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ORIGINAL ARTICLE

Evaluation of Oxidative Stress and Antioxidant Status in Pregnant Anemic Women

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Abstract The present study was conducted to investigate the oxidant–antioxidant status in iron deficient pregnant anemic women. One hundred thirty pregnant women with iron deficiency anemia (IDA) were divided into three groups, namely mild (50), moderate (50) and severe (30) anemic along with pregnant healthy women as controls (50). The complete blood count, plasma lipid peroxidation products, enzymatic and non-enzymatic antioxidants were measured according to respective protocols. The levels of complete blood count, iron, ferritin along with antioxidant enzymes namely catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase and reduced glutathione were significantly reduced in all IDA groups. However, the level of oxidized glutathione, lipid peroxides, protein carbonyls, conjugated dienes were found significantly increased in all anemic patients. Antioxidant vitamins, namely C, E and A were also found significantly decreased in IDA patients. On the basis of our results, it may be concluded that IDA tends to increase the pro-oxidant components, which may result in various complications including peroxidation of vital body molecules resulting in increased risk for pregnant women as well as fetus.

Keywords Iron deficiency anemia · Oxidants · Antioxidants · Pregnancy

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Introduction

Anemia during pregnancy is a commonest medical disorder that can have deleterious effects on mother and as well as fetus in the form of maternal morbidity and mortality, intrauterine growth retardation, poor weight gain, premature labor, preterm delivery and perinatal morbidity and mortality [1]. Among pregnant women at least half of all anemia cases have been attributed to iron deficiency. In India about 90% of anemia cases are reported to be due to iron deficiency, because high iron requirements during pregnancy are not easily fulfilled by dietary intake alone, especially when iron bioavailability is poor [2]. Because of religious reasons, poverty, or both, Indian population observes dietary patterns that are largely vegetarian [3]. There are also reports that iron deficiency can lead not only to anemia but it may also impair work performance, lead to an abnormal neurotransmitter function and result in altered immunological and inflammatory defenses [4].

Pregnancy is a stressful condition in which many physiological and metabolic functions are altered to a considerable extent. Consequently remarkable and dramatic events occur during this period for sustaining mother and fostering the growth and maintenance of fetus [5]. Pregnancy is a physiological state, which is accompanied by a high-energy demand and an increased oxygen requirement. Both of these may lead to increased oxidative stress. Oxidative stress may be defined as a condition where there is disturbance in the pro-oxidant antioxidant balance, which favors the former [6]. Lipid peroxidation is an oxidative process which occurs at low levels in all cells and tissues. Under normal conditions a variety of antioxidant mechanisms serve to control this peroxidative process [7]. The generation of free radicals is a normal physiological process but increased production of free radicals can act on lipids to causes lipid peroxidation. The cells have evolved a number of counter acting antioxidant defenses. Free radical scavenging mechanisms includes enzymatic and non-enzymatic antioxidants which limit the cellular concentration of free radical and prevent excessive oxidative stress. The aim of the present study was to assess the markers of oxidative stress and antioxidative enzymes in pregnant anemic women.

Materials and Methods

Subjects

The present study comprised of 130 pregnant anemic women [viz. mild (50), moderate (50) and severe (30)] aged between 20–40 years and pregnant healthy women as control (50) Hb as >11 g/dl ranging in same age. The subjects were selected amongst those attending the Department of Obstetrics and Gynaecology, Queen Mary's Hospital, Chhatrapati Shahuji Maharaj Medical University, Lucknow, U.P., India. Selected subjects were all consumers of normal mixed food, not taking any drug for preceding one month, which is a part of antenatal care. The inclusion criteria of anemic subjects were according to WHO, which defines mild anemia as Hb 10.0–10.9 g/dl, moderate as Hb 7.0–9.9 g/dl and severe as Hb < 7.0 g/dl [8].

Both groups were non-alcoholic, non-smoking and normotensive subjects having no history of metabolic diseases such as diabetes mellitus, malignancy, heart disease, or having infections such as tuberculosis, HIV, endocrine disorders.

Informed consent was obtained from each subject. The present study was approved by the Institutional Ethical Committee of Chhatrapati Shahuji Maharaj Medical University, Lucknow, India.

