Red Blood Cells Superoxide Dismutase Activity in Iron Deficiency Anemia

Fayez M. Hafez, Hoda M. A.Hassab, Zeinab E. Mourad[1] and Hamdy E. Ascalany

From the departments of Pediatrics and Clinical Pathology[1] Faculty of Medicine Alexandria University

Abstract:

In order to study the activity of superoxide dismutase (SOD) in red blood cells in children with iron deficiency anemia (IDA), thirty children suffering from IDA were studied. Ten matching normal healthy children served as controls. Complete blood count, iron profile, occult blood in stools and estimation of SOD activity were done. The results of this study showed that the SOD activity in anemic children was significantly higher than that of the control group (P<0.0005). However, no significant correlation was found between SOD activity and other parameters including reticulocyte count, serum ferritin, serum iron and total iron binding capacity (TIBC). The potential benefit of higher SOD activity in red cells from iron deficient children is to counteract the effect of oxidative stress.

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Introduction:

Despite the abundance of iron in the environment, iron deficiency is still the most common nutritional deficiency in the world and the most common cause of anemia world wide.(1) Iron deficiency anemia is a systemic disorder involving multiple systems rather than a pure hematologic condition associated with anemia. It results from lack of sufficient iron for synthesis of hemoglobin and shortening of red cell life span.(2) Previous studies suggested that the decreased red cell survival is secondary to an increased susceptibility of the red cell to oxidative stress.(3)

Oxidative damage to cells can be caused by the formation of superoxide radical (O_2), hydrogen peroxide radical (H_2O_2) and hydroxyl radical (OH). These oxidants lead to cell damage through lipid peroxidation of the polyunsaturated fatty acids of the cell wall.(4) The steady state formation of oxidants is balanced by a similar rate of their consumption by antioxidants that are both enzymatic and nonenzymatic. Superoxide dismutase enzyme (SOD) is one of the most important antioxidant enzymes in the red blood cell.(5) It is a copper-containing enzyme which is widely distributed throughout all aerobic organisms. Superoxide dismutase converts two molecules of superoxide into oxygen and hydrogen peroxide.

$$O_2 + O_2 + 2H \xrightarrow{SOD} O_2 + H_2O_2$$

This first step is of crucial importance to the red cell as it removes a free radical capable of initiating chain reactions causing oxidative stress to the cell thus leading to cell damage and shortening of the red cell life span.(6)

The aim of the present work was to study the activity of superoxide dismutase in red blood cells in children with iron deficiency anemia.

Subjects and Methods:

The present study was conducted on thirty children, suffering from iron deficiency anemia who attended the Damanhour teaching Hospital. Their age ranged from 10 months to 10 years. Sixteen of them were males and fourteen were females. Ten matching normal healthy children served as controls. None of the children received any form of iron therapy. All children were clinically free of other nutritional deficiencies, free from signs of acute illness or infections at the time of testing.

All studied children were subjected to:

- Thorough history taking & complete physical examination.
- A complete blood count including hemoglobin (Hb), hematocrit, total red cell count and red cell indices (MCV, MCH and MCHC).
- Measurement of serum iron,(7) total iron binding capacity(TIBC) (8) and serum ferritin.(9)
- Complete urine and stools analysis.
- Occult blood in stools.
- Estimation of SOD activity.(10)

Results:

The results of this study showed that all the anemic patients (100%) were complaining of progressive pallor. Six of them were complaining of irritability (20%), twelve were complaining of anorexia (40%), fifteen were suffering from decreased exercise tolerance (50%), and fourteen had a past history of recurrent respiratory tract infection (46%).

Physical examination showed enlarged spleen in 6 cases (20%) and systolic murmur in 7 cases (33%) (table I).

Stool examination for parasites revealed eggs of Enterobius Vermicularis in five subjects, while ten children showed ascaris eggs and six showed Entamoeba histolytica cysts in their stools. None of the patients had Hookworm ova or occult blood in the stools.

The mean values of hemoglobin, hematocrit and blood indices (MCV, MCH, MCHC) were significantly lower in anemic patients than the control group as shown in table II.

The mean serum iron and the mean serum ferritin were also significantly lower in anemic patients than the control group; while total iron binding capacity (TIBC) was significantly higher in anemic cases than in control group (table II).

The superoxide dismutase activity in anemic cases ranged from 900-3500 U/gm Hb with a mean of 1911.3 ± 804.3, while in controls it ranged from 500-750 U/gm Hb with a mean of 623.5 ± 72.57. The difference was statistically significant (table II). However, no significant correlation was found between SOD activity and other parameters including reticulocyte count ($r = 0.35$, $P>0.05$), serum ferritin ($r = -0.14$, $P>0.05$), serum iron ($r = 0.006$, $P>0.05$) and total iron binding capacity (TIBC) ($r = 0.25$, $P>0.05$) (table III).

Table I : Clinical findings of anemic cases.

Cases	Number (n=30)	%
Pallor	30	100%
Enlarged spleen	6	20%
Ejection systolic murmur	7	23.3%

Table II : Comparison between the mean values of Hb, Hct, blood indices, serum iron, TIBC, serum ferritin and SOD activity in anemic patients and control group.

