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ANALYSIS OF SERUM OXIDANT – ANTIOXIDANT STATUS IN PATIENTS WITH IRON DEFICIENCY ANEMIA (IDA)

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ABSTRACT

Iron deficiency is the most common deficiency worldwide, with negative effects on work capacity and on motor and mental development of infants, children, and adolescents, particularly in developing countries. Free radicals were generated due to the oxidative damage to the red blood cells and the exact pro-oxidant & antioxidant status in iron deficiency anemia was still ambiguous. Hence we have conducted this study not only to analyse but also to gain new insights into the oxidant-antioxidant status in patients diagnosed with iron deficiency anemia. This study was conducted in Saveetha Medical College & Hospital, Saveetha University with twenty patients diagnosed with iron deficiency anemia as study subjects and twenty age & sex matched healthy individuals as controls. Hemoglobin, ferritin, malondialdehyde, glutathione levels and the activity of the glutathione-s-transferase were measured in study subjects and compared to controls. There was a significant increase in malondialdehyde levels confirming the existence of oxidative stress in patients with iron deficiency anemia, compared to controls. The non-enzymatic and enzymatic antioxidants viz. glutathione and glutathione-s-transferase were beneficial in combating with the oxidative stress and this was evident with the decreased levels of the same in these patients compared to controls. The results of our study emphasize the use of antioxidants as a secondary therapy to reduce the oxidative damage in patients with iron deficiency anemia.

KEYWORDS: Iron deficiency anemia, oxidative stress, lipid peroxidation, ferritin, antioxidants, glutathione, and antioxidant enzymes

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INTRODUCTION

Lipid Peroxidation mediated by free radicals is considered to be the major mechanism of cell membrane destruction and cell damage. Free radicals are formed in both physiological and pathological conditions in mammalian tissues (1). The uncontrolled production of free radicals is considered as an important factor in the tissue damage induced by several pathophysiologies (2, 3). Anemia is a condition which is characterized by the less number of healthy red blood cells in the body, which provide oxygen to the tissues in our body (4, 5). Iron deficiency is the most common deficiency worldwide, with negative effects on work capacity and on motor and mental development of infants, children, and adolescents, particularly in developing countries (6, 7, 8, 9). Alteration in the oxidant – antioxidant profile is known to occur in anemia (10, 11). Antioxidants are the biochemical compounds which dispose, scavenge, and suppress the formation of free radicals, or oppose their actions (12) and two main categories of antioxidants are those whose role is to prevent the generation of free radicals and those that intercept any free radicals that are generated (13). They exist in both the aqueous and membrane compartment of cells and can be enzymes or non enzymes.

In the present study, the following parameters were assessed in plasma to elucidate the oxidant-antioxidant status in patients with Iron Deficiency Anemia (IDA). Malondialdehyde (MDA) levels were measured as Thio Barbituric Acid Reacting Substances (TBARS) which serves as an index of extent of lipid peroxidation. Glutathione (GSH) levels, which serves as non enzymatic antioxidant and the antioxidant enzyme, Glutathione-S -Transferase (GST) activity in plasma were estimated. GST is an enzyme involved in anti oxidant defense and also involved in detoxication.

MATERIALS AND METHODS

The study was conducted in twenty patients with clinically diagnosed Iron Deficiency Anemia (IDA). Twenty healthy individuals of matching age & sex were used as controls. This study was conducted at Departments of biochemistry and internal medicine, Saveetha MedicalCollege Hospital, Saveetha University, Thandalam, Chennai – 602 105, Tamilnadu, INDIA. The controls and patients were divided into two groups. Group 1:Twenty healthy age & sex matched controls.Group 2 :Twenty patients with clinically diagnosed Iron Deficiency Anemia (IDA).

Twenty clinically diagnosed patients who had not undergone any previous treatment for their Iron Deficiency Anemia (IDA) were chosen for the study from the outpatient department (OPD) of Saveetha Medical College Hospital, Saveetha University, Chennai. An equal number of age & sex matched healthy subjects were also investigated. The subjects were ranging in age 18 - 24 years. All the patients in the study were clinically diagnosed as patients with Iron Deficiency Anemia (IDA). The presence of Iron Deficiency Anemia (IDA) in patients was diagnosed by assessing blood hemoglobin level, and confirmed by assessing serum Ferritin level. None of these subjects were alcoholics or chronic smokers and were suffering from any condition producing oxidative stress like osteoarthritis, myocardial infarction, diabetes, etc. The Inclusion Criteria were: 1. Individuals (male / female) with > 18 years of age and 2. IDA defined by a Serum Ferritin < 25 ng/ml (or < 5ng/L). The exclusion criteria (14, 15) were: 1. Female subjects who are pregnant or intend to become pregnant, breastfeeding, within 3 months postpartum, or have a positive serum or urine pregnancy test, 2. Hemoglobin ≤7.0 g/dL, 3. Known causes of anemia other than iron deficiency (eg: hemolysis, vitamin B12 or folate deficiency, etc), 4. Subjects suffering from any other disorders which can produce oxidative stress (eg: osteoarthritis, myocardial infarction, inflammatory conditions, diabetes mellitus etc.), 5. Subjects < 18 years and 6. Subjects with Recent H/O fever for 1 month. Due permission was obtained from the institutional review board before the start of the study and the informed consents were also taken from the participants prior to study.

