

# Serum Antioxidant Status in Sickle Cell Disease Patients: Implications for Oxidative Stress and Disease Severity

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## Abstract

**Objectives:** The main aim of this subject is to determine the oxidative status of Iraqi sickle cell anemic patients and then correlated with various biomarkers.

**Methods:** In this study, blood samples from 100 sickle cell anemic subjects were analyzed, and then compared with control group which consisting of 50 individuals without sickle cell anemia was established. Various biochemical techniques were employed to measure different oxidative stress markers and inflammatory mediators. Serum samples were collected from blood to determine the levels of antioxidants such as catalase (CAT), glutathione peroxidase (G-Px), reduced glutathione (GSH), and lipid-peroxidation product malondialdehyde (MDA).

**Results:** The results revealed that the levels of serum antioxidant activity (CAT), GSH, and G-Px were significantly reduced with ( $P < 0.05$ ) in sickle cell anemic patients as compared with apparently control group. In contrast, the MDA level was significantly higher in sickle cell anemic patients than that found in the apparently control group.

**Conclusion:** In this work, there is an increased oxidative stress in sickle cell anemic patients, which is accompanied by a decrease in antioxidant activity and a rise in lipid peroxidation, leading to the intensification of sickle cell anemic symptoms in patients.

**Keywords:** Antioxidants, anemia, sickle cell, oxidative stress, reactive oxygen species

## Introduction

Sickle cell disease (SCD) is an inherited blood disorder brought on by a mutation in the B-globin gene, also known as hemoglobin subunit beta (HB-beta), which codes for a part of hemoglobin (Hb), the protein complex that makes up 70% of red blood cells (RBCs) and is in charge of carrying oxygen to all body organs.<sup>1,2</sup>

More than 300,000 babies each year were impacted; the United Nations (UN) and the World Health Organization (WHO) classify hereditary blood diseases like SCD as a global health concern. In addition to the United States and Europe, this illness is widespread in most of sub-Saharan Africa, the Middle East, India, the Caribbean, South and Central America, and several Mediterranean nations.<sup>3,4</sup>

Under some circumstances, such as dehydration, illness, or a lack of oxygen, the aberrant, sickled Hb (HbS) in SCD tends to polymerize in RBCs. RBCs are shaped like a sickle or a banana as a result of this process, as seen in [Figure 1](#), which also leads them to become hard and distorted. Sickle cell anemia phenotypic manifestation is a complicated pathophysiologic syndrome with various sources of pro-oxidant mechanisms and resulting chronic and systemic oxidative stress. Erythrocytes live in an environment of constant free radical production in healthy biological systems. To combat ROS, erythrocytes have a self-sustaining activity of antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (G-Px), reduced glutathione (GSH), and vitamins.<sup>5,6</sup>

Excessive ROS production overwhelms the blood's defenses and destroys biological macromolecules such as proteins, lipids, and DNA, altering the physical properties of RBC membrane, changing membrane permeability, and causing hemolysis.<sup>7</sup> However, reactive oxygen species (ROS) and

byproducts of their oxidative processes may be used to predict the severity of SCD.<sup>8</sup> The repeated polymerization and depolymerization of HbS molecules, on the other hand, results in increased levels of reactive oxygen species (ROS), which can cause a cyclic cascade characterized by blood cell adhesion, hemolysis, increased susceptibility to infections, chronic inflammatory diseases, and microvascular damage in organs, resulting in a decrease in quality of life and life expectancy.<sup>9</sup> In SCD patients, sickle erythrocytes are the primary source of pro-oxidants; the unstable, autooxidative HbS and rapid metabolic turnover caused by recurrent HbS polymerizations and depolymerizations stimulate ROS formation.<sup>10</sup>

Oxidative stress plays an important role in the pathophysiology of SCD and its effects.<sup>11</sup> The higher oxidative burden in SCD patients has been linked to a variety of factors. A high level of cell-free hemoglobin, a prolonged inflammatory state, increased HbS auto-oxidation, and iron overload are only a few of the processes.<sup>12</sup> According to previous studies,<sup>13</sup> higher levels of reactive oxygen species (ROS) have been linked to a variety of SCD-related problems. Furthermore, it has been proposed that an altered oxidant/antioxidant balance and increased oxidative stress have a role in the etiology of a variety of disorders in SCD patients.<sup>14</sup> ROS levels rise due to variables such as increased intravascular hemolysis and continuing inflammation.<sup>15</sup> Even though the body has its own system for combating excessive ROS, the antioxidant defense may be overwhelmed by the vast pool of ROS and may not effectively negate their effects in SCD patients.<sup>13</sup>

Despite the fact that various studies have looked at the pathophysiology of SCD in Iraq, there is very little information on oxidative stress. This study was carried out to bridge a knowledge gap and add to the body of knowledge on oxidative stress and antioxidants in SCD patients. The current study's goal was to examine blood antioxidant parameters in healthy

