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Research Article

Study of biochemical markers in iron deficiency anemia

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ABSTRACT

Background: The present study was aimed to study alterations in levels of oxidants and antioxidants in iron deficiency anemia (IDA).
Methods: 30 patients of IDA in age group of 20-50 and 30 healthy subjects were included for study. Serum Iron, TIBC & Hb% were estimated to diagnose IDA. Serum Malondialdehyde (MDA), Nitric oxide (NO) as an oxidants & Superoxide dismutase (SOD), vitamin E, vitamin C, Zinc (Zn) levels as antioxidants were estimated.
Results: Significant decreased levels of Hb%, serum iron, SOD, Vitamin C, vitamin E, Zn were found. TIBC, MDA, NO were significantly increased as compared to controls.
Conclusion: The normal adult erythrocytes can resist oxidative stress by several antioxidant defense systems. In IDA, oxidative stress causes lipid peroxidation and membrane damage. So antioxidants can be used as a marker for prevention of membrane damage due to oxidative stress.

Keywords: Oxidants, Antioxidants, IDA (Iron Deficiency Anemia)

INTRODUCTION

Iron deficiency anemia (IDA) is the most common cause of anemia throughout the world and one of the most common medical problems that confront the general physicians.[1] More than 2 billion people suffer from IDA worldwide.[2] If untreated, IDA would lead to a number of complications. Therefore, now more emphasis is being laid on the need of iron therapy.[1]

Along with other factors and reasons, several disease states are due to imbalance between the activities of oxidative agents and the antioxidant system within the cell, IDA is one of them.[3] Iron deficient subjects are known to have an increased susceptibility to infection.[3] A low serum iron along with an elevated TIBC (Total Iron Binding Capacity) is the criterion most often used to make a diagnosis of iron deficiency.[4]

Overwhelming effect of oxidants, impairment in the antioxidant defense system and reduced cellular immunity and myeloperoxidase activity were previously reported in-patients with IDA. All of these may contribute to inadequate erythrocyte survival in IDA.[5]

Very few studies have been done in human subjects regarding the IDA. Therefore, this study was aimed to evaluate the oxidants and antioxidants status in iron deficient anemic patients; this study provides with a base that we can look forward towards the status of antioxidants and the protection they may offer in this particular disease.

METHODS

Thirty patients of iron deficiency anemia in the age group of 20 to 50 years were studied. The control group included 30 healthy subjects, who had no prior history of

cardiovascular diseases, infectious diseases, cancer, inflammatory diseases, diabetes mellitus etc. which may induce the oxidative stress. 8 ml of venous blood sample was collected from antecubital vein from the subjects. Of the total blood sample, about 5ml of blood was collected in a dry, clean plain autoclaved bulb for the study. Serum lipid peroxide levels were determined in terms of MDA (Malondialdehyde) by Kei Satoh's method,[6] serum nitric oxide by Cadmium reduction method of Cortas and Wakid,[7] serum vitamin E by Baker and Frank Method, serum Iron[9] and TIBC (Total Iron Binding Capacity) by Ramasay's method, hemoglobin percentage was estimated by Drabkin method[6] and serum zinc was determined by using Atomic Absorption Spectrophotometer (AAS). The remaining blood sample (3ml) was collected in a bulb containing fluoride as an anticoagulant to study the parameters like plasma vitamin C (evaluated by 2:4 dinitrophenyl hydrazine method)[12] & RBC – Superoxide Dismutase (SOD) by the method of Kajari Das.[7]

RESULTS

Table 1 shows a significantly ($p < 0.01$) decreased Hb% levels in the IDA patients (6.75±2.36* gm%) as compared to the healthy controls (13.15±1.45 gm%). We also observed significantly ($p < 0.01$) decreased levels of iron in the IDA patients (35.23±13.52*µg/dl) as compared to the healthy individuals (106.3±26.92 µg/dl). There were also a significant ($p < 0.01$) increase levels of TIBC in the IDA patients (388.76±33.70*) as compared to the healthy individuals (316.96±16.01).

Table 1: Comparison of blood investigations in healthy controls and iron deficient anemic patients.

