



Status of malondialdehyde, catalase and superoxide dismutase levels/activities in schoolchildren with iron deficiency and iron-deficiency anemia of Kashere and its environs in Gombe State, Nigeria

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ABSTRACT

Background: Iron-deficiency anemia (IDA) or iron deficiency (ID) is by far the most common form of disorder affecting the cognitive development, physical growth and school performance of children in developing countries including Nigeria.

Objectives: In the present study, we aimed to examine whether IDA or ID, or both are associated with oxidative stress or otherwise by assessing the perturbations in oxidative stress markers including malondialdehyde (MDA), catalase (CAT) and superoxide dismutase (SOD).

Methods: Here, a total of eighty-one IDA, ID, and healthy control subjects of twenty-seven replicates each, were recruited and investigated. Human serum MDA, CAT and SOD levels were quantitatively analyzed using Enzyme-Linked Immunosorbant Assay.

Results: Mean serum MDA levels of IDA (5.10 ± 2.35 mmol/L) and ID (4.05 ± 1.35 mmol/L) groups were found to perturb significantly ($p < 0.05$), being higher than those of control (3.30 ± 0.95 mmol/L) subjects. Similarly, mean serum MDA levels of IDA (5.10 ± 2.35 mmol/L) group was found to be significantly ($p < 0.05$) higher when compared with ID (4.05 ± 1.35 mmol/L) subjects. Conversely, mean serum CAT and SOD activities of IDA (8.35 ± 2.21 ng/mL and 340.70 ± 153.65 ng/mL) group were found to differ significantly ($p < 0.05$), and those of ID (9.40 ± 1.47 ng/mL and 435.00 ± 144.75 ng/mL) subjects were found to perturb slightly ($p > 0.05$), being lower than those of control (10.40 ± 4.31 ng/mL and 482.12 ± 258.37 ng/mL) subjects.

Conclusions: Taken together, the results of the present study showed that lipid peroxidation was dramatically increased in both IDA and ID subjects in hydroperoxide-superoxide-dependent manner; in contrast, enzymatic antioxidant capacity was drastically decreased in both IDA and ID groups as evidenced by biochemical markers.

1. Introduction

Anemia is generally defined as a decrease in the concentration of hemoglobin in the blood below the normal level for the same sex and age (Tefferi, 2003; Cullis, 2011). Iron is an essential microelement that is required by virtually all cells in the human body most notably during hemoglobin synthesis (Tefferi, 2003; Asare et al., 2009; Takami and Sakaida, 2011; Altun et al., 2014; Zaka-ur-Rab et al., 2016). Each day 20–25 mg of iron is needed for erythropoiesis, most of which is obtained

from normal red blood cell turnover and hemoglobin catabolism (Moxness et al., 1996; Asare et al., 2009; Takami and Sakaida, 2011). Iron metabolism was formally reported to be largely regulated by hepcidin-25 (Rostoker, 2017, 2018). Iron and/or inflammation (IL-6) enhance the production of iron-regulating hormone (hepcidin-25) which in turn causes the reduction in the expression of ferroportin thereby inhibiting the export of iron out of hepatocytes, macrophages and enterocytes (Ganz and Nemeth, 2012; Rostoker, 2017, 2018). However, anemia, iron deficiency (ID), hypoxia, bleeding and erythropoietin were documented

