DOI: 10.7860/JCDR/2022/58439.17182

Original Article

Biochemistry Section

Assessment of Oxidative Stress Indicators among Patients with Sickle Cell Anaemia versus Non Sickle Cell Iron Deficiency Anaemia: A Case-control Study

RINA RAIBHAN WASNIK[1], MAHAVIR RAGHUNATH MUNDRA[2], PRASHANT VISHRAM PAUNIPAGAR[3], ARCHANA DHOK[4], ASHISH ANJANKAR[5], NILKANTH AKARTE[6]

ABSTRACT

Introduction: In sickle cell anaemia, haemolysed iron is reutilised, moreover, iron stores are increased owing to the blood transfusions, hence, iron deficiency is less common in sickle cell anaemia. But iron decompartmentalisation, due to unstable haemoglobin, is thought to be the cause for oxidative stress. Hence, iron deficiency anaemia and sickle cell anaemia need to be compared and studied to provide better treatment and awareness.

Aim: To compare the oxidative stress, antioxidants and iron indices among patients with Sickle Cell Anaemia (SCA) and Iron Deficiency Anaemia (IDA).

Materials and Methods: A case-control study was conducted at Jawaharlal Nehru Medical College, Wardha, Maharashtra, India, from January 2016 to December 2018. Total 240 SCA patients {120 SS (homozygous disease) and 120 AS (heterozygous trait)}, 120 patients with IDA and 120 age-matched and sex-matched controls were included in present study. Oxidative stress markers Malondialdehyde (MDA), Catalase, Superoxide Dismutase (SOD), vitamin C and vitamin E, zinc and iron indices {Iron, Total Iron Binding Capacity (TIBC) and serum ferritin} were analysed. Data was analysed using descriptive and inferential statistics using Analysis of Variance (ANOVA).

Results: The mean age of SS patients was 19.01±11.43, AS was 22.01±11.78 years, control was 22.63±12.17 years, and IDA was 18.50±11.46. There was an equal distribution of both males and females in all groups. The mean of haemoglobin in SS was 7.40 g/dL, AS was 10.31 g/dL, IDA was 8.63 g/dL, and control was 14.81 g/dL. The mean value of mean corpuscular volume for SS was 65.71 fL, AS was 65.64 fL, IDA was 72.38 fL, and control was 87.68 fL. Zinc for SS was 82.65 µg/dL, AS was 82.13 µg/dL, IDA was 43.33 µg/dL, and control was 103.99 µg/dL.

Conclusion: There is impairment in the oxidant and antioxidant status among patients with sickle cell anaemia with and without IDA.

Keywords: Antioxidants, Catalase, Ferritin, Malondialdehyde, Superoxide dismutase

INTRODUCTION

The stress negatively impact the mental and physical health of the people and influence the daily life routine. The biomarkers like Superoxide Dismutase (SOD) and catalase influence the level of oxidative stress. Oxidative stress is crucial in the pathogenesis of Sickle Cell Disease (SCD) and its consequences. Oxidative stress, which is frequently encountered by SCD patients as a result of the constant creation of reactive oxygen species (ROS), can result in endothelial dysfunction and acute inflammation. Antioxidant enzymes such as Superoxide Dismutase (SOD) and Catalase (CAT) are frequently protective [1].

Ferritin, a metabolic antioxidant produced during iron metabolism, is a high molecular weight protein that contains approximately 20% iron as storage [2]. The high molecular weight protein is present in all types of tissues in human. The major impact of these protein is identified in the hepatocytes and reticuloendothelial cells, which are responsible for the iron storage and provide iron supply to the body tissues [3]. Iron insufficiency is less common in anaemia as the iron generated by haemolysis is used more efficiently. The iron stores may also be restored as a result of repeated blood transfusions [4]. On the other hand, iron decompartmentalisation due to unstable haemoglobin is believed to be the cause for oxidative stress [5]. To provide a better management, in people with Sickle Cell Anaemia (SCA), iron homeostasis must be investigated. Thus, a cross-sectional comparison of iron deficiency anaemia and sickle cell anaemia is required [6]. In SCA patients, iron deficiency anaemia needs to be addressed, and additional supplements are cautiously provided in order to enhance the patient's health [7].

There are different types of antioxidants and markers which are considered for the analysis and identification of the SCA severity-zinc, vitamin C and E, serum iron, ferritin and Total Iron Binding Capacity (TIBC). The status of iron in the body leads to the stress due to lack of antioxidants in the blood cells and increase the severity of sickle cell diseases. Therefore, it can be considered that biochemical markers of oxidative stress in sickle cell anaemia is having a significant impact on the effects of antioxidants on the status of iron metabolism and consequent relief to the sickle cell anaemia patients [8].