The heparinised venous blood samples obtained from these subjects were used for the analysis. Hemoglobin level in blood can be estimated by cyanmethemoglobin method using Drabkin's solution, the standard method for measuring hemoglobin (Hb) in human blood is the well-recognized as recommended by the World Health Organization (WHO) (16). Serum from the venous blood samples obtained from these subjects was separated by centrifugation at 1,000 g for 15 minutes. Separated serum was used for the estimation of Ferritin, malondialdehyde, glutathione and glutathione-S-transferase (GST). Serum Ferritin levels were estimated by ECLIA (Electro Chemiluminiscence Immuno Assay) method (ECLIA method Elecsys 2010, Roche). MDA was determined as the measure of Thio Barbituric Acid Reactive Substances (TBARS) (17). GSH was estimated by the method of Beutler et al (18) using Di Thio Bis Nitro Benzoic acid (DTNB). and GST (EC 2.5.1.18) was measured by using 1-Chloro-2, 4-Dinitro Benzene (CDNB) (19). All reagents used were of analytical reagent grade. DTNB, CDNB and Thio Barbituric Acid were obtained from sigma chemicals, St.Louis, MO.

STATISTICAL ANALYSIS

Statistical analysis between group 1 (controls) and group 2 (patients with IDA) was performed by the independent - t-test using the SPSS package for windows, version 15. The data was expressed as mean ± SD and $P < 0.05$ was considered as significant.

RESULTS

The Mean ± SD of Hemoglobin (Hb) and Ferritin in controls and cases with Iron Deficiency Anemia were indicated in the table 1 and the Mean ± SD of Malondialdehyde (MDA), Glutathione, and Glutathione – S – Transferase in controls and patients with Iron Deficiency Anemia were indicated in the table 2.

Table 1
It shows the mean ± SD values of Hemoglobin (Hb) and Ferritin in controls and cases with Iron Deficiency Anemia.

	Controls (N=20) Mean±SD	Cases (IDA) (N=20) Mean±SD	P - Value
Hb (gm/dl)	12.67±0.41	9.80±1.15	.000
Ferritin (ng/ml)	66.87±10.31	6.76±4.33	.000

Table 2
It shows the mean ± SD values of Malondialdehyde (MDA), Glutathione, and Glutathione – S – Transferase in controls and patients with Iron Deficiency Anemia.

	Controls (N=20) Mean±SD	Cases (IDA) (N=20) Mean±SD	P - Value
MDA (nmoles/ml)	3.01±0.83	8.92±1.21	.000
GSH (mg/dl)	25.78±1.77	13.96±2.51	.000
GST (U/L)	8.05±1.03	3.89±0.79	.000

DISCUSSION

In the present study the lipid peroxidation product i.e. Malon Di Aldehyde (MDA) levels have been increased significantly in serum of the patients with iron deficiency anemia. Rise in MDA could be due to increased generation of Reactive Oxygen Species (ROS) due to the excessive oxidative damage generated in these patients. It is hypothesized that free radicals were produced due to the oxidative damage to the red blood cells (20). These oxygen species in turn can oxidize many other important biomolecules including membrane

lipids (21). Similar reports of elevated MDA levels have been reported in patients with anemia due to iron deficiency (22, 23).

We observed a significant decrease in the levels of serum glutathione (GSH, non enzymatic antioxidant defense system) in patients with iron deficiency anemia when compared to controls. The decrease in the levels of these non enzymatic antioxidant parameters may be due to the increased turnover, for preventing oxidative damage in these patients suggesting an increased defense against oxidant damage in these patients. Our results were supported by the studies conducted by Neelima et al (24). In contrast to our study, Gekova K et al reported increased levels of reduced glutathione (GSH) in iron deficiency anemic patients (25). The antioxidant enzymes, Glutathione – S – Transferases (GST) are a group of multifunctional proteins, which play a central role in detoxification of electrophilic chemicals & the hepatic removal of potentially harmful hydro phobic compounds from blood (26). We have observed a significant decrease in the levels of GST in iron deficiency anemia patients compared to controls. Similar reports of diminished antioxidant enzyme levels were observed in patients with iron deficiency anemia (23). The decreased activity of GST could be due to the scavenging property of this antioxidant enzyme to counter the effect against increased oxidative stress.

CONCLUSION

In Conclusion, Oxidative stress may be involved in Iron Deficiency Anemia. The results of our study have shown higher oxygen free radical production & decreased glutathione, supporting the higher oxidative stress hypothesis in IDA. So, the treatment with antioxidants in the initial stages of the disease may be useful as secondary therapy to prevent the oxidative damage in patients with Iron Deficiency Anemia.

CONFLICT OF INTEREST: NONE

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