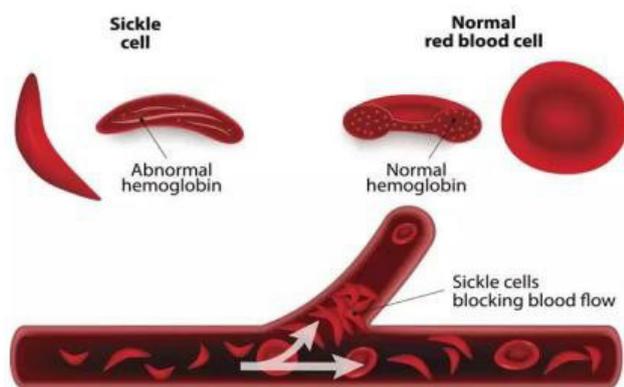


Fig. 1 Blood flow of sickle cell.<sup>16</sup>

Table 1. Methods employed for GSH level determination

Reagents	Test	Standard	Blank
Sample	100 µl	–	–
Phosphate buffer	900 µl	1000 µl	3000 µl
Hydrogen peroxide	2000 µl	2000 µl	–
Mix with vortex and incubate at 37°C for 2 min, after that, add:			
Vanadium reagent	2000 µl	2000 µl	2000 µl

After that, the tubes were kept at 25°C for 10 min. the changes in absorbance were recorded at 452 nm against the reagent blank.

controls and Iraqi SCA patients in clinical stability in order to learn more details about these patients' antioxidant systems.

## Materials and Methods

Participants with sickle cell disease (SCD) and age-matched healthy controls were included in this study. A consistent medical procedure was used to collect thorough demographic and clinical information on all subjects. The study comprised 50 SCD patients with age ranged between (15–60 years) and 50 healthy people as controls with matched age range. After describing the nature of the study to all participants and, for minors, their parents, informed written agreement was acquired. Blood samples (5 mL) were taken from each participant using gel-coated tubes for biochemical parameter analysis. The blood was centrifuged at  $2450 \times g$  for 10 minutes to extract the plasma, which was kept at  $-80^{\circ}\text{C}$  until use. The plasma antioxidants, including catalase (CAT), were quantified using a spectrophotometer in accordance with previously reported techniques, see Table 1.<sup>17</sup>

The GSH was determined by using a modified procedure that employed Ellman's reagent (DTNB). Standard and sample test tubes were prepared according to the protocol described by Kapoor and Kakkar.<sup>18</sup>

Reagents	Sample µL	Reagent blank µL	Standard µL
Sample	100	–	–
Standard	–	–	100
DDW	800	900	100
TCA	100	100	100

G-Px activity levels was determined using a previously described spectro-photometric method, as outlined by

Rotruck.<sup>19</sup> To measure MDA levels, 100 µl of sample was added to a test tube containing 2 ml of a working solution prepared as follows: 0.514 g of TBA, 25 g of TCA, and 0.5 ml of 1M HCl were mixed with 190 ml of distilled water (D.W.), followed by the addition of 1 g of SDS and completed to a volume of 200 ml. The sample was vortex and heated in a  $90^{\circ}\text{C}$  water bath for 50 min and then allowed to cool.

After centrifuging the sample for 5 minutes at 5000 rpm, the absorbance of the supernatant was measured spectrophotometrically at 532 nm against a reagent blank. The reagent blank was prepared in the same manner as above, except distilled water was substituted for the sample. Descriptive statistics were performed on the data for each group using IBM SPSS Statistics software, version 28.0 (IBM, SPSS, Chicago, Illinois, USA). Categorical variables were presented as  $n$  (%), while scale variables were expressed as mean  $\pm$  standard deviation. Analytical statistical tests were used to confirm significant differences in categorical variables among the parameters. Results with  $P$ -value  $< 0.05$  (two-tailed) were considered statistically significant.

## Results

In this study, blood samples were collected from a total of 50 sickle cell disease (SCD) patients comprising 27 females and 23 males with age range between 15 to 60 years. In addition, a control group of 50 individuals without SCD was included in the study, with 24 females and 26 males and age range of 15 to 60 years. The participants with SCD were assessed for their marital status and family history of SCD using a questionnaire, and the results are presented in Figures 2–4.

Table 2 presents significant differences in the levels of G-Px, MDA, and GSH among the study groups, with  $P$  values  $< 0.001$ , respectively. Sickle cell disease (SCD) patients exhibited a reduction in antioxidant defense mechanisms, leading to damage to cellular organelles and enzymes and an increase in lipid peroxidation.<sup>20</sup>

The amount of G-Px activity was considerably lower in SCD sufferers compared to healthy persons in the current investigation. In a similar way GSH activity was considerably lower in SCD participants as compared to healthy subjects, which is consistent with the findings of.<sup>21</sup> This reduction in G-Px and GSH activity might be attributed to an increase in reactive oxygen species (ROS), which leads to  $\text{H}_2\text{O}_2$  accumulation.  $\text{H}_2\text{O}_2$  is created by two electron transfers or by sickling, and it is eliminated by two major antioxidants, G-Px and CAT.