A vicious cycle of building cascade and cumulative effects of deoxygenation, polymerisation, ischaemia reperfusion, inflammation, and painful vaso-occlusion crises lead to increased synthesis of Reactive Oxygen Species (ROS) in SCD [9]. Evidence is found on marked increase in oxidative stress in both 'SS' and 'AS' types of sickle cell anaemia [9]. While, iron deficiency anaemia being more prone in the latter subtype [10]. Ferritin in children with sickle cell anaemia implies a relatively higher iron content in the reticuloendothelial cells [11].

As no similar study is done in the comprehensive role of the desired parameters in the Wardha region of central India, the present research was conducted to study the relationship of oxidative stress, antioxidants and iron status in sickle cell diseases patients along with sickle cell heterozygous cases in the region of Wardha and adjoining districts for comparative evaluation with non sickle cell iron deficiency anaemia.

MATERIALS AND METHODS

This case-control study was conducted from January 2016 to December 2018 in the Department of Biochemistry at Jawaharlal Nehru Medical College and its Teaching Hospital Acharya Vinoba Bhave Rural Hospital (AVBRH), Wardha, Maharashtra, in coordination with Paediatrics, General Medicine, Community Medicine and Pathology Departments. Present study was carried, as per the ethical guidelines with Institutional Ethical Clearance {DMIMS(DU) IEC/2015-16/1565}.

Inclusion criteria: All the patients attending Outpatient Department/ Inpatient Department of Medical college and Hospital based on the haemoglobin electrophoresis using Marringo technique [12] and Nalbundian solubility test [13] as well as haematological parameters carried by Sysmex blood counter were included in the study. Iron deficiency anaemia cases were diagnosed on the basis of low Haemoglobin (Hb), low Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), and Mean Corpuscular Haemoglobin Concentration (MCHC) were further confirmed by low serum ferritin levels [14,15]. For diagnosis of iron deficiency in SCD serum ferritin below 25 ng/mL and low MCV were considered as most useful screening test [16]. IDA patients were ruled out for SCA by Hb electrophoresis and solubility test.

Exclusion criteria: Patients with chronic diseases, ischemic heart disease, diabetes mellitus and sickle cell patients with acute crises or recent blood transfusion within three months were excluded from the study.

Sample size calculation: The study sample size was calculated using the formula:

$$n=\frac{4pq}{E^2}$$

Where, p is prevalence of oxidative stress among SCD, was considered as 22.5% [17], q is 100-p, E is anticipated relative precision (20% of p).

Therefore, the sample (n):

$$n=\frac{4\times22.5\times77.5}{4.5^2}=344$$

As there are three groups, each group should be consisting minimum of 115 participants. Total 480 subjects after written informed consent were enrolled in study. Out of total 480 subjects:

- SS homozygous trait (n=120): Sickle cell anaemia cases.
- AS heterozygous trait (n=120): Sickle cell anaemia cases.
- Iron Deficiency Anaemia (IDA) (n=120): Non sickle cell disease.
- Control (n=120): Age and sex matched normal healthy individuals.

Study Procedure

Under aseptic precaution 4 mL of venous blood was collected in plain as well as 4 mL in heparin bulb and analysed for following parameters:

- Complete Blood Count (CBC) parameter were analysed by sysmex cell counter.
- The concentrations of iron and TIBC in serum were measured by Garcic A, Ferrozine method, using Human diagnostics Worldwide-Wiesbaden-Germany kit using ROBOnik prietest eXP biochemistry analyser [18].
- Serum ferritin was estimated by Enzyme-Linked Immunosorbent Assay (ELISA) method [19], using DiaMetra Milano-Italy Diagnostics UK, kits using B4B Diagnostic divisions ELISA reader.
- Zinc was measured using, Centronic India Diagnostic Pvt. Ltd. kit using ROBONik prieteste XP biochemistry analyser [20].
- Superoxide Dismutase (SOD) [21] was measured using Randox kit using ROBONik prieteste XP biochemistry analyser.
- Ascorbic acid (vitamin C) level was measured by the method of Aye Kyaw (1978) [22].
- Vitamin E was assayed by colorimetric method using B4B Diagnostic divisions ELISA reader [23].
- Malondialdehyde (MDA): Serum MDA was estimated by Thiobarbituric acid assay using ELICO SL 244 UV-VIS/BL 198 BIO spectrophotometer and result obtained was expressed in mmol/L [24].
- Catalase was assayed by commercially available Cayman's Catalase Assay kit using Robonik Elisa reader for determining formaldehyde concentration [25].