Serial No.	Parameters	Healthy controls	IDA
1.	Hb% (gm%)	13.15±1.45	6.75±2.36*
2.	Iron (µg/dl)	106.3±26.92	35.23±13.52*
3.	TIBC (µg/dl)	316.96±16.01	388.76±33.70*

Table 2: Comparison of oxidant levels in healthy controls and iron deficient anemic patients.

Serial No.	Parameters	Healthy controls	IDA
1.	MDA (nmol/ml)	2.68±0.57	5.06±1.98*
2.	NO (µmol/l)	51.33±3.76	61.96±11.91*

Table 2 shows a significantly ($p < 0.01$) increased MDA levels in the IDA patients (5.06±1.98* nmol/ml) as compared to the healthy controls (2.68±0.57nmol/ml). We also observed significantly ($p < 0.01$) increased levels of NO in the IDA patients (61.96±11.91*µmol/l) as compared to the healthy individuals (51.33±3.76 µmol/l).

Table 3 shows a significantly ($p < 0.01$) decreased SOD levels in the IDA patients (1.78±0.51* units/mg of Hb) as compared to the healthy controls (3.22±0.26 units/mg of Hb). We also observed significantly ($p < 0.01$) decreased levels of Vitamin C (0.421±0.18* mg/dl) & Vitamin E (0.486±0.12* mg/l) in the IDA patients as compared to levels of Vitamin C (1.20±0.21 mg/dl) & Vitamin E (1.41±0.38 mg/l) in healthy individuals. Zinc levels were highly significantly ($p < 0.05$) decreased in IDA patients (0.686±0.240** mg/l) in comparison with healthy controls (0.81±0.15 mg/l).

Table 3: Comparison of antioxidant levels in healthy controls and iron deficient anemic patients.

Serial No.	Parameters	Healthy controls	IDA
1.	SOD (units/mg of Hb)	3.22±0.26	1.78±0.51*
2.	Vitamin C (mg/dl)	1.20±0.21	0.421±0.18*
3.	Vitamin E (mg/l)	1.41±0.38	0.486±0.12*
4.	Zinc (mg/l)	0.81±0.15	0.686±0.240**

Values expressed in mean±SD
*P<0.01 (significant)
**P<0.05 (highly significant)

DISCUSSION

In the present study, the IDA patients were screened for serum MDA, as a measure of lipid peroxide index and found increased levels when compared with controls ($p<0.001$).

The causes of increased oxidative stress and decreased antioxidant defense in iron deficiency anemia have not been completely explained, although a significant increase in lipid peroxidation has been found.

Our results were in accordance with Bartal M et al,[13] Vives Corrons et al,[14] Acharya J et al.[15] The low MCHC (Mean Corpuscular Hemoglobin Concentration), characteristic of IDA, may contribute to the excess of intracellular oxidating agents and to membrane damage.[14] Susceptibility of RBC's to lipid peroxidation was increased when expressed per gm of Hb.[16]

The present study revealed significant increase in the levels of NO in study subjects than those of healthy controls ($p<0.001$).

Iron deficiency anemia increases NO production and elevated NO concentrates might be due to less

concentration of iron in the subjects indicating increased NO production may contribute to lipid peroxidation.[17] Thus our result matches with Choi JW et al.[17]

Significantly lowered RBC-SOD activity was seen in the study group (IDA) subjects as compared with controls ($p<0.001$). Our results were corroborated with Erdal Kurtoglu et al,[15] Mehmet Isler et al.[5] Thus, in their study the SOD activity in anemic patients were less than that of control groups, which might be caused by insufficient nutrition and oxidative stress under hypoxic condition. It is well known that reactive oxygen species, especially hydrogen peroxide inhibits SOD activity may contribute to free radical propagation.[5]

As compared to control subject's vitamin C was significantly depleted in IDA patients ($p<0.001$). Decrease in the levels of Vitamin C compared with control subjects might be due to increased utilization to trap the oxy radicals and failed regeneration of oxidized Vitamin E. Thus, ascorbic acid is of certain beneficial effect in alleviating the state of absorption.[35]