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to interfere with iron metabolism leading to downregulation of hepcidin-25 synthesis thereby upregulating the expression of ferroportin, a protein that regulates the export of iron out of those cells (Ganz and Nemeth, 2012; Rostoker, 2017; Ueda and Takasawa, 2018). Inflammation in chronic kidney diseases was reported to increase ferritin and hepcidin-25 irrespective of iron status which in turn reduce iron availability (Rostoker, 2018; Ueda and Takasawa, 2018). In the absence of inflammation, association between ferritin and hepcidin-25 is robust to predict iron status (Ueda and Takasawa, 2018; Rostoker, 2018). In addition, ID exposes cells, especially the red blood cells, to cell death either via cell necrosis or via apoptosis, or both (Hallberg, 2001; Cullis, 2011; Shinto et al., 2018) likely due to lipid peroxidation by polyunsaturated lipids embedded in the cell membranes of the red blood cells (Asare et al., 2009; Takami and Sakaida, 2011; Samhan-Arias et al., 2011; Zaka-ur-Rab et al., 2016). Lipid peroxidation is not surprising to associate with cell necrosis or apoptosis (Ven-Den-Berg et al., 1992; Asare et al., 2009; Takami and Sakaida, 2011; Samhan-Arias et al., 2011; Altun et al., 2014; Zaka-ur-Rab et al., 2016; Shinto et al., 2018). Iron-deficiency anemia (IDA) is by far the most common form of anemia affecting the cognitive development, physical growth and school performance of children in developing countries such as Nigeria due mainly to less iron in the diet, less absorption and/or iron recycling or blood loss (Hallberg, 2001; WHO, 2001; Krishnamoorthy et al., 2010; Onimawo et al., 2010; Akodu et al., 2016). According to report by World Health Organization (WHO), IDA is the result of a wide variety of causes that often coexist together with other micronutrient deficiencies (Tefferi, 2003; Hallberg, 2001; WHO, 2001; Krishnamoorthy et al., 2010). Although iron deficiency is the major contributor to IDA as formally reported by WHO, deficiencies of other essential micronutrients such as vitamin C, folic acid and vitamin B₁₂ (Hallberg, 2001; WHO, 2001; Jeremiah et al., 2007; Krishnamoorthy et al., 2010; Onimawo et al., 2010; Akodu et al., 2016) and to some extent infectious diseases such as malaria, tapeworm infestation and schistosomiasis (Anumudu et al., 2008) as well as socioeconomic factors such as maternal education, gender norms, and low income have been thought to be associated with IDA (Mahmud et al., 2013). In Nigeria, prevalence of anemia among schoolchildren ranges from 62% to 82.5% of which IDA accounts for 50% (Jeremiah et al., 2007; Anumudu et al., 2008; Niki et al., 2008). Therefore, iron deficiency in human cells especially in the red blood cells, which account for 30% of the estimated cells in human, can lead to membrane damage associated with free radicals generation (Weiss, 1980; Van-Der-Zee et al., 1987; Ven-Den-Berg et al., 1992; Niki et al., 2008). Formation of free radicals due to iron deficiency coupled to anemia precipitates the imbalance between pro-oxidants and antioxidants capacity (Weiss, 1980; Van-Der-Zee et al., 1987; Ven-Den-Berg et al., 1992; Altun et al., 2014; Zaka-ur-Rab et al., 2016). Thus, IDA is thought to be associated with oxidative stress as formally reported in animal models, pregnant women and adults (Ven-Den-Berg et al., 1992; Nagababu et al., 2008; Altun et al., 2014; Zaka-ur-Rab et al., 2016; Akodu et al., 2016). In IDA and ID conditions, the enzymatic mechanisms of free radical scavenging system are functionally diminishing (Altun et al., 2014; Zaka-ur-Rab et al., 2016). Studies on status of oxidative stress markers in IDA subjects have been extensively reported (Macdougall, 1972; Weiss, 1980; Van-Der-Zee et al., 1987; Acharya et al., 1991; Ven-Den-Berg et al., 1992; Moxness et al., 1996; Tekin et al., 2001; Isler et al., 2002; Kavakli et al., 2004; Jeremiah et al., 2007; Anumudu et al., 2008; Nagababu et al., 2008; Niki et al., 2008; Asare et al., 2009; Krishnamoorthy et al., 2010; Takami and Sakaida, 2011; Samhan-Arias et al., 2011; Bay et al., 2013; Mahmud et al., 2013; Altun et al., 2014; Zaka-ur-Rab et al., 2016; Shinto et al., 2018). However, data on oxidative stress markers in IDA or ID among schoolchildren appears to be limited and more often than not contradictory (Macdougall, 1972; Acharya et al., 1991; Tekin et al., 2001; Isler et al., 2002; Kavakli et al., 2004; Krishnamoorthy et al., 2010; Bay et al., 2013; Altun et al., 2014; Zaka-ur-Rab et al., 2016).

Although low levels of essential micronutrients among children in Nigeria are dramatically increasing at an alarming rate, IDA or ID is a silent public health problem in Gombe State irrespective of Kashere and its environs. In addition, to the best of our knowledge, literatures about changes in oxidative stress markers in ID, IDA or normal subjects in children, adolescents and youth of Kashere community have been neither documented nor reported. Thus, the present study aims to investigate these gaps by taking into cognizance the oxidative stress markers including MDA, CAT and SOD of primary schoolchildren aged 6–12 years in Kashere and its environs.

2. Materials and methods

2.1. Chemicals

Human MDA ELISA kit (Catalogue No: EKHU-0372); Human SOD ELISA kit (Catalogue No: EKHU-1716) and Human CAT ELISA kit (Catalogue No: EKHU-1714) were of the highest commercially analytical grade and were purchased from Melsin Medical Co., Limited, China. Iron Nitro PAPS Monoreagent was also of the highest commercially analytical grade and was purchased from Centronic GmbH Co., Germany.

2.2. Apparatus

ELISA plate shaker – incubator; microplate washer (RT-2600C), microplate reader (RT-2100C), Sysmex hematology analyzer and spectrophotometer were of the highest commercially analytical grade and were obtained from Sigma-Aldrich Co., USA.