Normal range for all the parameters are given in [Table/Fig-1] [18-25].

Parameters	Normal range
Iron, TIBC (µg/dL)	Iron: 37-148, TIBC:274-385
Serum ferritin (ng/mL)	10-350
Zinc (µg/dL)	70-115
Superoxide dismutase (U/mL)	164-240
Vitamin C (mg/dL)	0.6-2
Vitamin E (µg/mL)	8-15
Malondialdehyde (µmol/L)	2-5
Catalase (nmol/min/mL)	32-68

[Table/Fig-1]: Reference ranges for various parameters used in the study [18-25].

STATISTICAL ANALYSIS

For the data analysis of the study, different types of tests were used such as Z, Chi-square and Pearson's correlation coefficient. Statistical Package for Social Sciences (SPSS) version 25.0 software was used for statistical analysis. The graph and pad prism 5.0 and p-value ≤0.05 was considered as level of significance.

RESULTS

The gender distribution within the sample of the study involved 240 (50%) males and 240 (50%) females. There were 60 each for both males and females in SS, AS, IDA and control group [Table/Fig-2]. Haemoglobin level was low among SS group followed by IDA and AS as compared to controls, whereas MCV, MCH and MCHC were the lowest for both SS and AS group followed by IDA and control group [Table/Fig-3].

Variables	SS (Mean±SD)	AS (Mean±SD)	IDA (Mean±SD)	Control (Mean±SD)	p-value
Mean age (in years)	19.01±11.43	22.01±11.78	18.50±11.46	22.63±12.17	>0.05
Gender distribution					
Male	60	60	60	60	>0.05
Female	60	60	60	60	

[Table/Fig-2]: Age and gender distribution.

Variables	SS (Mean±SD)	AS (Mean±SD)	IDA (Mean±SD)	Control (Mean±SD)	p-value (Chi-square test)
Haemoglobin (g/dL)	7.40±1.58	10.31±1.44	8.63±0.87	14.81±1.6	<0.001
MCV (fL)	65.71±5.78	65.64±5.63	72.38±4.87	87.68±4.26	<0.001
MCH (pg)	29.55±1.56	29.71±1.46	25.57±0.90	30.45±1.74	<0.001
MCHC (g/dL)	33.84±1.75	33.73±1.79	32.05±1.17	33.05±1.10	<0.001

[Table/Fig-3]: Comparison of haematological parameters.

The mean serum iron was highest in control group, followed by the IDA, AS and SS group. The TIBC level was highest among IDA group followed by SS, AS, and was lowest among controls. The difference for mean serum iron, TIBC and ferritin among groups was found significant [Table/Fig-4]. Mean vitamin C and vitamin E

Indicators	SS (n=120) (Mean±SD)	AS (n=120) (Mean±SD)	IDA (n=120) (Mean±SD)	Control (n=120) (Mean±SD)	p-value (Chi-square test)
Serum Iron (µg/dL)	63.75±11.77	64.99±11.15	82.75±10.85	114.65±32.14	<0.001
Total iron binding capacity (µg/dL)	364.28±32.96	360.69±35.89	499.11±23.31	321.18±44.94	<0.001
Ferritin (ng/mL)	94.16±3.96	95.89±2.43	40.40±13.04	117.09±39.17	<0.001

[Table/Fig-4]: Comparison of iron indices.

Indicators	SS (n=120) (Mean±SD)	AS (n=120) (Mean±SD)	IDA (n=120) (Mean±SD)	Control (n=120) (Mean±SD)	p-value (Chi-square test)
Superoxide dismutase (U/mL)	318.81±11.4	252.39±14.19	215.61±8.02	199.28±12.03	<0.01
Zinc (µg/dL)	82.65±4.25	82.13±4.47	43.33±4.05	103.99±3.26	<0.001
Vitamin C (mg/dL)	0.442±0.0243	0.638±0.0257	0.586±0.1184	0.926±0.0445	<0.001
Vitamin E (µg/mL)	8.62±.63	10.22±.45	6.48±.59	11.47±.58	<0.001
Malondialdehyde (mmol/L)	4.15±0.09	2.45±0.16	4.11±0.75	1.81±0.11	<0.001
Catalase (nmol/min/mL)	34.68±0.56	34.58±0.53	36.33±1.39	39.69±0.14	<0.001

[Table/Fig-5]: Comparison of antioxidant's enzymes, vitamins and oxidative stress markers.

level was significantly higher among controls followed by the AS group. Superoxide dismutase and zinc level was significantly higher among controls followed by the SS group and was lowest among IDA group [Table/Fig-5].