Sample Collection

Six ml venous blood was taken from each subject at the time of recruitment and divided into three aliquots. Two ml blood was transferred to an EDTA containing evacuated tube used to determine hemoglobin (Hb), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), Red blood cell (RBC) counts, reduced glutathione (GSH) and oxidized glutathione (GSSH). Two ml of whole blood was also transferred into heparin containing tube and then centrifuged; plasma separated and used for the estimation of lipid peroxide levels (LPO), protein carbonyl contents (PC), conjugated dienes (CD) and iron. Remaining 2 ml of venous blood was also centrifuged at 3000 rpm for 15 min, serum separated and used for the estimation of vitamin A, C, and ferritin. The RBCs was lysed by mixing chilled water and RBC lysate was used for the estimation of antioxidant enzymes namely catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR).

Biochemical Estimation

Blood haemoglobin was determined by using the cyanomethemoglobin method [9]. Haematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), red blood cell counts were determined by using Sysmax A-380 automated cell counter. The concentration of iron in plasma was measured with flame atomic absorption spectrophotometer (Perkin Elmar AAS-700 Ueberlinger, Germany) [10]. Catalase activity was determined spectrophotometrically by the method of Aebi [11]. SOD activity was determined spectrophotometrically according to the method of McCord and Fridovich [12]. GPx was assayed by the method of Pagila and Valentine [13]. The GR was assayed by the method of Hazelton and Lang [14]. Total protein of RBC sample was determined by the method of Lowry et al. [15]. GSH and GSSG were estimated by Ellman [16]. Lipid peroxide was estimated according to method of Ohkawa et al. [17]. Ascorbic acid (vitamin C) levels were estimated as described by Beulter [18]. α-Tocopherol (vitamin E) and Retinol (vitamin A) were measured by high-performance liquid chromatography (HPLC) as per the modified method of Omu et al. [19]. Conjugated dienes (CD) were measured by the method of Racknagel and Ghosal [20]. The protein oxidation was measured by estimating the protein carbonyl (PC) levels by the method of Liu et al. [21].

Statistical Analysis

Data were first subjected for normal distribution and homogeneity of variance testing. Due to skewed

distribution and heterogeneous variance between groups in most of the studied variables, data were thus analyzed by using non parametric tests. The biochemical parameters were described by mean sum of ranks, median and inter-quartile range (Q25–Q75). The groups were compared by Kruskall–Wallis: H analysis of variance (ANOVA) and the significance of sum of mean ranks between the groups were done by Z test. The association between variables was assessed by Spearman rank order correlation. A two-tailed probability (P) value $P < 0.05$ was considered to be statistically significant. The statistical analysis was performed on STATISTICA version 6.0.

Results

Demographic Characteristics

A total of 180 cases were enrolled in the present study. There were 50 subjects each in control, mild, and moderate groups while 30 were in severe group. The four groups were similar with respect to age, weight, and parity and their duration of conception were also almost similar (Table 1). All the subjects were of same socio-economic backgrounds.

Blood Parameters

The blood parameters of all the four groups are summarized in Table 2. Our results data show that the median values of all blood parameters decreased linearly with severity (from control severe) i.e. the levels were lowest in severe anemic group and were highest in control group. Kruskall–Wallis (H) ANOVA revealed that the blood parameters differed significantly ($P < 0.01$) between the groups (Table 2). The mean sum of ranks (levels) of Hb, Hct, MCV, MCH, RBC, Fe and Ferritin of severe group lowered significantly ($P < 0.05$ or $P < 0.01$) from respective control, mild and moderate groups, except MCV, RBC and Ferritin which did not ($P > 0.05$) differed with respective moderate groups. Similarly, the levels of all blood parameters of moderate group were found to be significantly ($P < 0.01$) low as compared with respective controls and mild groups except MCV, MCH and Ferritin which did not ($P > 0.05$) differed with respective mild groups. The levels of all blood parameters of mild group were also found to be significantly ($P < 0.05$ or $P < 0.01$) low as compared with respective control groups except for MCV. The level of MCV of control group and mild group was found to be statistically the same ($P > 0.05$).

Enzymatic and Non-Enzymatic Antioxidant Parameters

The enzymatic and non-enzymatic antioxidant parameters of all the four groups are summarized in Table 3. Like blood parameters, the median values of enzymatic and non-enzymatic antioxidant parameters were decreased linearly with severity except GSSG. Kruskall–Wallis (H) ANOVA revealed that the enzymatic and non-enzymatic antioxidant parameters differed significantly ($P < 0.05$ or $P < 0.01$) between the groups (Table 3). The levels of CAT, SOD, GPx, GR, GSH, Vit. C, Vit. E and Vit. A of severe anemic group were significantly ($P < 0.05$ or $P < 0.01$) low as compared with their respective controls, however, in mild and moderate groups except for GPx, GSH and Vit. C which did not ($P > 0.05$) differed with respective moderate groups, rest of the results were same. The level of Vit. A of severe group also not ($P > 0.05$) differed with mild group. Similarly, the levels of all above parameters of moderate group also found to be significantly ($P < 0.05$ or $P < 0.01$) low as compared to respective control and mild groups except GR, Vit. E and Vit. A, which did not ($P > 0.05$) differed with respective mild groups. The levels of all these parameters of mild group were also found to be significantly ($P < 0.01$) low as compared to respective control groups except for SOD, GPx, GR, Vit. E and Vit. A. In contrast, the level of GSSG of severe anemic group was