Depleted Vitamin E was observed in IDA patients as compared with controls ($p<0.001$). Our results were in accordance with Lorna G. Macdougall et al.[18] The most significant role of Vitamin E in cellular metabolism was thought to be that of an antioxidant, preventing the peroxidative breakdown of membrane lipids. Red cells from subjects with low serum vitamin E concentrations ($p<0.05$ on gm per 100ml) have been noted to lyses readily on exposure to H_2O_2.[18] Also the reduced level of vitamin E was observed in anemic patients due to the possible involvement of Vitamin E with heme synthesis.[19]

Decreased serum Zinc levels were found in IDA subjects while comparing with controls ($p<0.05$). Our study matches with Ece et al,[20] Mikhailova L et al[21] and Prasad AS.[22] Zinc is essential for normal iron metabolism.[23] Study of iron supplementation among anemic patients effectively increase iron status while adding zinc to iron supplementation.[24] Thus, the administration of zinc along with iron presumably increased production of proteins and globin related to hematopoiesis in the bone marrow.[25]

Serum iron levels were significantly depleted as compared to control groups ($p<0.001$). Our study observation matches with Rajadhyakshya RC,[26] and Gupta P.[27] Iron deficiency may result from dietary inefficiency, impaired absorption, and increased requirements or due to chronic blood loss.[28] Thus, iron deficiency is the commonest cause of microcytic anemia.[29]

Concentration of hemoglobin was decreased in IDA patients as compared to controls ($p<0.001$). Our results were in coherent with Rajadhyakshya GC et al.[30] Heme is synthesized in the cytoplasm of maturing erythroid cells and reticulocytes. Heme synthetase, a mitochondrial enzyme catalyzes the formation of heme by insertion of iron into protoporphyrin IX.[31] This iron in IDA is decreased which further leads to decrease in Hb synthesis.[28]

Statistically significant elevation in the levels of TIBC in IDA subjects was seen when compared with control subjects. These results match with Rajadhyakshya GC et al,[31] Ralph Carmel et al,[32] Khubchandani RP.[33] Once iron is translocated across the epithelial barrier, it is coupled to transferring, which delivers iron to tissues throughout the body. Each transferring molecule can bind two atoms of iron. The aggregate binding sites of all the transferring in the circulation comprise the Total iron Binding Capacity (TIBC) of plasma. Normally 20% to 45% of irons binding sites are filled. Specific receptors to the plasma membranes of cells organize transferring. This leads to internalization of the protein and release of iron into the cell cytoplasm. Thus, in anemia increased free iron is confluence with increased TIBC.[34]

CONCLUSION

In abridgement, present study reveals alterations in the levels of iron, Hb and TIBC, along with the augmented free radicals and impaired antioxidant potential.

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REFERENCES

1. Amin RB, Bal S, Dewan B, Langade D, Mathew S, Sudarshan S. An open trial to assess ferric hydroxide polymaltose complex plus folic acid (orofer) in IDA. The Physicians Update; JAMA India;4(10):53-56.
2. Shaha A. Iron deficiency anemia Part I. Indian J Med Sci: Feb 2004; 58(2) : p79-81.
3. A.L.A. Ferre, et al. Lipid peroxidation antioxidant enzymes and glutathione levels in human erythrocytes exposed to colloidal iron hydroxide in vitro. Braz J Med Biol Res: June 1999: 32(6): p 689-694.
4. R.P. Khubchandani et al. Management of iron deficiency anemia: Journal of applied medicine Jan 1988: p-55-56.
5. Mehmet Isler et al. Superoxide dismutase and glutathione peroxidase in erythrocytes of patients with iron deficiency anemia: Effects of different treatment modalities. Croatian Medical journal; 2002; 43(1): p16-19.
6. Kei Satoh. Determination of serum lipid peroxide. Clinica Chemica Acta; 1978; 37: p-90.
7. Kajari Das et al. A modified spectrophotometric assay of superoxide dismutase using nitrite formation by superoxide radicals. Indian journal of