Statistically significant mean difference serum ferritin levels was noted among IDA and other groups. The mean difference in ferritin levels was especially significant among the IDA in comparison with normal individuals. The difference in the mean levels of serum iron was statistically significant among the IDA and other groups, while there was no significant variation in the mean difference noted among the individuals with SS and AS. The mean difference among the IDA and control group was significantly variable than any of the other groups. This mean difference was noted to be statistically significant (p-value <0.001) [Table/Fig-6].

The mean difference in the levels of superoxide dismutase was significantly variable among each of the four groups. Statistically

Parameter	Group	Group comparison	Mean difference (95% CI)	p-value
Ferritin (ng/mL)	SS	AS	-1.73 (-10.89 to 7.42)	0.917
		IDA	53.75 (26.90 to 80.60)	<0.001
		Control	-22.93 (-100.49 to -54.63)	<0.001
	AS	SS	1.73 (-7.42 to 10.89)	0.917
		IDA	55.48 (29.35 to 81.61)	<0.001
		Control	-21.20 (-28.26 to-14.14)	<0.001
	IDA	SS	-53.75 (-80.60 to-26.90)	<0.001
		AS	-55.48 (-81.61 to-29.35)	<0.001
		Control	-76.68 (-158.01 to-4.65)	<0.001
	Control	SS	22.93 (100.49 to 54.63)	<0.001
		AS	21.20 (14.14 to 28.26)	<0.001
		IDA	76.68 (4.65 to 158.01)	<0.001
Serum Iron (µg/dL)	SS	AS	-1.24 (-4.16 to 1.68)	0.956
		IDA	-19.00 (-21.88 to-16.12)	<0.001
		Control	-50.91 (-57.06 to-44.74)	<0.001
	AS	SS	-1.24 (-4.16 to 1.68)	0.956
		IDA	-17.76 (-20.56 to-14.96)	<0.001
		Control	-49.66 (-55.78 to-43.54)	<0.001
	IDA	SS	-19.00 (-21.88 to-16.12)	<0.001
		AS	17.76 (14.96 to 20.56)	<0.001
		Control	-31.90 (-38.00 to-25.80)	<0.001
	Control	SS	-50.91 (-57.06 to-44.74)	<0.001
		AS	49.66 (43.54 to 55.78)	<0.001
		IDA	31.90 (25.80 to 38.00)	<0.001
Total iron binding capacity (µg/dL)	SS	AS	3.58 (-8.22 to 15.39)	0.826
		IDA	-134.83 (-146.64 to-123.02)	<0.001
		Control	43.09 (31.28 to 54.90)	<0.001
	AS	SS	-3.58 (-15.39 to 8.22)	0.862
		IDA	-138.41 (-150.22 to-126.60)	<0.001
		Control	39.51 (27.70 to 51.32)	<0.001
	IDA	SS	134.83 (123.02 to 146.64)	<0.001
		AS	138.41 (126.60 to 150.22)	<0.001
		Control	177.93 (166.12 to 189.73)	<0.001
	Control	SS	-43.09 (-54.90 to-31.28)	<0.001
		AS	-39.51 (-51.32 to-27.70)	<0.001
		IDA	-177.93 (-189.73 to-166.12)	<0.001

[Table/Fig-6]: Pair-wise comparative study of iron indices.

significant mean difference in the zinc and catalase levels was noted among IDA and other groups. The mean difference in zinc and catalase levels was especially significant among the IDA in comparison with normal individuals. The difference in the mean levels of vitamin C and vitamin E was statistically significant among all the groups, while there was no significant variation in the mean difference of malondialdehyde among the individuals with SS and AS. The mean difference among the IDA and control group was significantly variable than any of the other groups. This mean difference was noted to be statistically significant (p-values <0.001) [Table/Fig-7].

DISCUSSION

The study was conducted to explore the role of various indicators including iron metabolites, enzymes and antioxidants. The study was successfully and conclusively able to establish the relationship of these parameters with reference to the homozygous and heterozygous sickle cell disorder and iron deficiency anaemia in comparison with the normal individuals. The present study findings correlate with those of Gabra N et al., where a significant decrease in ferritin levels were observed in homozygous SCA compared to control [26]. Lower ferritin among SCA might be due to increased mobilisation and utilisation of ferritin iron for new compensatory erythropoiesis in view of exaggerated haemolysis.

When sickle cell patients were compared to healthy controls, the GSH system, which is made up of reduced and total glutathione, revealed a severe deficit [27]. Vitamins E and C are also a part of this system. The current study examined zinc, vitamin C and vitamin E where significant decrease was found in SS patients compared to control. The study conducted by Antwi-Boasiako C et al., and Gizi A et al., have shown similar results [28,29].