2.3. Study location and study subject

This research was carried out at the Health Services Unit, Federal University of Kashere, Gombe State, Nigeria. The study was approved by the Ethical Research Committee of the University (Approval No.: FUK/R/AS/PF/0992) and State Universal Basic Education Board (SUBEB) (Approval No.: SUBEB/003/GM/16), Gombe State, prior to the experimentation. Also, all human-subject experiments were performed with the informed consent of the subjects – according to the guidelines of the Health Services Unit, Federal University of Kashere, Human Subject Use Committee. The cut-off value for assessing anemia in this research was considered in accordance with WHO recommendation (blood hemoglobin concentration < 11.5 g/dL and < 12 g/dL) for children between 6 – 11 years and 12 years respectively (WHO, 2001). Serum iron concentration was taken as < 37 µg/dL and < 59 µg/dL for female and male children respectively while serum ferritin concentration was taken as < 10 ng/mL for female children and < 16 ng/mL for male children.

2.3.1. Exclusion criterion

Acute bleeding, anemic children who needed blood transfusion, preschool children and schoolchildren above 12 years, primary schoolchildren aged 6–12 years other than those in Kashere and its environs, female primary schoolchildren aged 6–12 years in Kashere and its environs experiencing menstrual period, history of smoking, alcoholic drinking and blood transfusion within 6 months before the study were excluded.

2.3.2. Sample collection

Five (5) mL of venous blood was collected from IDA, ID and healthy control subjects according to the anthropometric data below (Table 1) in plain tubes (Becton Dickenson, USA). The sera obtained after centrifugation for 20 min at the speed of 2000–3000 rpm were kept at – 20 °C freezer until use. Activities/levels of human serum CAT, SOD and MDA were quantitatively analyzed using Enzyme-Linked Immunosorbent Assay (Voller et al., 1978; Liddell, 2003) at Chemical Pathology

Table 1

Anthropometric measurements of the study subjects aged 6–12 years.

Group	female:male	Age (yr)	Weight (kg)	Height (m)	BMI (kg/m ²)
IDA	11:16	8.37 ± 2.02	24.71 ± 7.21	1.23 ± 0.12	16.07 ± 1.95
(n = 27)					
ID	11:16	8.48 ± 1.74	24.04 ± 4.86	1.23 ± 0.10	15.89 ± 1.13
(n = 27)					
Control	11:16	8.78 ± 2.03	25.04 ± 6.26	1.23 ± 0.13	16.28 ± 1.45
(n = 27)					

Values are expressed as mean ± standard deviation of 27 replicates; BMI = body mass index; IDA = iron-deficiency anemia; ID = iron-deficiency.

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2.4. Methods of MDA, CAT and SOD assay

2.4.1. Assay procedure for SOD

2.4.1.1. Test principle. The kit uses enzyme linked immunosorbent assay-double antibody sandwich principle to assay superoxide dismutase (SOD) level in the sample. The microelisa stripplate provided in this kit has been coated by purified SOD antibody to make solid-phase antibody, then SOD is added to wells and combined with SOD antibody labeled by HRP, become antibody - antigen - enzyme-antibody complex. After washing completely to remove the uncombined enzyme, chromogen solution A and chromogen solution B are added and the color of the liquid changes into the blue. And at the effect of acid, the color finally becomes yellow. The color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of SOD in the samples is then determined by comparing the O.D. of the samples to the standard curve (Voller et al., 1978; Liddell, 2003).

2.4.1.2. Procedure. Standard reagent density and standard densities for SOD are thus: 540 ng/mL and 360 ng/mL, 240 ng/mL, 120 ng/mL, 60 ng/mL and 30 ng/mL. This assay recognizes recombinant and natural SOD by CV (%) < 15%, and also has high sensitivity and excellent specificity for detection of SOD with assay range and sensitivity of 12 ng/mL – 480 ng/mL and < 6 ng/mL respectively. Human SOD ELISA kit (Catalogue No: EKHU-1716) was purchased from Melsin Medical Co., Limited, China.

2.4.2. Assay procedure for CAT

The test principle and procedure for CAT estimation is similar to above except that standard reagent density is 22.5 ng/mL and standard densities are 15 ng/mL, 10 ng/mL, 5 ng/mL, 2.5 ng/mL and 1.25 ng/mL. This assay recognizes recombinant and natural CAT by CV (%) < 15%, and also has high sensitivity and excellent specificity for detection of CAT with assay range and sensitivity of 0.6 ng/mL – 20 ng/mL and < 0.25 ng/mL respectively. Human CAT ELISA kit (Catalogue No: EKHU-1714) was purchased from Melsin Medical Co., Limited, China.

2.4.3. Assay procedure for MDA

The test principle and procedure for MDA estimation is similar to that of SOD explained thus far except that standard reagent density is 9 mmol/L and standard densities are 6 mmol/L, 4 mmol/L, 2 mmol/L, 1 mmol/L and 0.5 mmol/L. This assay recognizes recombinant and natural MDA by CV (%) < 15%, and also has high sensitivity and excellent specificity for detection of MDA with assay range and sensitivity of 0.3 mmol/L – 7 mmol/L and < 0.1 mmol/L respectively. Human MDA ELISA kit (Catalogue No: EKHU-0372) was purchased from Melsin Medical Co., Limited, China.