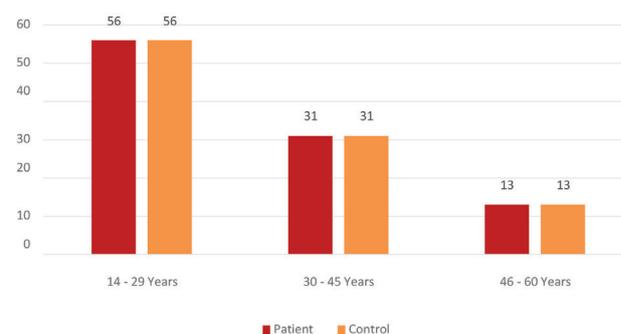


Fig. 2 Frequency of the study population in cases of sickle disease compared to control group according to age groups ( $N = 100$ ) of the age group.



Fig. 3 Frequency of the study population in cases of sickle disease compared to control group according to marital status and gender (N= 100).

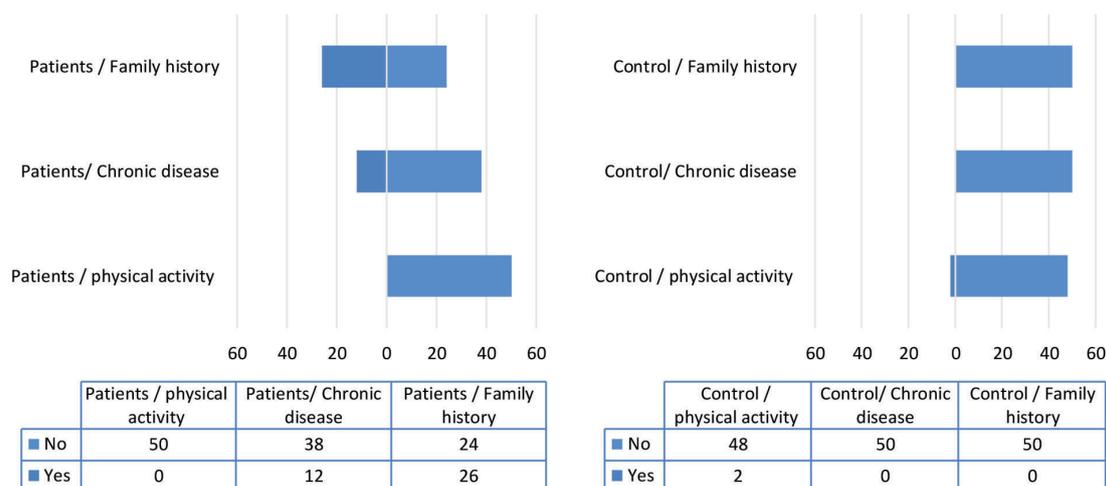


Fig. 4 Frequency of among family history, chronic disease and physical activity of the study population in (A) cases of sickle disease compared to (B) control group (N = 100).

Table 2. Mean differences of biomarkers in cases of sickle disease compared to control group

Variables	Patient (N = 50) Mean ± SD	Control (N = 50) Mean ± SD	P value
CAT, U/L	0.33 ± 0.13	0.36 ± 0.14	0.204[NS]
G-Px, U/L	334.41 ± 112.63	400.51 ± 72.53	<0.001[S]
MDA, U/L	2.16 ± 1.50	1.44 ± 0.44	0.002[S]
GSH, U/L	18.85 ± 2.56	22.93 ± 2.80	<0.001[S]

Results are presented as mean ± SD, P < 0.05 considered significantly different, [S] = Significant, [NS] = non-significant-test.

The current study's findings are consistent with prior studies, indicating that the lower levels of GSH and G-Px are attributable to increased oxidative stress.

In contrast, MDA levels in the serum of sickle cell anemia (SCA) patients were considerably higher than in healthy controls. This rise in MDA levels might be attributable to increased ROS generation in SCA patients, and MDA levels provide

information about the amount of oxidative damage in cells, which is consistent with the findings of.<sup>22,23</sup> Other studies, however, have found a statistically significant reduction in MDA in older SCA patients. Furthermore, no association was observed between age, SCA patients, and healthy people.<sup>24</sup>

In Table 3, Based on gender groups, only MDA levels showed a significant difference; the P value was <0.01 and also consistent with the study of.<sup>20</sup> While based on age groups, no significant differences were found in the levels of measured biomarkers with P values > 0.05, as presented in Table 4.