Parameters	Group	Group comparison	Mean difference (95% CI)	p-value
Superoxide dismutase (U/mL)	SS	AS	66.42 (34.33 to 98.51)	<0.001
		IDA	103.20 (75.03 to 131.37)	<0.001
		Control	119.53 (86.28 to 152.78)	<0.001
	AS	SS	-66.42 (-98.51 to -34.33)	<0.001
		IDA	36.78 (4.67 to 68.89)	<0.001
		Control	53.11 (16.46 to 89.76)	<0.001
	IDA	SS	-103.20 (-131.37 to -75.03)	<0.001
		AS	-36.78 (-68.89 to -4.67)	<0.001
		Control	16.33 (12.15 to 44.81)	<0.001
	Control	SS	-119.53 (-152.78 to -86.28)	<0.001
		AS	-53.11 (-89.76 to -16.46)	<0.001
		IDA	-16.33 (-44.81 to -12.15)	<0.001
Zinc (µg/dL)	SS	AS	0.51 (-11.63 to 12.67)	0.756
		IDA	39.31 (27.77 to 50.87)	<0.001
		Control	-21.34 (-31.89 to -10.79)	<0.001
	AS	SS	-0.51 (11.63 to -12.67)	0.756
		IDA	38.80 (26.93 to 50.67)	<0.001
		Control	-21.86 (-32.76 to -10.96)	<0.001
	IDA	SS	-39.31 (-50.87 to -27.77)	<0.001
		AS	-38.80 (-50.67 to -26.93)	<0.001
		Control	-60.66 (-70.89 to -50.43)	<0.001
	Control	SS	21.34 (10.79 to 31.89)	<0.001
		AS	21.86 (10.96 to 32.76)	<0.001
		IDA	60.66 (50.43 to 70.89)	<0.001
Vitamin C (mg/dL)	SS	AS	-0.20 (-0.27 to -0.13)	<0.001
		IDA	-0.14 (-0.39 to -0.09)	<0.001
		Control	-0.48 (-0.58 to -0.40)	<0.001
	AS	SS	0.20 (0.13 to 0.27)	<0.001
		IDA	0.05 (0.03 to 0.07)	<0.001
		Control	-0.29 (-0.39 to -0.19)	<0.001
	IDA	SS	0.14 (0.09 to 0.39)	<0.001
		AS	-0.05 (-0.07 to -0.03)	<0.001
		Control	-0.34 (-0.59 to -0.09)	<0.001
	Control	SS	0.48 (0.40 to 0.58)	<0.001
		AS	0.29 (0.19 to 0.39)	<0.001
		IDA	0.34 (0.09 to 0.59)	<0.001
Vitamin E (µg/mL)	SS	AS	-1.59 (-3.13 to -0.07)	<0.001
		IDA	2.14 (0.44 to 3.84)	<0.001
		Control	-2.85 (-4.54 to -1.16)	<0.001
	AS	SS	1.59 (0.08 to 3.13)	<0.001
		IDA	3.73 (2.28 to 5.20)	<0.001
		Control	-1.26 (-2.70 to -0.20)	<0.001
	IDA	SS	-2.14 (-3.84 to -0.44)	<0.001
		AS	-3.73 (-5.20 to -2.28)	<0.001
		Control	-4.99 (-6.62 to -3.36)	<0.001
	Control	SS	2.85 (1.16 to 4.54)	<0.001
		AS	1.26 (0.20 to 2.70)	<0.001
		IDA	4.99 (3.36 to 6.62)	<0.001
Malondialdehyde (µmol/L)	SS	AS	1.70 (1.35 to 2.07)	<0.001
		IDA	0.04 (-1.45 to 1.53)	0.850
		Control	2.34 (2.06 to 2.62)	<0.001
	AS	SS	-1.70 (-2.07 to -1.35)	<0.001
		IDA	-1.66 (-3.18 to -0.16)	<0.001
		Control	0.64 (0.25 to 1.01)	<0.001
	IDA	SS	-0.04 (-1.45 to 1.53)	0.850
		AS	1.66 (0.16 to 3.18)	<0.001
		Control	2.30 (0.81 to 3.79)	<0.001
	Control	SS	-2.34 (-2.62 to -2.06)	<0.001
		AS	-0.64 (-1.01 to -0.25)	<0.001
		IDA	-2.30 (-3.80 to -0.81)	<0.001
Catalase (nmol/min/mL)	SS	AS	0.10 (-1.42 to 1.62)	0.758
		IDA	-1.65 (-4.60 to -1.30)	<0.001
		Control	-5.01 (-6.15 to -3.88)	<0.001
	AS	SS	-0.10 (-1.42 to 1.62)	0.758
		IDA	-1.76 (-4.68 to -1.18)	<0.001
		Control	-5.11 (-6.19 to -4.03)	<0.001
	IDA	SS	1.65 (1.30 to 4.60)	<0.001
		AS	1.76 (1.18 to 4.69)	<0.001
		Control	-3.36 (-6.11 to -0.61)	<0.001
	Control	SS	5.01 (3.88 to 6.15)	<0.001
		AS	5.11 (4.03 to 6.19)	<0.001
		IDA	3.36 (0.61 to 6.11)	<0.001

