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ORIGINAL ARTICLE

Evaluation of malondialdehyde and glutathione peroxidase changes in iron deficiency anemia patients and their correlation with body iron status and haematological indices

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Iron deficiency anemia (IDA) is a prevalent disorder among women of reproductive age. This study aims at investigating whether the activities of glutathione peroxidase (GPx) and malondialdehyde (MDA) levels can be affected during iron deficiency anemia among women of reproductive age. In this study, 30 women with IDA, 30 with iron deficiency and 30 healthy controls were studied. The concentrations of serum malondialdehyde were measured and the activities of GPx were evaluated in all the three groups. The mean serum concentration of MDA was significantly higher in IDA group than ID group and healthy group women (5.18±0.46 vs 3.4±0.34 and 2.75±0.33 μmol/L) respectively. The mean GPx activity in IDA groups was significantly lower than healthy group women (1.4±0.2 vs 2.91±0.12 and 2.99±0.24 (U/gHb)). The concentration of MDA in IDA and ID group was negatively correlated with levels of haemoglobin (Hb), mean corpuscular volume (MCV) and serum ferritin ($p<0.05$). In healthy control subjects; there was a negative correlation between MDA concentration and Hb, MCV and serum ferritin ($p<0.01$). Furthermore, the activity of GPx and these factors levels showed significant positive correlation in every group ($p<0.01$ and $p<0.05$). Our findings showed that lipid peroxidation increases in women with ID and IDA but the activity of GPx decreases in IDA but not in ID. Moreover, there was no significant correlation between serum free iron levels with MDA concentration and GPx activity.
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Keywords: Anemia, Iron deficiency, Malondialdehyde, Glutathione peroxidase, Lipid peroxidation.

INTRODUCTION

Iron deficiency anemia is the most prevalent nutritional deficiency in the world, influencing people of all ages in both industrialized and developing societies (World Health Organization, 2001). Iron deficiency does not only hinder the production of hemoglobin, but also the production of other proteins containing Fe^{2+}, such as cytochromes, myoglobin, catalase, and peroxidase (Acharya *et al.*, 1991; Rodrigues *et al.*, 2017). The body has various antioxidants such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) which can counteract the deleterious effects of reactive oxygen species (ROS) and protect from cellular and molecular damage (Baccin *et al.*, 2009).

Disturbance of the antioxidant defense system and reduced cellular immunity and myeloperoxidase activity have been previously reported in patients with iron deficiency anemia (IDA) (Rodrigues *et al.*, 2017). Based on the nature of iron toxicity (Yesilbursa *et al.*, 2001), iron deficiency (ID) would not be expected to increase lipid peroxidation, unless a decrease in the iron dependent enzymes

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impairs the antioxidant mechanisms of red blood cells (RBC). It is therefore unclear whether the ability of RBC to undergo lipid peroxidation is increased or decreased during IDA in menstruating women (Yesilbursa *et al.*, 2001; López-García *et al.*, 2018).

A disturbance of the balance between formation of ROS and the rate at which they are scavenged by enzymatic and non-enzymatic antioxidants is referred to as oxidative stress (Zaka-Ur-Rab *et al.*, 2016). Prime targets of ROS are the polyunsaturated fatty acids (PUFA) in cell membranes causing lipid peroxidation, which may lead to damage of the cell structure and function (Koskenkorva-Frank *et al.*, 2013). Certain aldehydes such as malondialdehyde (MDA), the end product of lipid peroxidation (LPO), arising from the free radical degradation of PUFA, can cause cross-linking in lipids, proteins and nucleic acids. All of these may contribute to inadequate RBC survival (Nouri *et al.*, 2017). The aim of this study was to investigate whether the malondialdehyde (MDA) levels and activities of glutathione peroxidase (GPx) can be affected during iron deficiency anemia in women of reproductive age.

MATERIAL AND METHODS

Study design

In this case-control study 90 subjects, at Golestan Hospital, were included in the study (People aged between 21-47 years who do not take iron tablets). The study was approved by the human research ethics committee at Ahwaz Jondishapour University of Medcial Science and informed consent was obtained from each volunteer before participation.

The groups were characterized as follows: thirty anaemic women of reproductive age (21-47 years) were recruited as IDA group as per the criteria for IDA (Looker et al., 1997). Thirty women with low levels of haematologic factors such as Hb and HCT were included in the ID group. We also included thirty healthy women without any symptoms of iron deficiency anemia in the control group. Selection of participants was within each group based on non-probabilistic method and the participants were selected recruited at different times. This study was conducted from March 2016 to June 2017 at Golestan hospital affiliated with Ahwaz Jondishapour University of Medical Science. The number of people who completed the study in each group was thirty. Information of subjects was obtained by a structured questionnaire from every subject. After taking a brief biography, Patients taking iron tablets and also patients over the age of 47 were excluded from the study.

