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# Oxidative Stress in Iron Deficiency Anemia

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**Original Research Article**

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**Abstract:** Iron deficiency anemia is widely prevalent nutritional disorder worldwide, resulting in oxidative stress which in turn leads to cardiovascular diseases, cancer, diabetes mellitus and ageing. Oxidative stress and iron levels were studied in 84 subjects, 36 of whom were anemic (Hb <11 gm/dl) and 48 healthy subjects who were taken as controls. In these subjects serum iron, Total Antioxidant Capacity, superoxide dismutase and malondialdehyde levels were estimated. In the present study it was found that anemic subjects had higher oxidative stress as compared to controls and female anemia subjects were more affected than males. Preventive action may help in reducing the morbidities caused by the oxidative stress.
**Keywords:** Anemia, iron deficiency, oxidative stress.

## INTRODUCTION

Although, iron is the fourth most abundant micromineral in the crust of earth [1], iron deficiency anemia (IDA) still remains the commonest nutritional disorder worldwide [2] and 2 billion people suffer from IDA [3]. According to the National Family Healthy Survey 2004-05, the prevalence of IDA in India is 78.9% among children below 3 years, 55% in women and 24% in men [4].

A low serum iron level with raised TIBC (Total Iron Binding Capacity) is the criteria used for diagnosis of iron deficiency [5]. Oxidative stress resulting from IDA affects the production of iron containing antioxidant proteins, tilting the balance to the oxidative side [6].

Imbalance in the state between reactive oxygen species (ROS) and antioxidant system causes irreversible damage to the cell leading to oxidative stress. Oxidation of proteins, lipid, carbohydrates and DNA can be due to effect of free radicals on the cell and its organelles and these free radicals are neutralized by antioxidant systems of the body [7]. The antioxidant balance is shifted towards the oxidative side in patients with IDA who have increased oxidants [8] and this affects the production of hemoglobin and iron containing proteins like cytochromes, myoglobin, catalase and peroxidase. The enzymes of the antioxidant defense system get functionally defective and free radicals produced, trigger oxidative damage. However, data on oxidative and antioxidant defenses in IDA is limited and often contradictory [9].

Analysis of antioxidative capacity is considered as a useful marker in medical diagnosis and treatment of several diseases, like cardiovascular diseases, cancers, diabetes mellitus and ageing [10]. The cumulative action of all the antioxidants present in the body is provided by analyzing Total Antioxidant Capacity (TAC) and is considered better than the simple sum of antioxidant measurements [11]. In patients with IDA, TAC was significantly lower and oxidant activity was much higher ($p<0.05$), as compared to healthy controls [12].

All organisms possess effective enzymatic and non-enzymatic antioxidant (AO) systems, which counter act to ROS mediated harmful oxidative reactions [13]. Superoxide dismutase (SOD) is one of the most important AO enzymes in the red blood cell. It is a copper-containing enzyme widely present in all aerobic organisms [14]. The damaging ROS is removed by SOD by dismutation of superoxide radicals leading to formation of hydrogen peroxide and oxygen in extra and intracellular compartments (13) and includes Cu, Zn-SOD, mitochondrial Mn-SOD, and extracellular Cu, Zn-SOD [15].

Lipid peroxidation leads to production of Malondialdehyde (MDA) which is found to be elevated in oxidative stress conditions. Lipid peroxides being formed by oxidation of polyunsaturated fatty acids, being unstable undergo decomposition to form reactive carbonyl compounds [16] and MDA which is

considered to be the most important marker for monitoring oxidative stress [17].

Even though anemia is highly prevalent in India and iron deficiency state is expected to be associated with oxidative stress as reported in certain studies abroad, there is paucity of data regarding the same in Indian scenario. So, the present study was conducted to evaluate total antioxidant capacity (TAC), RBC superoxide dismutase (SOD) status and lipid peroxidation, MDA levels in young adults with IDA.

## MATERIALS AND METHODS

After ethical clearance, 36 samples of IDA adult subjects and 48 healthy subjects were collected from out-patient department of A.B. Shetty Memorial Institute of Dental Sciences, Manglore. Informed consent was obtained from each participant and the objectives of the study were fully explained. A venous sample of 5 ml was collected from each subject. The subjects with health related problems like renal and liver dysfunction, or any other chronic disease were excluded from the study. Status of iron, SOD, TAC and oxidative stress among the subjects were analyzed using Systronics PC based double beam spectrophotometer (model 2202). The hemoglobin (Hb) levels were found using Rapid semi-autoanalyzer Star 21plus. TAC levels was estimated by Phosphomolybdenum method [18], SOD levels by Nitro Blue Tetrazolium salt (NBT) method [19], MDA levels by TCA-TBA method [20] and serum iron by Bathophenanthroline method [24]. The statistical analysis was done using SPSS 11.0 for windows software.