[Table/Fig-7]: Comparative analysis of enzymatic antioxidant/antioxidant vitamins.

Zinc reduces oxidative stress. In patients with SCD, deficiency of zinc could be due to excessive haemolysis and excess excretion which results from oxidative stress, increase demand and less intake in adolescents. The decrease in vitamin C and E could be due to deficiency resulting from inadequate intake, increased demand, and increased excretion [30,31].

Other studies reported that SCA patients have lower leukocyte and vitamin C levels than healthy cases [32]. From this study it can be considered that the level of vitamin C has a direct impact on the approach of an individual and affecting the level of serum ferritin concentrate. Proper diet and intake of vitamin C, is helpful for the individual to avoid such issues and maintaining the healthy life style.

The high levels of MDA get accumulated and disturbs Red Blood Cell (RBC) phospholipid bilayer [33,9]. The oxidation of phospholipids hampers the function of plasma as well as internal organelle and also membrane damage contributes towards irreversible sickling [34]. Macrophagic erythrophagocytosis due to excess MDA [35] and as HbS is exposed to more endogenous oxidants further increase the cascade of lipid peroxidation [36]. This enhances the oxidative stress and contributes to pathogenies of sickle cell diseases.

A few studies showed that MDA levels were increased in SCA as compared with control. According to Titus J et al., there was a significant difference in the mean levels of superoxide dismutase in all groups, which is consistent with the results of the current investigation [9,33]. It can be concluded that superoxide level affects their risk of developing SCD, and a larger level of this component increases that risk. Common RBC components like superoxide dismutase and catalase aid in disease prevention. A rise in SOD may neutralise the flow of superoxide ions that expose sickled RBCs to more hydrogen peroxide [29].

Catalase levels were found to be significantly lower in patients with SCD (SS and AS) compared to control. Similar findings were reported by other too [37,38]. Biswal S et al., reported decreased GPx levels in children with SCD [38]. Catalase is involved in neutralisation of H_2O_2 formed as a result of action of SOD. Catalase plays a major role in neutralisation of H_2O_2 without using cellular reducing equivalents. Decreased levels observed in the present study could be the result of prolonged oxidative stress present in these patients. Superoxide dismutase and catalase are common components of RBCs that help in the protection from disorders. Increase in SOD might dismutate the increase flux of superoxide ions which exposes sickled RBCs to higher hydrogen peroxide [29].

Limitation(s)

Interventional study with conjugational therapeutic use of antioxidants and long term follow-up with large number of subjects could have enhanced the evidence generated by the present research work.

CONCLUSION(S)

Findings of the present study, suggest that there is impairment in the oxidant and antioxidant status among patients with sickle cell anaemia with and without IDA. Correction of these parameters through use of antioxidant vitamins to counter the effect of oxidants produced could be useful in correcting the oxidative stress and help in prevention of the complications of SCD in which oxidative stress plays an important role. Future research should focus on the interventional studies with follow-up on direct or indirect impact of oxidative stress and iron parameters, therapeutic evaluation of antioxidants of persons affected with sickle cell disorder and their carriers. The analysis of extended disorders is also required, in the future studies to understand the changes in β-globulin gene encoding for normal as well as HbS with various associated repressors and depressors for haem synthesis.