2.5. Determination of serum iron and blood hemoglobin and hematocrit levels/counts

Iron in the serum was calorimetrically measured by the Nitro-PAPS method (Makino et al., 1988) – Monoreagent at 578 nm. Hemoglobin and hematocrit levels/counts in the blood were quantitatively analyzed using Sysmex hematology analyzer machine.

2.6. Statistical analysis

The results obtained were statistically evaluated using One-Way Analysis of Variance (ANOVA). Differences were considered statistically significant at $p < 0.05$ followed by Least Significant Difference (LSD) to determine where the difference among the three (3) groups actually lies.

3. Results

Anthropometric measurements including female to male ratio, age, weight, height and body mass index across the IDA, ID and control groups of 27 replicates each are given in Table 1. Mean age, weight, height and body mass index of IDA (mean age = 8.37 ± 2.02 ; mean weight = 24.71 ± 7.21 ; mean height = 1.23 ± 0.12 ; mean body mass index = 16.07 ± 1.95); ID (mean age 8.48 ± 1.74 ; mean weight 24.04 ± 4.86 ; mean height 1.23 ± 0.10 ; mean body mass index = 15.89 ± 1.13); control (mean age = 8.78 ± 2.03 ; mean weight = 25.04 ± 6.26 ; mean height = 1.23 ± 0.13 ; mean body mass index = 16.28 ± 1.45) groups were statistically ($p > 0.05$) insignificant and virtually the same.

3.1. Status of human serum activities/levels of CAT, SOD and MDA

The levels of MDA in the serum across the study groups are presented in Fig. 1. When mean serum levels of MDA in IDA (5.10 ± 2.35 mmol/L) and ID (4.05 ± 1.35 mmol/L) subjects are compared with control (3.30 ± 0.95 mmol/L) subjects, the difference is statistically significant ($p < 0.05$). Importantly also, when mean serum levels of MDA in IDA (5.10 ± 2.35 mmol/L) subjects are compared with ID (4.05 ± 1.35 mmol/L) subjects, the difference is statistically significant ($p < 0.05$). Mean serum MDA levels of IDA and ID (5.10 ± 2.35 mmol/L and 4.05 ± 1.35 mmol/L) groups were found to perturb significantly ($p < 0.05$) being higher than those of control (3.30 ± 0.95 mmol/L) subjects. Equally also, mean serum MDA levels of IDA (5.10 ± 2.35 mmol/L) group was significantly ($p < 0.05$) higher when compared with ID (4.05 ± 1.35 mmol/L) subjects. Therefore, based on these data, free radicals generation via partly lipid peroxidation is significantly elevated in IDA subjects. Furthermore, the activities of CAT and SOD in the serum across the study groups are

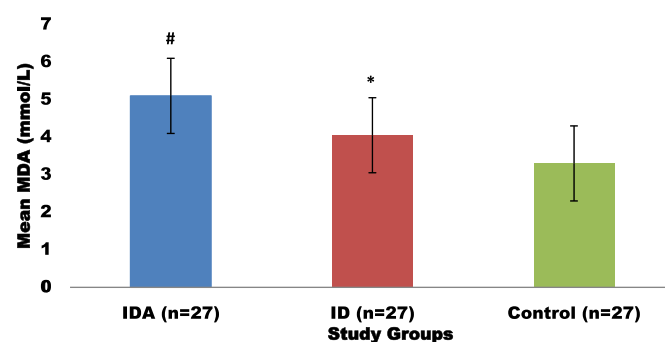


Fig. 1. Levels of mean serum MDA in iron-deficiency anemia, iron deficiency and healthy control subjects. Values are expressed as mean ± standard deviation of 27 replicates; # = significant difference at $p < 0.05$ when IDA group is compared with ID and control groups; * = significant difference at $p < 0.05$ when ID group is compared with control group; IDA = iron-deficiency anemia; ID = iron-deficiency; MDA = malondialdehyde.