## RESULTS

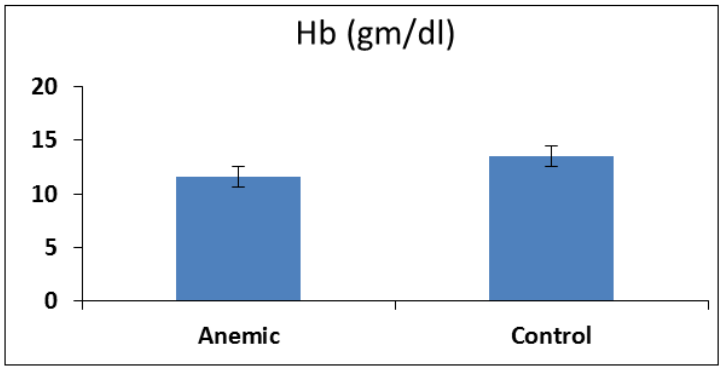
Of the eighty four subjects (56 males and 28 females) were taken for the study. Thirty six subjects were found to be anemic based on hemoglobin level (11.59±0.91 gm/dl) while forty eight formed the control group. 32.1% of the males and 64.3% of the females were anemic. Further, oxidative stress was calculated by using MDA as a parameter and antioxidant status was found using SOD and TAC levels. Iron status was also evaluated for all subjects.

**Table-1: Descriptive Statistics of 84 subjects**

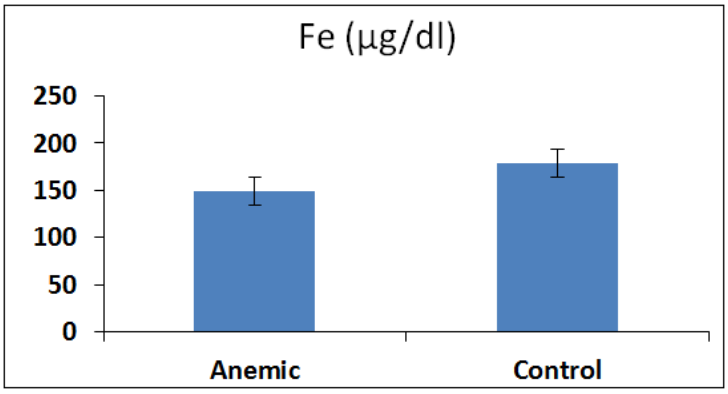
| Variables | N | Mean±SD |
|---|---|---|
| Hb (gm/dl) | 84 | 12.66±1.21 |
| Fe (µg/dl) | 84 | 165.24±42.10 |
| TAC (µg/ml) | 84 | 125.87±26.20 |
| SOD (U/gHb) | 84 | 1243.64±182.09 |
| MDA (µM/L) | 84 | 2.90±0.90 |

**Table-2: Student's unpaired t-test for significant difference between mean values of anemic and control groups**

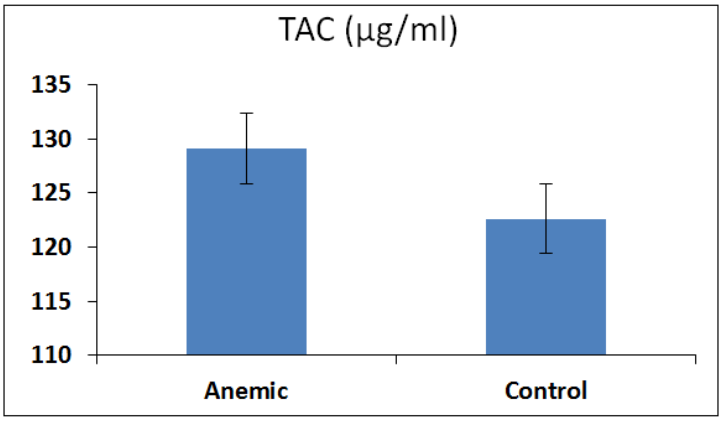
| Variables | Mean±SD | | *p* Value |
|---|---|---|---|
| | Anemic (n=36) | Control (n=48) | |
| Hb (gm/dl) | 11.59±0.91 | 13.55±0.52 | <0.05 |
| Fe (µg/dl) | 148.83±32.74 | 178.80±44.41 | <0.05 |
| TAC (µg/ml) | 129.12±22.35 | 122.62±19.40 | <0.05 |
| SOD (U/gHb) | 1227.89±151.02 | 1259.39±121.62 | <0.05 |
| MDA (µM/L) | 3.47±0.12 | 2.33±0.75 | <0.05 |