REFERENCES

[1] da Silva DGH, Ricci Jr O, de Almeida EA, Bonini Domingos CR. Potential utility of melatonin as an antioxidant therapy in the management of sickle cell anaemia. J Pineal Res. 2015;58(2):178-88.
[2] Biswal S, Rizwan H, Pal S, Sabnam S, Parida P, Pal A, et al. Oxidative stress, antioxidant capacity, biomolecule damage, and inflammation symptoms of sickle cell disease in children. Hematology. 2019;24(1):01-09.
[3] Al-Naama LM, Mea'ad KH, Mehdi JK. Association of erythrocytes antioxidant enzymes and their cofactors with markers of oxidative stress in patients with sickle cell anaemia. Qatar Med J. 2015;2015(2):14.
[4] Alayash AI. Oxidative pathways in the sickle cell and beyond. Blood Cells Mol Dis. 2018;70:78-86.
[5] Kuross SA, Rank BH, Hebbel RP. Iron compartments associated with sickle RBC membranes: A mechanism for the targeting of oxidative damage. Prog Clin Biol Res. 1989:319:601-10.
[6] Baliga S, Chaudhary M, Bhat SS, Bhatiya P, Thosar N, Bhansali P, et al. Determination of total antioxidant capacity of saliva in sickle cell anemic patients-A cross-sectional study. Indian Soc Pedod Prev Dent. 2020;35(1):14.
[7] Dosunmu-Ogunbi AM, Wood KC, Novelli EM, Straub AC. Decoding the role of SOD2 in sickle cell disease. Blood Adv. 2019;3(17):2679-87.
[8] Detterich JA, Liu H, Suriany S, Kato RM, Chalacheva P, Tedla B, et al. Erythrocyte and plasma oxidative stress appears to be compensated in patients with sickle cell disease during a period of relative health, despite the presence of known oxidative agents. Free Radic Biol Med. 2019;141:408-15.
[9] Titus J, Chari S, Gupta M, Parekh N. Pro-oxidant and anti-oxidant status in patients of sickle cell anaemia. Indian J Clin Biochem. 2004;19(2):168-72.
[10] Mohanty MB, Mukherjee RB, Colah M, Wadia K. Iron deficiency anaemia in sickle cell disorders in India. Indian J Med Res. 2008;127:366-69.
[11] Akodu SO, Diaku-Akinwumi IN, Kehinde OA, Serum Iron Status of Under-Five Children with Sickle Cell Anaemia. Hindawi Publishing Corporation. Anaemia. 2013;(9):254765.
[12] Marengo-Rowe AJ. Rapid electrophoresis and quantitation of haemoglobin on cellulose acetate. J Clin Pathol. 1965;18(6):790-92.
[13] Nalbandian RM, Nichols BM, Camp FR, Lusher JM, Conte NF, Henry RL, et al. Test-a rapid inexpensive technique for the detection of haemoglobin S and non-S sickling haemoglobin. Clin Chem. 1971;17:1028-32.
[14] Loannou GN, Spector J, Scott K, Rockey DC. Prospective evaluation of a clinical guideline for the diagnosis and management of iron deficiency anaemia. Am J Med. 2002;113(4):281-87.
[15] Skikne BS, Punnonen K, Caldron PH, Bennett T, Rehu M, Gasior GH, et al. Improved differential diagnosis of anaemia of chronic disease and iron deficicency anaemia: A prospective multicenter evaluation of soluble transferrin receptors and the sTfR/log ferritin index. Am J Hematol. 2011:86(11):923-27.
[16] Vichinsky E, Kleman K, Embury S, Lubin B. The diagnosis of iron deficiency in case of sickle cell disease. Blood. 1981;58(5):963-68.
[17] Deshmukh P, Garg BS, Garg N, Prajapati NC, Bharambe MS. Prevalence of sickle cell disorders in rural Wardha. Indian J Community Med. 2006;31:26-27.
[18] Garcic A. A high-sensitive simple determination of serum iron using chromazurol. B Clin Chim Acta. 1979;94:115-19.
[19] Van Oost BA, Willekens FL, Neerbos BRV, Beld BVD. Implication of using different tissue ferritins as antigen for ferritin in serum four radioimmunoassay kits compared. Clin Chem. 1982;28(12):2429-33.
[20] Johnsen O, Eliasson R. Evaluation of a commercially available kit for the colorimetric determination of zinc. Int J Androl. 1987;10(2):435-40.
[21] Arthur DJR, Boyne R. Superoxide dismutase and Glutathione peroxidase activities in neutrophils from selenium deficient and copper deficient cattle. Life Sci. 1985;36:1569-75. Doi: 10.1016/0024-3205(85)90381-9.
[22] Kwaw A. A simple colorimetric method for ascorbic acid determination in blood plasma. Clin Chim Acta. 1978;86(2):153-57.
[23] Jargar JG, Hattiwale SH, Das S, Dhundasi SA, Das KK. A modified simple method for Determination of serum α-tocopherol (Vitamin E). J Basic Clin Physiol Pharmacol. 2012;23(1):45-48.
[24] Ledwozyw A, Michalak J, Strepien A, Kadziolka A. TBA-TCA assay by spectrophotometric determination of MDA. Clin Chim Acta. 1986;155:275-84.
[25] Johansson LH, Borg LAH. A spectrophotometric method for determination of catalase activity in small tissue samples. Anal Biochem. 1988;174(1):331-36.
[26] Garba N, Ifeanyichukwu MO, Amilo GI, Babadoko AA, Audu I. Evaluation of iron status in adult sickle cell anaemia patients in Zaria, North Western Nigeria. Trans Biomed. 2016;64(7):01-04.
[27] Chaves NA, Alegria TGP, Dantas LS, Netto LES, Miyamoto S, Domingos CRB, et al. Impaired antioxidant capacity causes a disruption of metabolic homeostasis in sickle erythrocytes. Free Radic Bio Med. 2019;141:34-46.
[28] Antwi-Boasiako C, Dankwah GB, Aryee R, Hayfron-Benjamin C, Aboagye G, Campbell AD. Correlation of lipid peroxidation and nitric oxide metabolites, trace elements, and antioxidant enzymes in patients with sickle cell disease. J Clin Lab Anal. 2020;34(7);e23294.
[29] Gizi A, Papassotiriou I, Apostolakou F, Lazaropoulou C, Papastamataki M, Kanavaki I, et al. Assessment of oxidative stress in patients with sickle cell disease: The glutathione system and the oxidant-antioxidant status. Blood cells Mol Dis. 2011;46(3):220-25.
[30] Prasad AS. Zinc in human health: Effect of zinc on immune cells. Mol Med. 2008;14(5-6):353-57.
[31] Hasanto RMW. Zinc and antioxidant vitamin deficiency in patients with severe sickle cell anaemia. Ann Saudi Med. 2006;26(1):17-21.
[32] Chiu D, Vichinsky E, Ho SL, Liu T, Lubin BH. Vitamin C deficiency in patients with sickle cell anaemia. Am J Pediatr Hematol Oncol. 1990;12(3):262-67.
[33] Bhoi S, Shah S, Goel AK, Dhingra A, Mishra PK. Oxidative stress in sickle cell disease-A tertiary hospital experience in Western Odisha. Int J Med Sci Public Health. 2014;3(8):970-73.
[34] Jain SK. The accumulation of malondialdehyde, a product of fatty acid peroxidation, can disturb amino phospholipid organization in the membrane bilayer of human erythrocyte. J Biol Chem. 1984;259:3391-94.
[35] Cappo C, Bongrand P, Benoliel AM, Depieds R. Non-specific recognition in phagocytosis: Ingestion of aldehyde-treated erythrocytes by rat peritoneal macrophages. Immunology. 1979;36:501-08.
[36] Kuross SA, Hebbel RP. Nonheme iron in sickle erythrocyte membranes: Associations with phospholipids and potential role in lipid peroxidation. Blood. 1988;72(4):1278-85.
[37] Al-Naama LM, Mea'ad KH, Mehdi JK. Association of erythrocytes antioxidant enzymes and their cofactors with markers of oxidative stress in patients with sickle cell anaemia. Qatar Med J. 2015;2015(2):14.
[38] Biswal S, Rizwan H, Pal S, Sabnam S, Parida P, Pal A, et al. Oxidative stress, antioxidant capacity, biomolecule damage, and inflammation symptoms of sickle cell disease in children. Hematology. 2018;24(1):01-09.