shown in Fig. 2 (A) and (B) respectively. When mean serum levels of CAT and SOD in IDA (8.35 ± 2.21 ng/mL and 340.70 ± 153.65 ng/mL) subjects are compared with ID (9.40 ± 1.47 ng/mL and 435.00 ± 144.75 ng/mL) and control (10.40 ± 4.31 ng/mL and 482.12 ± 258.37 ng/mL) subjects, the difference is statistically significant ($p < 0.05$). When mean serum levels of CAT and SOD in ID (9.40 ± 1.47 ng/mL and 435.00 ± 144.75 ng/mL) group is compared with control (10.40 ± 4.31 ng/mL and 482.12 ± 258.37 ng/mL) group, there is no significant difference ($p > 0.05$). Mean serum CAT and SOD activities of IDA (8.35 ± 2.21 ng/mL and 340.70 ± 153.65 ng/mL) subjects were found to differ significantly ($p < 0.05$), being lower than those of ID (9.40 ± 1.47 ng/mL and 435.00 ± 144.75 ng/mL) and control (10.40 ± 4.31 ng/mL and 482.12 ± 258.37 ng/mL) subjects. Mean serum CAT and SOD activities of ID (9.40 ± 1.47 ng/mL and 435.00 ± 144.75 ng/mL) subjects were slightly ($p > 0.05$) lower when compared with the control (10.40 ± 4.31 ng/mL and 482.12 ± 258.37 ng/mL) group. Thus, antioxidant defense mechanism, in accordance with our results, is more implicated in IDA subjects than it does in ID subjects.

3.2. Status of serum iron and blood hemoglobin levels as well as hematocrit counts

The levels of serum iron and blood hemoglobin and hematocrit counts across the study groups are presented in Fig. 3(A), (B) and (C) respectively. When mean levels/counts of iron, hemoglobin and hematocrit in the serum and the blood of IDA (25.78 ± 13.36 µg/dL; 9.63 ± 1.61 g/dL and $28.92 \pm 4.33\%$) subjects are respectively compared with ID (37.47 ± 11.54 µg/dL; 13.10 ± 0.63 g/dL and $38.38 \pm 7.92\%$) and control (57.44 ± 15.33 µg/dL; 13.29 ± 0.77 g/dL and $36.70 \pm 1.62\%$) subjects, the difference is statistically significant ($p < 0.05$). In addition, when mean serum iron levels of IDA (25.78 ± 13.36 µg/dL) group are compared with ID (37.47 ± 11.54 µg/dL) group, there is significant difference ($p < 0.05$). There is no difference in mean blood hemoglobin and hematocrit levels/counts between ID (13.10 ± 0.63 g/dL and $38.38 \pm 7.92\%$) and control (13.29 ± 0.77 g/dL and $36.70 \pm 1.62\%$) subjects statistically ($p > 0.05$). Mean serum iron and blood hemoglobin and hematocrit levels/counts of IDA (25.78 ± 13.36 µg/dL; 9.63 ± 1.61 g/dL and $28.92 \pm 4.33\%$)

subjects were found to differ statistically ($p < 0.05$) being lower than those of ID (37.47 ± 11.54 µg/dL; 13.10 ± 0.63 g/dL and $38.38 \pm 7.92\%$) and control (57.44 ± 15.33 µg/dL; 13.29 ± 0.77 g/dL and $36.70 \pm 1.62\%$) subjects. Mean serum iron levels of ID (37.47 ± 11.54 µg/dL) group were found to be significantly ($p < 0.05$) being lower when compared with control (57.44 ± 15.33 µg/dL) subjects, even though mean blood hemoglobin and hematocrit levels/counts levels between ID (13.10 ± 0.63 g/dL and $38.38 \pm 7.92\%$) and control (13.29 ± 0.77 g/dL and $36.70 \pm 1.62\%$) subjects were nearly the same. Henceforth, iron deficiency coupled with anemia in agreement with our findings is more deleterious in bringing about lipid peroxidation with a concomitant reduction in antioxidant defense capacity.

4. Discussion

Proper understanding of perturbations in oxidative stress markers including MDA, CAT and SOD in primary schoolchildren with IDA or ID can be of clinical significance against loss of cognitive development and poor school performance of children in developing countries including Nigeria. Decrease in iron levels brings the body to an insufficient amount of not only hemoglobin to support red blood cells (RBCs) production but also iron-containing proteins, catalase, cytochromes, among others, to some extent (Ven-Den-Berg et al., 1992; Isler et al., 2002; Nagababu et al., 2008; Zaka-ur-Rab et al., 2016). Although iron deficiency may be likely due to physiological demands in growing children, adolescents and pregnant women, the underlying pathology needs to be checked (WHO, 2001; Jeremiah et al., 2007; Anumudu et al., 2008; Niki et al., 2008; Onimawo et al., 2010; Akodu et al., 2016). Erythropoiesis needs to be controlled so there is a balance between RBC production and destruction (Tefferi, 2003; Cullis, 2011; WHO, 2001). New cells are being made at a rate of more than 2 million per second in healthy people (Tefferi, 2003; Cullis, 2011). This process is controlled not only hormonally (Ganz and Nemeth, 2012; Rostoker, 2017) but also depends on adequate supplies of iron, amino acids, vitamin B₁₂ and folic acid as well as other micro-nutrients (WHO, 2001; Tefferi, 2003; Jeremiah et al., 2007; Anumudu et al., 2008; Niki et al., 2008; Krishnamoorthy et al., 2010; Onimawo et al., 2010; Cullis, 2011; Akodu et al., 2016). Iron is needed for transport