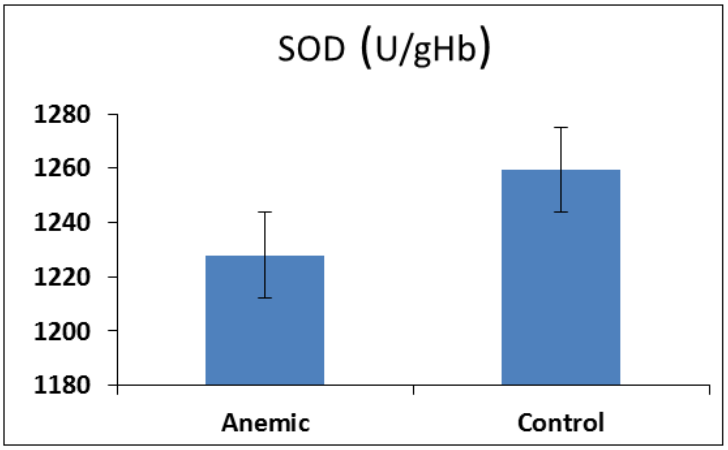

**Graph-1: Comparison between mean Hb levels in anemic & control groups**



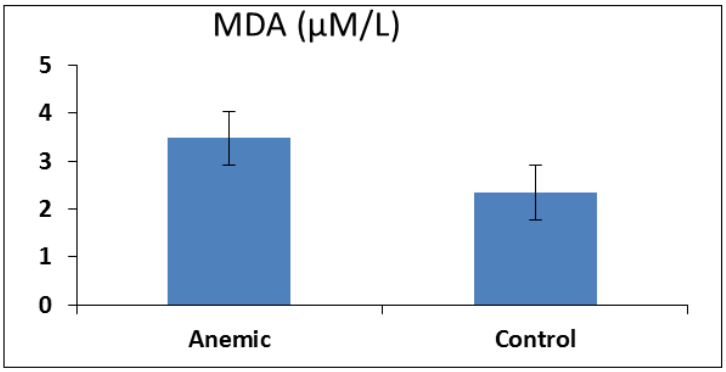

**Graph-2: Comparison between mean iron levels in anemic & control groups**




**Graph-3: Comparison between mean TAC levels in anemic & control groups**




**Graph-4: Comparison between mean SOD levels in anemic & control groups**




**Graph-5: Comparison between mean MDA levels in anemic & control groups**

## DISCUSSION

It was found that there was a significant association between gender and Hb levels ($p<0.05$). In case of females from iron deficiency anemia antioxidant status was lower than normal (graph 3), thus resulting in higher oxidative stress indicated by MDA levels (graph 5), compared to their male counterparts who had normal antioxidant status. Also in case of females, significantly higher level of lipid peroxidation was found, possibly due to lower levels of vitamin C and TAC. However, in case of males, the level of lipid peroxidation was significantly lower even in cases of anemic subjects.

SOD levels were determined in all the subjects and it was found that male and female subjects suffering from anemia had a considerably lower range of SOD as compared to controls (graph-4). TAC values were lower in female subjects with anemia as compared to males with anemia, further leading to higher oxidative stress (graph-3). Even though oxidative stress was found to be elevated in anemic subjects but the results were found to be inconclusive.

The current study also showed that there is no significant correlation between the levels of SOD or MAD and the age of the subjects, as was shown earlier by Dittmar M, Knuth M, Beineke M and Epe B [21]. However, our observation was carried out in 84 subjects and this number is not very large to show any statistical significance. Hence by increasing the scale of study (subject numbers) in the future, a more significant conclusion may be drawn. There were no subjects above the age of 70 years and this could possibly be a factor which led to deviation from the earlier experiments.

According to the World Health Organization (WHO), IDA is especially prominent in south Asia [22] and up to 88% of pregnant and 74% of non-pregnant woman are anemic in India. Iron status can be known by measurements of hemoglobin, serum ferritin, serum iron and transferrin (total iron–binding capacity) [23]. However, these measurements have their limitations; they can indicate iron deficiency and the severity of anemia is differentiated by the severity of the reduction in hemoglobin levels.

## CONCLUSIONS

This type of study may help the biotechnologist to genetically modify the nutritional factors in plants, bacteria, fortification of food etc. so that IDA can be prevented at an early stage, may be from infancy and in a cheaper way as this type of anemia is prevalent in the developing countries.

The relationship of increased DNA damage with low serum iron, hemoglobin micronutrient level seen in IDA suggest that DNA damage could be due to iron deficiency which also causes oxidative stress. Dietary factors, hookworm infestation, malaria, increased demand due to physiological status aggravate deficiency of iron. Iron fortified salt to increase the hemoglobin level have been tried. Strategies involving genes that could increase the availability of iron and other micronutrients in an ideal dose and available in commonly consumed food stuff has to be explored. A change in dietary habit and nutritional education of the population needs to be tried.

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