Sample collection

Nine milliliters of venues blood samples obtained from median cubital vein of all subjects and aliquoted into three tubes: Three millilitres (3mls) was taken in standard tubes containing ethylenediaminetetraacetic acid (EDTA) for estimation of Hb, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), RBC count and GPx activity. Three millilitres (3mls) of whole blood was taken in heparinised tubes which were centrifuged (1800 rpm for 10 minute) and the serum was analysed for MDA and iron. Three millilitres (3mls) were incubate at 37°C and centrifuged in order to carry out serum ferritin assay. After sampling, the samples were placed on ice and immediately transferred to the laboratory for testing.

Biochemical Analysis

Blood hemoglobin concentration was measured using the cyanomethemoglobin method using Randox kits (Randox-Laboratories, USA). The concentration of iron in serum was measured by flame atomic absorption spectroscopy (Perkin Elmer AAS-700 Ü̈berlingen, Germany). Total iron binding capacity (TIBC) levels were measured by automatic analyser (Kodak Ektachem 500) using the colorimetric method and standard kits. Serum ferritin level was determined by enzyme-linked immunosorbent assay (ELISA) using human ferritin enzyme immunoassay test kit (IBL Immunobiological Laboratories, Hamburg, Germany). Serum MDA concentrations were assayed by measurement of thio barbituric acid reactive substances (TBARS) (Kei, 1978). The GPx activity was measured by the coupled method of

Paglia and Valentine using t-butyl hydroperoxide as a substrate (Paglia and Valentine, 1967).

Statistical analysis

The data are expressed as mean ± SD except correlation coefficients. Since the distribution of biochemical variables were normal, correlation was assessed by the Pearson's correlation analysis. P-value of 0.05 was selected as the point of minimal statistical significance. Analysis of variance calculated by One-way ANOVA for comparison group average using SPSS software (Statistical Package for the Social Sciences, version 20.0, SPSS Inc, Chicago, IL) for windows. Mean values of the groups were compared using the Tukey post hoc test.

RESULTS

Information of subjects such as mean age, weight, body mass index (BMI) and pregnancy history were provided in Table 1. These parameters were almost similar and did not show any significant difference. Also, the blood indices including mean Hb, TIBC, ferritin, RBC count, MCV, MCH, MCHC, serum free iron levels and HCT in the three groups are shown in Table2.

A significant difference in GPx activity between IDA and ID group as well as between IDA and healthy group were observed. GPx activity in control group was higher than other groups (Figure 1).

We also compared serum MDA concentration in the three groups and found that these values were significantly higher, in IDA group than in ID group or as in healthy control group (5.18±0.46 μmol/L, 3.4±0.34 μmol/L and 2.75±0.33 μmol/L respectively; Pvalue<0.05) (Figure 2).

Table 3 shows the correlation between blood factors and GPx activity as well as serum MDA concentration in all of the subjects. In this regard, in the IDA group, hemoglobin level and TIBC were positively correlated with MDA concentration (r= 0.203 and r=0.121 respectively) although statistically not significant (p>0.05) and a

Table 1: Age, number of pregnacies and anthropometric characteristics of study participants

Parameters	Group 1 (Apparent Healthy)	Group 2 (ID)	Group 3 (IDA)
Age (year)	34 ±6	32 ± 7	33 ±8
Weight (kg)	70.7 ±7	71.5 ±5	70.6 ±6
Height (cm)	164.5 ±2.8	164.8 ±3.2	163.7 ±2.5
BMI (kg/ m2)	26.08±0.92	26.28±1	26.34±0.78
Number of Pregnancies	2.9±1.1	3.0±1.0	2.9±1.2

Data presented as mean ± standard deviation, ID: Iron deficiency; IDA: iron deficiency anemia