PARTICULARS OF CONTRIBUTORS:

1. Assistant Professor, Department of Biochemistry, Datta Meghe Medical College, Nagpur, Maharashtra, India.
2. Associate Professor, Department of Biochemistry, Datta Meghe Medical College, Nagpur, Maharashtra, India.
3. Professor and Head, Department of Biochemistry, ESIC Medical College, Gulbarga, Karnataka, India.
4. Professor and Head, Department of Biochemistry, Jawaharlal Nehru Medical College, Wardha, Maharashtra, India.
5. Professor, Department of Biochemistry, Jawaharlal Nehru Medical College, Wardha, Maharashtra, India.
6. Emeritus Professor, Department of Biochemistry, Jawaharlal Nehru Medical College, Wardha, Maharashtra, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Prashant Vishram Paunipagar,
Professor and Head, Department of Biochemistry, ESIC Medical College,
Sedam Raod, Gulbarga-584106, Karnataka, India.
E-mail: drprashantesic@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: Jun 22, 2022
- Manual Googling: Nov 08, 2022
- iThenticate Software: Nov 11, 2022 (6%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:

- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Jun 14, 2022**
Date of Peer Review: **Jul 14, 2022**
Date of Acceptance: **Nov 15, 2022**
Date of Publishing: **Dec 01, 2022**