Table 1 Characteristic of the pregnant women in all groups

Characteristics	Control ($n = 50$)	Mild ($n = 50$)	Moderate ($n = 50$)	Severe ($n = 30$)
Age (years)	26.7 ± 0.13	25.2 ± 0.21	25.8 ± 0.19	24.7 ± 0.17
Weight (kg)	53	50	50	47
Height (cm)	153.7	154.8	151.3	152.2
Literacy (%) illiterate and				
<10th grade	76	70	74	73
>10th grade	24	30	26	27
Parity (%)				
1	20	24	18	20
≥2	80	76	82	80
Gestational age (wk)	21.3 ± 0.09	19.7 ± 0.11	22.2 ± 0.14	22.6 ± 0.11

There were no significant differences between the groups

Table 2 Blood parameters summary of pregnant anemic women

Variables	Groups	Mean sum of ranks	Median	Inter quartile range (Q25–Q75)	Kruskall–Wallis: H (3, $N = 180$)	P
Hb (g/dl)	Control	155.50	13.10	12.50–14.50	166.67	0.000
	Mild	105.44[a]	10.50	10.30–10.70		
	Moderate	55.56[ab]	8.40	7.50–9.10		
	Severe	15.50[abc]	6.85	6.70–6.90		
Hct (%)	Control	148.49	35.85	34.70–37.70	136.47	0.000
	Mild	104.22[a]	32.65	29.80–34.40		
	Moderate	61.22[ab]	28.50	26.50–29.70		
	Severe	19.78[abc]	22.65	21.60–24.70		
MCV (fl)	Control	119.23	85.90	79.50–92.40	39.73	0.0000
	Mild	100.63	81.35	71.20–91.80		
	Moderate	76.56[a]	75.80	66.40–85.20		
	Severe	48.97[ab]	71.90	64.30–75.30		
MCH (pg)	Control	123.12	30.55	28.50–33.40	43.05	0.0000
	Mild	94.14[a]	29.25	25.10–31.50		
	Moderate	80.65[a]	27.10	22.70–31.40		
	Severe	46.48[abc]	25.65	22.10–27.40		
RBC ($x10^{12}$/L)	Control	143.93	4.86	4.59–5.15	110.12	0.000
	Mild	104.87[a]	4.23	3.93–4.61		
	Moderate	52.17[ab]	3.67	3.49–3.94		
	Severe	41.38[ab]	3.61	3.54–3.77		
Fe (mg/dl)	Control	146.39	48.20	44.50–50.50	131.39	0.000
	Mild	106.53[a]	37.30	34.20–42.70		
	Moderate	59.51[ab]	26.25	20.50–29.50		
	Severe	22.28[abc]	18.90	17.90–20.50		
Ferritin (μg/L)	Control	155.50	28.50	26.40–31.50	123.87	0.000
	Mild	86.19[a]	11.40	9.50–14.20		
	Moderate	60.74[a]	9.40	6.10–11.40		
	Severe	38.95[ab]	4.60	3.60–9.50		

Alphabet in superscript "a" in comparison with Control; "b" in comparison with Mild; "c" in comparison with Moderate. Alphabet in bold font are significant at $P < 0.01$; light font are significant at $P < 0.05$ and groups without alphabet are insignificant ($P > 0.05$)

found to be significantly ($P < 0.05$ or $P < 0.01$) higher than the respective control, mild and moderate groups. The level of GSSG of moderate group was also found to be significantly ($P < 0.01$) high than the respective control and mild groups while its level was not ($P > 0.05$) found different between control and mild group.

Oxidative Stress Parameters

The oxidative stress parameters of all the four groups are summarized in Table 4. Results show that all oxidative parameters increase linearly with severity of anemia i.e. the levels were highest in severe anemic group and lowest in control group. Kruskall–Wallis (H) ANOVA revealed that the oxidative stress parameters differed significantly ($P < 0.05$ or $P < 0.01$) between the groups (Table 4). The levels of LPO, PC and CD of severe group were found to be significantly ($P < 0.05$ or $P < 0.01$) higher than the respective controls, mild and moderate groups except for PC and CD which did not differ ($P > 0.05$) with respective moderate groups. Similarly, the level of LPO and PC of moderate group was also found to be significantly ($P < 0.01$) higher than the respective control and mild groups. The level of LPO of mild group was also significantly ($P < 0.01$) high than the control group.

