprotein oxidative damage [33]. Depending on SOD levels, significant changes in LDL levels were reported in both the SCD and SCT patients. Higher LDL levels in SCD were associated with below-normal SOD levels, indicating increased lipid peroxidation and OS. In SCT, LDL levels were highest when SOD levels were normal or below normal, and lowest when SOD levels were above-normal (Table 4). These data suggest that maintaining reduced LDL levels and minimizing

cardiovascular risk in those individuals is dependent on normal SOD function [32]. Overall, our findings underline the need to control OS and ensure individuals with SCD and SCT have enough antioxidant levels in their bodies. It may be possible to improve lipid profiles and decrease the risk of cardiovascular disorder by adjusting SOD levels. More studies are needed to better understand these relationships and develop targeted therapies that improve clinical outcomes.

5. Limitations : There are numerous limitations to this study. Initially, the results' generalizability was limited by the small sample size. Second, because the study was conducted solely at one medical site, selection bias may have been present. Finally, dietary consumption and environmental conditions these a few possible confounding variables that were not taken into account.

6. Conclusions : The findings of the study displayed that participants with SCD and SCT had elevated OS because they had considerably lower levels of VE, Se, and SOD when compared to HC. Elevated concentrations of TC, TG, and VLDL were also reported in SCD and SCT subjects, with HDL levels significantly lower than in HC. The results suggest that OS and dyslipidemia are prevalent in SCD and SCT individuals, highlighting the potential need for antioxidant and lipid-lowering therapies in managing these conditions. These biomarkers interact by exacerbating OS and lipid imbalances, which collectively contribute to the pathophysiology of SCD.

Acknowledgements: Conflict of Interest

The authors do not disclose any conflicts of interest. Ethical Consideration

According to the research committee's decision numbered (142/2023) on May 7, 2023, the current research was fully approved by the ethical consideration committee of the Training and Human Development Unit, Thi-Qar Health Department, Ministry of Health and Environment, Iraq.

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