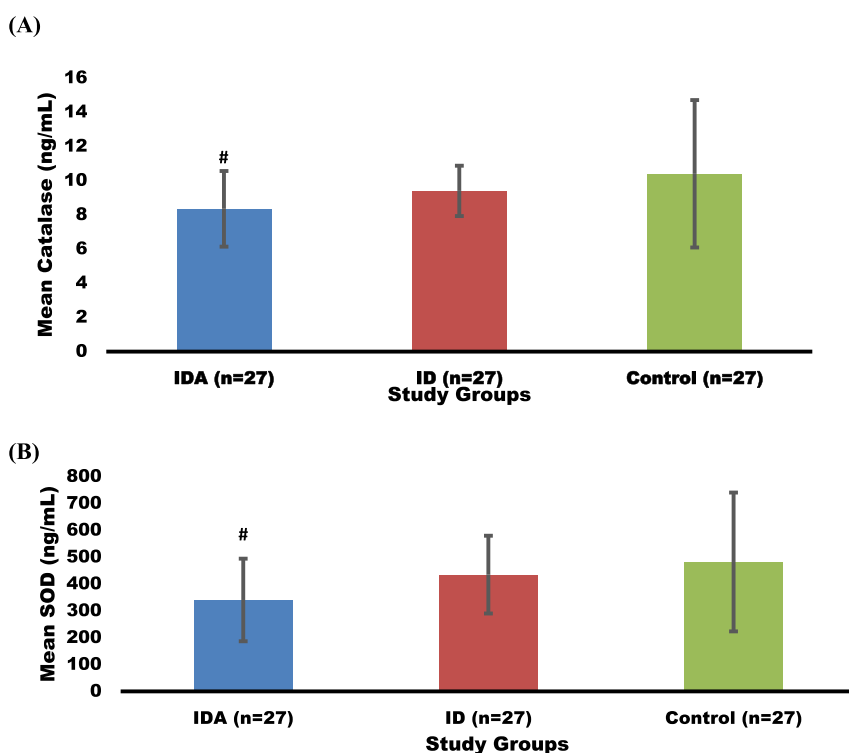


Fig. 2. Levels of catalase and SOD in the serum among iron-deficiency anemia, iron deficiency and healthy control subjects. Status of mean serum catalase activities across the study subjects. Values are expressed as mean $\pm$ standard deviation of 27 replicates; # = significant difference at $p < 0.05$ when IDA group is compared with ID and control groups; IDA = iron-deficiency anemia; ID = iron-deficiency (A). Status of mean serum SOD activities across the study subjects. Data are expressed as mean $\pm$ standard deviation of 27 replicates; # = significant difference at $p < 0.05$ when IDA group is compared with ID and control groups; IDA = iron-deficiency anemia; ID = iron-deficiency; SOD = superoxide dismutase (B).