Table 2: Comparison between haematological indices among group

Parameters	Group 1 (Apparent Healthy)	Group 2 (ID)	Group 3 (IDA)
RBCs count (million RBC/μl of blood)	5.09±0.27	4.99 ± 0.29	4.59 ± 0.59[ab]
Hb (g/dl)	12.86±0.23	10.38± 0.22[a]	7.38 ± 0.28[ab]
Hct (%)	44.33±3.46	40.17 ± 2.17[a]	34.63 ± 2.36[ab]
MCHC (g/dl)	29.13±2.57	30.16 ± 1.7	21.41 ± 1.66[ab]
MCH (pg/cell)	24.70±1.43	24.89 ± 1.5	16.49 ± 1.27[ab]
MCV (fl/cell)	85.3 ± 7.37	82.75 ± 6.3	77.34 ± 7.2[ab]
TIBC (μg/dl)	96.3 ± 3.44	148.25± 7.2[a]	251.98 ± 16.55[ab]
Serum ferritin (ng/ml)	139.86 ± 4.13	78.78 ± 4.73[a]	12.58 ± 2.31[ab]
Serum iron (μg/dl)	110.33± 5.2	32.2 ±3.9a	76.66 ± 5.8[ab]

ID: Iron deficiency; IDA: iron deficiency anemia; Values are mean±SD; n =30 in each group; aP<0.05 versus healthy group; bP<0.05 versus ID group

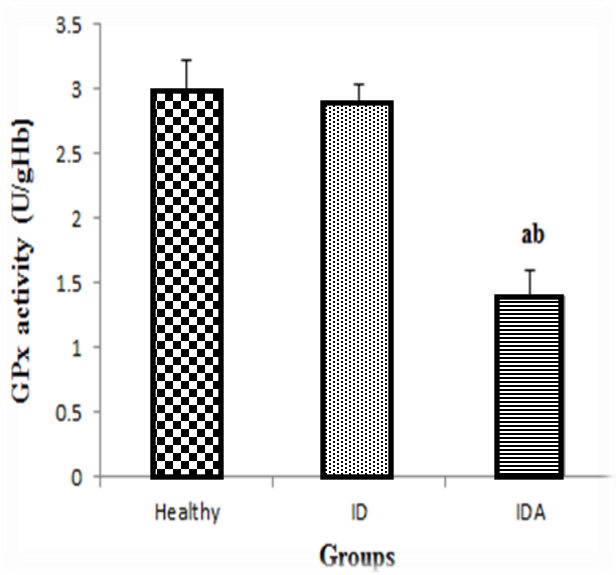

Figure 1. Differences between GPx activity in IDA, ID and Healthy groups

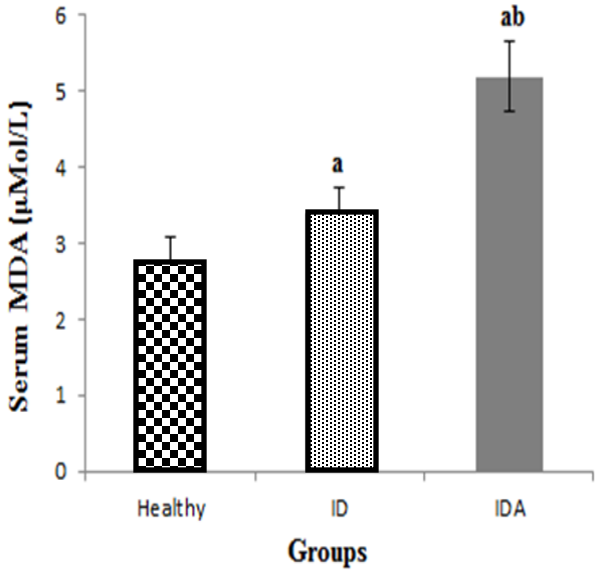

Figure 2. Differences between MDA concentration in IDA, ID and Healthy groups

Table 3. Correlation between haematological factors and MDA concentration and GPx activity in three groups

Parameters	Group 1 (Healthy)		Group 2 (ID)		Group 3 (IDA)	
	MDA (µmol/L)	GPx (U/gHb)	MDA (µmol/L)	GPx (U/gHb)	MDA (µmol/L)	GPx (U/gHb)
TIBC (µg/dl)	0.074	0.263	-0.037	0.016	0.121	0.346
Hb (g/dl)	0.391*	-0.041	0.057	-0.145	0.203	0.153
MCV (fl/cell)	-0.206	0.183	0.234	-0.11	-0.083	-0.01
Serum iron (µg/dL)	-0.265	-0.038	-0.195	0.315	-0.131	-0.029
Serum ferritin (ng/ml)	-0.304	0.251	0.115	0.064	-0.386*	0.231

ID: Iron deficiency; IDA: iron deficiency anemia; *: Significant at p<0.05.

significant negative correlation (r= -0.386, p< 0.05) was observed between ferritin and MDA concentration. There was also a positive correlation between hemoglobin, TIBC and serum ferritin level with GPx activity (r= 0.153, r=0.346, and r=0.231 respectively) in the IDA group, although statistically not significant (p>0.05). In IDA, ID and healthy groups there was no significant correlation between serum free iron levels and MDA concentration. There was also no significant correlation between GPx activity and serum free iron levels in IDA, ID and control groups.