Biochemistry and Biophysics; June 2000; 37: p201-4.
8. Baker and Frank. Varley's Practical Clinical Biochemistry, 6th edition, Heinemann Professional publishing: 902.
9. H Varley. Practical Clinical Biochemistry; 4th edition: p472.
10. Varley. Ramasays Dipyridy1 method for total iron binding capacity. Practical Clinical Biochemistry; 4th edition: p475.
11. Cyanomethemoglobin method, How'ks physiological chemistry; Oser, 14th edition: p1906.
12. Caraway W. T. Carbohydrates in Tietz N. L. Ed; Fundamentals of Clinical Chemistry. W. B. Saunders Company: p173-6.
13. M Bartal et al. Iron deficiency anemia: recovery from in vitro oxidative stress. Acta Hematol ; 1993;90: p94-98.
14. Vives Corrons JL et al. Increased susceptibility of microcytic red blood cells to in vitro oxidative stress. Eur. J. Haematol; 1995;55:p327-331.
15. Kumerova A. et al. Anemia and antioxidants defense at the red blood cells. Mater Med Pol; Jan-Jun 1998; 30(1-2): p12-5.
16. Acharya J et al. Red cell lipid peroxidation & antioxidant anzymes in iron deficiency. Eur J. Haematol; Oct. 1991; 47(4): p287-91.
17. Choi JW et al. Iron deficiency anemia increases nitric oxide production in healthy adolescents. Ann. Hematol; Jan 2002; 81(1);p1-6.
18. Lorna G. Madougall. Red cell metabolism in iron deficiency anemia. The Journal of Pediatrics; May 1972; 80 (S) : p775-782.
19. Machlin et al. Vitamin E, A comprehensive Treatise 1980;1:p 289-372.
20. Ece A et al. Increased serum copper and decreased serum zinc levels in children with iron deficiency anemia Bio Trace Elem Res; 1997 winter 59 (1-3): p 31-9.
21. Mikhailova L. et al. Iron, copper and Zinc content in healthy persons and iron deficiency anemia patients. Vutr Boles, 1881; 20 (6): p114-21.
22. Prasad A.S. Zinc deficiency. BMJ; 2003; 326: P409-410.
23. Zinc is essential for normal iron metabolism & prevention of anemia, but high levels of zinc can depress iron & lead to anemia. J. Am. Coll. Nutr: Jun 1998; 17(3); P 291-5.
24. Moshynska Ovetal. Hemoglobin – induced lipid peroxidation in anemia. Lik Sprava; Jul – Aug 2001; 4: p39-43.
25. Soroku Nishiyama et al. Zinc & IGF-I concentrations in pregnant women with anemia before and after supplementation with iron and /or zinc. Nutrition; 1999; 18(3): p261-67.
26. G.C. Rajadhyakashya et al. Evaluation of Efficacy and Tolerability of Iron Polymaltose complex Tablets in Iron Deficiency Anemia, During pregnancy. JAMA; Dec. 2000; 3 (12): p 53-98.
27. Sharma D et al. Maternal and Perinatal outcome in Anemia during pregnancy. The Indian Practionar; May 2005; 57(5); p 295-98.
28. Cortan et al. Robbins pathologic Basic of Disease. 6th edition: p627-630.
29. Natural News. Zinc, not iron, may be the answer to anemia. Journal of the American college of Nutrition; June 1999; 19(3): P261-7.
30. Drew Provan et al. Red cells II: acquired anemia's and polycythaemia. The lancet : April 8, 2000; 355: p 1260-68.
31. Willam J. Thomas et al. Free erythrocyte prophyrin : Hemoglobin ratios, serum ferritin and transferring saturation levels during treatment of infants with iron deficiency anemia; p455-461.
32. Ralph Carmel et al. Iron deficiency occurs frequently in patients with pernicious anemia; JAMA : Nov. 1987 [2(2)] : p141-43.
33. Anish Desai et al. Review of iron polymaltose complex in the management of iron deficiency anemia: JAMA- The Physians Update; 4(8): p 85-90.
34. Dilip Pawar et al. Iron polymaltose complex: A review of the novel concept in iron delivery in anemia during pregnancy: JAMA India. The Physician Update; 4(8): p80-83.
35. Hawary MF et al. Ascorbic acid effect on intestinal iron absorption in different types of anemia's. Egypt Paediatr Assoc; Apr 1975; 23(2): p 145-9.

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