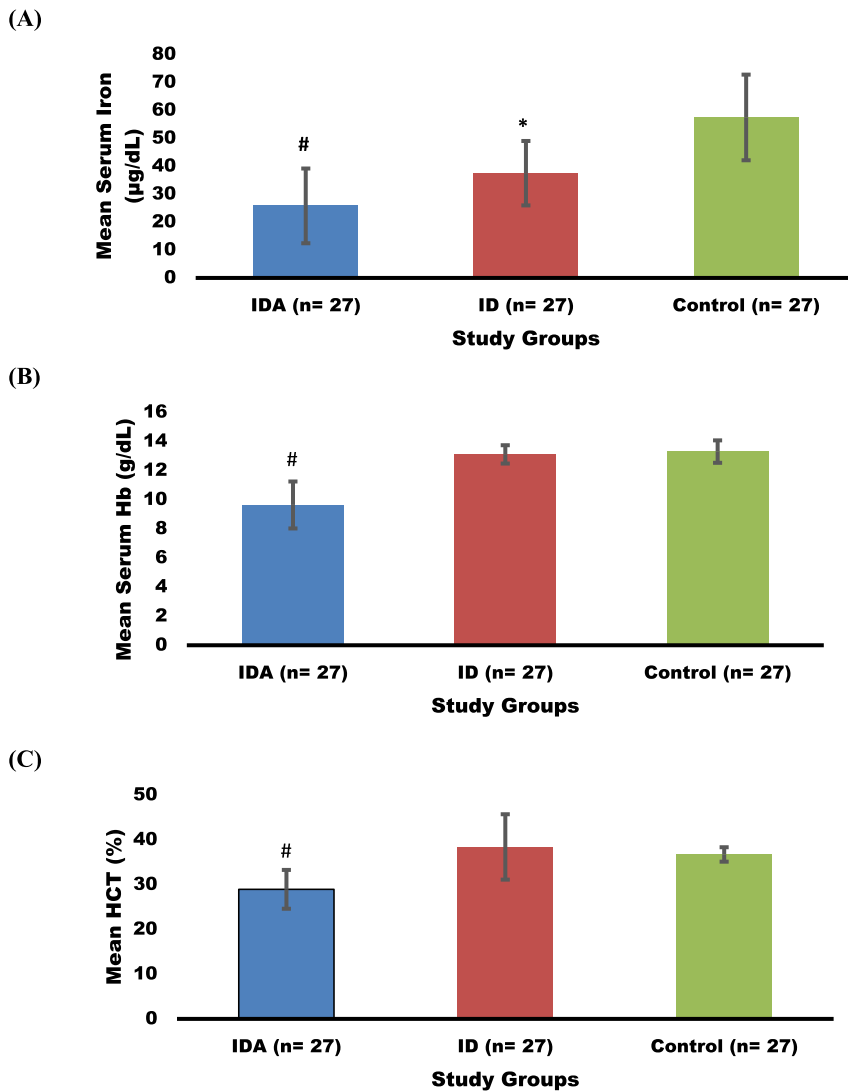


Fig. 3. Levels/counts of mean serum iron, hemoglobin and hematocrit in iron-deficiency anemia, iron deficiency and healthy control subjects. (A) Levels of iron in the serum across the study subjects. (B) Levels of hemoglobin in the blood across the study subjects. (C) Hematocrit counts across the study subjects. Values are expressed as mean $\pm$ standard deviation of 27 replicates; # = significant difference at $p < 0.05$ when IDA group is compared with ID and control groups; * = significant difference at $p < 0.05$ when ID group is compared with control group; IDA = iron-deficiency anemia; ID = iron deficiency; HCT = hematocrit.

of oxygen and synthesis of hemoglobin, myoglobin, iron-containing proteins, catalase, cytochromes, among others (Isler et al., 2002; Tefleri, 2003; Nagababu et al., 2008; Cullis, 2011; Altun et al., 2014; Zaka-ur-Rab et al., 2016). Iron is by far documented to participate in Fenton reaction and/or Haber-Weiss reaction by reacting *in vivo* with poorly reactive, less potent reactive oxygen species (ROS) including superoxide anion radical and hydrogen peroxide to hydroxyl radical which in turn damages DNA, lipids and proteins directly (Weiss, 1980; Thomas et al., 1985; Van-Der-Zee et al., 1987; Acharya et al., 1991; Ven-Den-Berg et al., 1992; Isler et al., 2002; Nagababu et al., 2008; Zaka-ur-Rab et al., 2016). Hydroxyl radical is by far the most dangerous free radical ever identified (Weiss, 1980; Van-Der-Zee et al., 1987; Ven-Den-Berg et al., 1992; Nagababu et al., 2008). Paradoxically, on the basis of our findings (Fig. 1), although iron is deficient, lipid peroxidation in IDA and ID subjects was significantly ($p < 0.05$) induced when compared with healthy control subjects. In the same manner, lipid peroxidation in IDA subjects was significantly being higher than those in ID subjects. The significant ($p < 0.05$) elevation in serum levels of MDA, which appears to be an indicator of lipid peroxidation, in IDA and ID groups even though iron is deficient could be attributed to the fact that lipid peroxidation is possible in a hydroperoxide-superoxide-dependent manner that bypasses Fenton reaction and/or Haber-Weiss reaction (Hodgson and Fridovich, 1975; Weiss, 1980; Thomas et al., 1985; Van-Der-Zee et al., 1987; Acharya et al., 1991; Ven-Den-Berg et al., 1992; Vives-Corróns et al.,

1995; Tekin et al., 2001; Isler et al., 2002; Nagababu et al., 2008; Krishnamoorthy et al., 2010; Altun et al., 2014). However, the mechanism by which lipid peroxidation is favored in this fashion is not fully understood (Thomas et al., 1985; Acharya et al., 1991; Vives-Corróns et al., 1995; Tekin et al., 2001). Equally also, the significantly ($p < 0.05$) observed difference in MDA levels between IDA and ID subjects might be attributed to loss of deformability, cytosolic calcium elevation and increase membrane stiffness in RBCs (Weiss, 1980; Van-Der-Zee et al., 1987; Thomas et al., 1985; Acharya et al., 1991; Ven-Den-Berg et al., 1992; Vives-Corróns et al., 1995; Nagababu et al., 2008; Altun et al., 2014). In corroboration with the findings of the present study, elevation in MDA levels was formally reported to lower erythrocyte deformability (Acharya et al., 1991; Ven-Den-Berg et al., 1992; Vives-Corróns et al., 1995; Nagababu et al., 2008; Altun et al., 2014). Importantly, however, lipid peroxidation via hydroxyl radical generation may be possible in conditions such as iron deficiency and iron deficiency coupled with anemia (Acharya et al., 1991; Ven-Den-Berg et al., 1992; Vives-Corróns et al., 1995; Kavakli et al., 2004; Bay et al., 2013; Ganz and Nemeth, 2012; Rostoker, 2017; Ueda and Takasawa, 2018; Rostoker, 2018). This may be due partly to indirect release of iron from ferritin in a hydroperoxide-superoxide-dependent manner which conversely triggers the production of hydroxyl radical (Thomas et al., 1985; Ven-Den-Berg et al., 1992; Nagababu et al., 2008; Ganz and Nemeth, 2012; Rostoker, 2017; Ueda and Takasawa, 2018; Rostoker, 2018). Interestingly,