Correlation

The correlations between all observed variables are summarized in Table 5. All variables showed significant ($P < 0.05$ or $P < 0.01$) correlation with each other except Vit. A and CD which did not correlate significantly ($P > 0.05$) with most of the variables. The Hb, Hct, MCV, MCH, RBC, Fe, Ferritin, CAT, SOD, GPx, GR, GSH, Vit. C, Vit. E and Vit. A showed significant ($P < 0.01$) and negative correlation with severity, while GSSG, LPO, PC and CD showed significant ($P < 0.05$ or $P < 0.01$) and positive correlation.

Table 3 Enzymatic and non-enzymatic antioxidant parameters summary of pregnant anemic women

Variables	Groups	Mean sum of ranks	Median	Inter quartile range (Q25–Q75)	Kruskall–Wallis: H (3, $N = 180$)	P
CAT (U/mg protein)	Control	146.98	55.25	48.50–62.10	119.80	0.000
	Mild	98.94[a]	40.90	40.10–49.60		
	Moderate	65.03[ab]	40.15	35.70–45.20		
	Severe	24.75[abc]	33.60	28.50–36.20		
SOD (U/mg protein)	Control	122.81	1.22	0.95–1.35	71.88	0.0000
	Mild	108.15	1.12	0.98–1.21		
	Moderate	78.51[ab]	0.98	0.84–1.12		
	Severe	27.22[abc]	0.67	0.59–0.86		
GPx (U/mg protein)	Control	128.80	37.05	33.10–41.70	90.28	0.000
	Mild	116.52	37.70	31.40–40.10		
	Moderate	58.30[ab]	28.45	26.30–31.70		
	Severe	36.97[ab]	25.90	24.30–27.80		
GR (U/mg protein)	Control	115.19	29.65	22.90–33.70	31.56	0.0000
	Mild	94.85	26.60	22.30–30.60		
	Moderate	86.81[a]	26.20	22.20–28.90		
	Severe	48.25[abc]	22.20	17.50–24.50		
GSH (μM)	Control	143.08	419.55	398.40–426.50	107.37	0.000
	Mild	102.23[a]	377.50	367.40–407.50		
	Moderate	61.64[ab]	359.50	341.50–374.90		
	Severe	31.42[ab]	345.80	334.20–355.80		
GSSG (μM)	Control	44.92	143.20	133.20–153.20	129.98	0.000
	Mild	60.35	149.00	144.20–156.40		
	Moderate	124.33[ab]	175.40	168.70–184.20		
	Severe	160.33[abc]	199.45	188.40–211.40		
Vit. C (mg/dl)	Control	141.66	1.29	1.22–1.36	109.47	0.000
	Mild	106.21[a]	1.13	0.93–1.23		
	Moderate	59.01[ab]	0.74	0.64–1.09		
	Severe	31.53[ab]	0.66	0.58–0.78		
Vit. E (mg/dl)	Control	125.92	0.96	0.87–1.12	66.12	0.0000
	Mild	101.13	0.82	0.69–1.09		
	Moderate	80.04[a]	0.80	0.67–0.91		
	Severe	31.18[abc]	0.53	0.48–0.67		
Vit. A (mg/dl)	Control	107.80	30.40	28.40–33.50	11.94	0.0076
	Mild	95.00	30.40	23.40–35.30		
	Moderate	80.30[a]	28.45	23.50–30.90		
	Severe	71.17[a]	28.40	20.40–32.40		

Alphabet in superscript "a" in comparison with Control; "b" in comparison with Mild; "c" in comparison with Moderate. Alphabet in bold font are significant at $P < 0.01$; light font are significant at $P < 0.05$ and groups without alphabet are insignificant ($P > 0.05$)

Discussion

Pregnancy is a physiological state accompanied by a high-energy demand and an increased oxygen requirement. Various compensatory adaptive changes, including increased ventilation for enhanced oxygen demand, occur with advancing pregnancy to meet the increasing requirements for proper bodily functions of mother to fulfill the needs of the fetus [22]. Such a condition may be responsible for raised oxidative stress in pregnancy. Decreased erythrocyte survival, which is secondary to an increased susceptibility to oxidant damage, has been reported in IDA.