In order to investigate the interplay between the measured biomarkers and their potential role in the progression of the case study, a multivariable linear regression model was utilized to analyze the response relationship between parameters. The results indicated that serum CAT levels were significantly and positively correlated with G-Px and GSH levels, while exhibiting a negative correlation with MDA levels. These findings were consistent with the results reported by previous studies,<sup>25,26</sup> with p-values <0.001. Furthermore, a weakly significant correlation was observed between G-Px and CAT and GSH activity levels. Additionally, serum GSH levels showed

Table 3. Mean differences of biomarkers in cases of sickle disease compared to control groups based on gender

Variables	Male (N = 50) Mean ± SD	Female (N = 50) Mean ± SD	P value
CAT U/L	0.32 ± 0.13	0.36 ± 0.13	0.135[NS]
GPX U/L	363.00 ± 102.22	371.74 ± 98.50	0.664[NS]
MDA U/L	2.10 ± 1.27	1.51 ± 0.97	<b>0.011[S]</b>
GSH U/L	20.89 ± 3.49	20.89 ± 3.28	0.997[NS]

Results are presented as mean ± SD,  $P < 0.05$  considered significantly different, [S] = Significant, [NS] = non-significant. T-test.

Table 4. Correlations of the Biochemical parameters among patients' groups

Variables	CAT	GPX	MDA	GSH
CAT, U/L	1	<b><math>r = 0.3</math></b> <b><math>P = 0.003</math></b>	$r = -0.91$ $P = <0.001$	<b><math>r = 0.4</math></b> <b><math>P = &lt;0.001</math></b>
G-Px, U/L	<b><math>r = 0.3</math></b> <b><math>P = 0.003</math></b>	1	$r = -0.1$ $P = 0.540$	<b><math>r = 0.2</math></b> <b><math>P = 0.017</math></b>
MDA, U/L	$r = -0.2$ $P = 0.119$	$r = 0.1$ $P = 0.540$	1	$r = 0.1$ $P = 0.998$
GSH, U/L	<b><math>r = 0.4</math></b> <b><math>P = &lt;0.001</math></b>	<b><math>r = 0.2</math></b> <b><math>P = 0.017</math></b>	$r = 0.1$ $P = 0.998$	1

Results are presented as mean ± SD,  $P < 0.05$  considered significantly different, [S] = Significant, [NS] = non-significant. ANOVA test.

significant positive correlations with CAT and G-Px levels (all  $P < 0.05$ ). The detailed relationships between the parameters are presented in Table 3.

The CAT level in SCD subjects has been a subject of inconsistency in various studies. While a few studies have reported a decrease in CAT activity in SCD patients, as seen in our research, others have seen a rise in CAT levels.<sup>27,28</sup> Elevated CAT levels might be ascribed to a preventative function to scavenge  $H_2O_2$ , whereas lower levels could be related to catastrophic and sustained oxidative stress, where it has been reported by that low level of serum total antioxidant and inhibition of serum GSH, GPX and CAT are involved in many diseases according to this study.<sup>29</sup>

## Discussion

The results of this study indicate that the presence of HbS in erythrocyte of SCD subjects not only causes biochemical alterations of biomolecules but also is responsible for increases in free radical production. The imbalance between overproduction of free radicals and inability of cellular content of antioxidants to eliminate leads to the oxidative stress in patients of SCD,<sup>30</sup> so antioxidants are important in scavenging free

radicals and non-radical oxidants as well as protecting cells from oxidative stress.<sup>31</sup> Antioxidants, which include both tiny molecules like GSH and thioredoxin and enzymes like SOD, GPx, and CAT, counteract the stress induced by reactive radicals, also known as oxidative stress, and protect cellular biomolecules.

According to the antioxidant defense mechanisms analysis of the current study's results and because of the mechanisms of oxidative stress in sickle cell disease, the plasma biomarker plasma concentration assay was able to differentiate between sickle cell anemia patients and healthy controls. While sickle cell disease patients in this study had significantly higher serum MDA levels than the controls, the erythrocyte CAT level in HbSS in the steady state was significantly lower, and another outcome of the study was that SCD patients had significantly lower mean erythrocyte GSH, GPx levels compared with controls group.

## Conclusion

There is an increase in oxidative stress in sickle cell anemic patients, which is accompanied by a decrease in antioxidant activity and an increase in lipid peroxidation, leading to an intensification of sickle cell anemic symptoms in patients, according to this study.

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## Compliance with Ethical Standards

## Conflict of Interest

The authors warrant that they don't have any competing interests to declare.

## Ethical Approval

All procedures involving human participants in research projects were conducted in compliance with the ethical standard of the research committee of Kerbala University, as well as the 1964 Helsinki declaration and any revisions or other ethical standards deemed equivalent.

## Informed Consent

Consent to participate in the study was received from each individual person who took part in the research. ■

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