accumulation of hydrogen peroxide was reported to cross the erythrocyte membrane and rapidly exposes the hemoglobin to produce free radical species including hydroxyl radical (Weiss, 1980; Van-Der-Zee et al., 1987; Ven-Den-Berg et al., 1992; Nagababu et al., 2008). Conversely, accumulation of hydrogen peroxide may be attributed to decrease in catalase activity (Macdougall, 1972; Hodgson and Fridorivh, 1975; Isler et al., 2002). Interestingly our findings suggested the reduction in catalase activity which appears to be statistically significant ($p < 0.05$) in IDA subjects when compared with ID and control groups (Fig. 2 (A)). These findings were in concordance with the fact that hemoglobin is by far the suitable substrate for hydrogen peroxide (Macdougall, 1972; Hodgson and Fridorivh, 1975; Ven-Den-Berg et al., 1992; Isler et al., 2002). However, slight ($p > 0.05$) decrease in catalase activity was observed in ID subjects. Moreover, the reduction in catalase activity in IDA and ID subjects when compared with healthy control subjects could have been likely due to iron requirement for catalase biosynthesis (Rockey and Cello, 1993; Isler et al., 2002; Ueda and Takasawa, 2018). Additionally, our findings (Fig. 2 (B)) demonstrated a significant ($p < 0.05$) reduction in SOD levels of IDA subjects when compared with ID and control subjects. In contrast, there was a slight ($p > 0.05$) reduction in SOD activities of ID subjects when compared with control group. Antioxidant capacity of RBCs was reported to be implicated in IDA subjects with a significant decline in the activities of antioxidant enzymes such as SOD and catalase (Macdougall, 1972; Hodgson and Fridorivh, 1975; Bartal et al., 1993; Gutteridge, 1994). Decline in SOD activities in both IDA and ID subjects is not surprising presumably due to decrease in catalase activity leading to the accumulation of hydrogen peroxide that in turn feedback inhibits SOD activity (Isler et al., 2002; Zaka-ur-Rab et al., 2016). Apparently, inactivation of SOD activity caused by accumulation of hydrogen peroxide would in turn lead to accumulation of superoxide anion radical that ultimately favors the lipid peroxidation in hydroperoxide-superoxide-dependent manner (Macdougall, 1972; Hodgson and Fridorivh, 1975; Acharya et al., 1991; Bartal et al., 1993; Gutteridge, 1994; Vives-Corrons et al., 1995). In this regard, oxidative stress could have been the underlying pathology of anemia coupled with iron deficiency (Ven-Den-Berg et al., 1992; Nagababu et al., 2008) as evidenced by our data (Fig. 3 (A), (B) and (C)). Correspondingly, induced autoxidation of hemoglobin brought about by production of superoxide anion radicals may as well be attributed to decrease in RBCs' half-life which in turn leads to subsequent variations associated with IDA (Ven-Den-Berg et al., 1992; Rocky and Cello, 1993; Nagababu et al., 2008).

Considering our sample size, highly-accurate detection of variations in levels/activities of the studied oxidative stress markers, may be hindered. Ethically, a total of 81 subjects of 27 replicates were approved by the Ethical Research Committee of the University. In addition, relevant parameters such as oxidative stress index, total antioxidant capacity, non-enzymatic antioxidant defenses including glutathione reduced form and vitamin A, C and E, total oxidative stress, among others, formally reported by other studies were not assayed in the present study.

5. Conclusion

All together, the findings of present study revealed that oxidative stress was increased in primary schoolchildren with IDA and ID in hydroperoxide-superoxide-dependent fashion as evidenced by induced elevation in MDA levels, whereas enzymatic antioxidant capacity of CAT and SOD activities was drastically lowered. Thus, perturbations in MDA, CAT and SOD levels/activities across the study groups can suggest that IDA and ID in primary schoolchildren are not only stress-induced but also oxidative stress may be the underlying pathology of both IDA and ID.

Further studies should be conducted to assess the levels of C-reactive protein (CRP), IL-6 and hepcidin-25 in IDA and ID conditions with a view to excluding the inflammation out.

Declarations

Author contribution statement

Sani Sharif Usman, Musa Dahiru: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Bashir Abdullah: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data.

Shariff Bilal Abdullahi: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Usman Muhammad Maigari: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Abdullahi Ibrahim Uba: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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