DISCUSSION

Previous studies reported that antioxidant capacity of RBC is reduced under oxidative stress condition (Isler *et al.*, 2002; Altun *et al.,* 2014; Alférez *et al.,* 2015). RBC plays a crucial role in effective antioxidant defense system and produce highly active antioxidant enzymes, such as SOD, CAT and GPx. The leading causes of increased oxidative stress and decreased antioxidant defense in IDA have not been clearly illustrated, but researchers have found a significant increase in lipid peroxidation (Altun *et al.,* 2014; Chaudhary *et al.,* 2014; Alférez *et al.,* 2015).

In addition to deficiency of antioxidant defense system activity and increased lipid peroxidation in

RBCs of patients with IDA, it has been found that the pentose phosphate pathway activity was also reduced in IDA (Isler *et al.,* 2002). In this study we observed increase in the levels of MDA (as a by-product of lipid peroxidation) in serum of patients with IDA and iron deficiency non-anemic subjects compared with healthy control women. These findings are relevant to oxidation status and antioxidant defense system activity in these subjects. Also, the results of our study showed a significant decrease in GPx activity in IDA compared to iron deficient non-anemic subjects and control group subjects. Production of antioxidant enzymes such as CAT, GPx and SOD is dependent on body iron stores (Kurtoglu *et al.,* 2003; Zaka-Ur-Rab *et al.,* 2016).

On the other hand, significant decrease in the levels of serum MDA and increase of GPx activity in anemic patients after treatment by iron supplementation has been reported (Kurtoglu *et al.,* 2003; Sundaram *et al.,* 2007). It is not exactly understood whether increasing production of free radicals or the decreasing activity of the antioxidant system is the main cause for these observations. According to these data we can assume that during iron deficiency conditions, production of antioxidant enzymes including GPx decreases because of decreased body iron stores. Madhikarmi and Murthy (2014) showed decreased GPx activity in patients with IDA, and suggested that iron is essential for RBC GPx activity in anemia (Madhikarmi and Murthy, 2014). We observed a significant increase in serum free iron concentration in IDA group in comparison to ID group which may be due to haemolysis resulting from IDA. A previous study (Chaudhary *et al.,* 2014) reported that RBC in IDA were more susceptible to oxidation. Amirkhizi *et al.* (2006) reported that those in the highest tertile of serum iron were at least twice as likely to have higher serum MDA levels.

The generation of free radicals is an important factor in oxidative damage to microcytic RBC (Altun *et al.,* 2014). Free iron ions can act as free radicals (Fenton's reaction) leading to exacerbation of lipid peroxidation (Yesilbursa *et al.,* 2001). Yetgin *et al.* (1992) found that selenium concentration was significantly lower in IDA. GPx is a selenium-dependent enzyme and may be affected by selenium deficiency (Yetgin *et al.,* 1992). Significant correlation was reported between selenium concentration, and GPx activity (Perona *et al.,* 1977; Madhikarmi and Murthy, 2014). Other studies have also shown decreased activities of antioxidant enzymes, such as SOD, GPx, and CAT, in patients with IDA (Madhikarmi and Murthy, 2014; Alférez *et al.,* 2015). Kurtoglu *et al.,* (2003) showed that the indicators of increased oxidative status were significantly higher, whereas the antioxidant enzymes were significantly lower in IDA patients compared to controls. We did not observe any correlation between MDA concentrations with free iron concentrations in subjects of IDA, ID and control groups. However, a negative correlation between MDA concentration and the ferritin level in IDA group was observed. There was also no direct correlation between GPx and iron levels. The same results were obtained with regard to Hb and TIBC.

CONCLUSION

Based on these results, lipid peroxidation increases in women with ID and IDA but the activity of GPx decreases in IDA but not in ID. Moreover, there was no significant correlation between serum free iron levels with MDA concentration and GPx activity. Therefore, natural antioxidants can help patients with ID and IDA to prevent of lipid peroxidation and to restore of GPx activity in these patients. Given the contradicting results of this and other studies, larger-scale studies are recommended to confirm the detrimental effect of free iron ions as an oxidant factor in patients with iron deficiency.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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