	Cases (n=30)	Controls (n=10)	t-value	P value
Hemoglobin conc. Range Mean ±SD	 5-9 7.76 ± 0.82	 12-15 13.38 ± 0.95	 16.63	 0.0006*
Hematocrit (Hct) Range Mean±SD	 18-30 26.13 ± 2.7	 36-45 39.1 ± 3.10	 11.79	 0.0004*
MCV (fl) Mean±SD	66.26 ± 6.4	82.6 ± 4.9	8.39	0.0003*
MCH (pg) Mean±SD	20.63 ± 2.73	31.6 ± 2.63	16.23	0.001*
MCHC (gm%) Mean±SD	29.23 ± 1.406	36.5 ± 2.01	8.92	0.0003*
Serum Iron (ug/dl) Range Mean±SD	 80-110 93.7 ± 11.04	 14-30 20.17 ± 4.72	 26.2	 0.000*
TIBC (ug/dl) Range Mean±SD	 260-300 281.77 ± 12.2	 420-650 513 ± 77.7	 19.02	 0.000*
Serum Ferritin (ng/ml) Range Mean±SD	 1.4-8 4.40 ± 2.11	 25-80 48.1 ± 16.2	 36.2	 0.000*
SOD activity (U/gram Hb) Range Mean±SD	 900-3500 1911.3 ± 804.3	 500-750 623.5 ± 72.57	 8.66	 0.0005*

**highly significant*

Table III : Correlation between SOD activity, reticulocyte count (RET),Ferritin, Iron as well asTIBC.

Parameter	r value	P value
SOD # RET	0.35	>0.05*
SOD # Ferritin	-0.14	>0.05*
SOD # Iron	-0.006	>0.05*
SOD # TIBC	0.25	>0.05*

** not significant*

Discussion:

Iron deficiency anemia continues to be the most common specific nutritional deficiency in the world. Despite the advances in infant feeding during the last decades, it failed to eliminate iron deficiency as a public health problem.[1]

In the present study, the mean superoxide dismutase (SOD) activity was found to be significantly higher in the studied cases than the control group. This is in accordance with Jansson et al.,[11] Ozsaylus,[12], Acharya et al.[13] and Bartal et al.[14]

The potential benefit of higher SOD activity in red cells from iron deficient children is to counteract the effect of oxidative stress that can cause lipid peroxidation and damage the red cell. Superoxide dismutase enzymatically converts superoxide radical (O_2) to Hydrogen peroxide (H_2O_2) and thereby impairs its spontaneous conversion to singlet oxygen, [15] and hydroxyl radical.

Furthermore, the red cells had an increased susceptibility to hemolysis on exposure to H_2O_2 in vitro.[16] Although SOD increases the production of H_2O_2, which can result in oxidative damage to the red cell, but it is still less harmful to the red cells than singlet oxygen and hydroxyl radical that would accumulate to a lesser degree with an elevation of SOD activity.

Some of the patients have a reduced red cell survival, demonstrated by radioactive tagging studies.[17] The direct cause for the shortened survival is not known.

Increased oxidative stress to red cells is supported by Yip et al.[3] who found increased malonyl dialdehyde (MDA) formation in iron deficient red cells. Furthermore, abnormal accumulation of hydrogen peroxide was reported. This H_2O_2, formed in red cells, is normally reduced to oxygen and water by the enzymes glutathione peroxidase and catalase, and both enzymes have been found to be decreased in red cells of iron deficiency anemic child.[17]

Rao et al., in 1996,[18] reported that an assay of lipid peroxidation and activity of antioxidant enzymes in iron deficiency anemic cells showed that malonyl dialdehyde (MDA) production was elevated as an indication of oxidative stress and increased level of superoxide dismutase activity as proved by the present study.

Another important function for the SOD enzyme is to help conversion of ferrous to ferric iron. Ferrous iron results in the generation of the hydroxyl radical, a key intermediate in many of the pathways that propagate additional free radicals. Therefore, a higher SOD activity in iron deficient red cells is likely to constrain oxidative damage.

In iron deficiency anemia there is an increase protoporphyrin concentration. In the red cell, protoporphyrin is an oxidative compound and may contribute to the decreased red cell survival in iron deficiency.[17]

Another possible explanation for high SOD activity in iron deficient red cells could be the differences in cell age. Many red cell enzymes decrease in activity with increasing red cell age. Bartosz et al., [19] reported that the young red cells have a higher content of SOD per gram Hb than older ones. Also, in investigating the effect of the reticulocytosis on SOD level, he found that both the reticulocyte-rich and the reticulocyte-poor fractions were highest in SOD in the iron deficient cells. These findings suggest that the increased SOD activity in iron deficient red cells is not a function of reticulocytosis, and this was in agreement with our results, as there was no significant effect of reticulocytic count on SOD activity (table III).

Acharya et al., [13] reported that the increased red blood cell SOD activity in iron deficiency anemia might be explained by the younger RBC population and the reductions in the glutathione peroxidase and catalase activities, by the microcytic hypochromic changes and the lack of availability of iron respectively. Bartal et al., [14] reported that red blood cells in iron deficiency anemia have a decreased activity of essential antioxidant enzymes as catalase and glutathione peroxidase. They

examined the effect of in-vitro exposure to oxidative agents in iron deficiency anemic red cells and their recovery capacity and demonstrated that iron deficiency anemic cells are more susceptible to oxidation but have good capacity for recovery.

Because our endogenous antioxidant defenses are not completely effective, it seems reasonable to propose that dietary antioxidants are particularly important to diminish the cumulative effects of oxidative damage over the long human life span, and that they account for some of the beneficial effects of fruits, grains and vegetables. Iron therapy and treatment of the cause are the main principles of management of iron deficiency anemia. Further clinical trials are needed to evaluate the effect of addition of antioxidants as adjuvant in the therapy of iron deficiency anemia.

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