Our results showing decreased level of haemoglobin might be due to the fact that iron is an essential constituent of heme and when its levels are low, as reported by us, it may lead to decreased haemoglobin synthesis. The present study had also shown that the levels of Hct, MCV, MCH, red blood cell counts Fe and ferritin found decreased in all anemic women groups. Ferritin is the main iron storage compound and novel marker of iron depletion in the body

Table 4 Oxidative stress parameters summary of pregnant anemic women

Variables	Groups	Mean sum of ranks	Median	Inter quartile range (Q25–Q75)	Kruskall–Wallis: H (3, $N = 180$)	P
LPO (nmole MDA/mg protein)	Control	42.15	2.21	2.04–2.44	109.37	0.000
	Mild	76.89[a]	2.56	2.34–2.95		
	Moderate	109.72[ab]	3.17	2.75–3.54		
	Severe	161.73[abc]	4.05	3.71–4.23		
PC (nmole/mg protein)	Control	57.36	1.29	1.22–1.48	57.18	0.0000
	Mild	74.90	1.46	1.14–1.76		
	Moderate	110.76[ab]	1.84	1.44–2.21		
	Severe	137.97[ab]	2.11	1.84–2.22		
CD (μM)	Control	80.05	44.10	37.40–50.50	10.35	0.0158
	Mild	83.01	43.40	33.50–59.30		
	Moderate	93.15	44.70	33.90–65.30		
	Severe	115.98[ab]	51.55	48.20–63.10		

Alphabet in superscript "a" in comparison with Control; "b" in comparison with Mild; "c" in comparison with Moderate. Alphabet in bold font are significant at $P < 0.01$; light font are significant at $P < 0.05$ and groups without alphabet are insignificant ($P > 0.05$)

Unit: LPO (lipid peroxide, nmole MDA/mg protein); PC (protein carbonyl, nmole/mg protein) and CD (conjugated diene, μM)

[23]. In the present study, we observed increased level of plasma lipid peroxidation products (LPO, PC and CD) in pregnant women with IDA, which may be attributed to over production of reactive oxygen species (ROS) or a deficiency of antioxidant defense. Although previous studies have suggested that IDA may be related to increased lipid peroxidation [24, 25], however, its mechanism has not been completely clarified. Antioxidant enzymes are the major defense system of cells in normal aerobic reactions [26]. Although, erythrocytes possess highly efficient antioxidant enzymes, such as CuZn–SOD and GPx compared to other cell types [27], though as our results showed that women with IDA have lower CuZn–SOD activity than healthy control. Our results are in accordance with earlier such reports [25]. Decreased SOD activity in IDA may be linked to increased oxidative stress, because it is well known that ROS, especially hydrogen peroxide (H_2O_2), inhibit SOD activity [28]. CAT and SOD are metaloproteins and accomplish their antioxidant function by enzymatically detoxifying the peroxides (–OOH), H_2O_2 and $\bar{O}_2$ respectively. CAT has been suggested to provide important pathway for H_2O_2 decomposition into H_2O and O_2. We also found that CAT activity was significantly decreased in IDA groups as compared with controls. This finding was in agreement with those of Acharya et al. [29] who also reported decreased CAT activity in patients with IDA. CAT is an iron-dependent enzyme and is not unexpected to be decreased in iron deficiency. The positive relationship, which we observed between Hb and ferritin concentration with CAT activity was also confirmed in the present study.

Similarly, GPx activity in IDA groups was decreased when compared with controls. This finding is in accordance with the finding of Yetgin et al. [30], who reported decreased GPx activity in children with iron deficiency. Decreased activity in IDA may be due to perturbed pentose phosphate pathway, as IDA may have restricted the availability of NADPH, a co-factor for GPx functioning [27]. GSH plays a pivotal role in protection of cells against oxidative stress. It can act as a non-enzymatic antioxidant by direct interactions of SH group with ROS or it can be involved in the enzymatic detoxification reactions for ROS as a coenzyme [31]. Many studies have reported a decrease in GSH level in IDA. Similarly in our study we also observed significantly depletion of GSH in all anemic groups when compared with control. Significant perturbations were also observed in the oxidized form of GSH, i.e. GSSG.

Furthermore, the levels of vitamin C, E and A were found decreased significantly in pregnant IDA women. The decrease in endogenous ascorbic acid may be due to its extensive use as antioxidant to protect the gastrointestinal tract from the free radical damage during iron repletion and increased levels of lipid peroxidation products [32]. The same may be true in case of vitamin E as its serum levels were also found decreased in IDA. It is a chain-breaking antioxidant involved in the inhibition of propagation of free radicals generation during IDA. We also observed that vitamin A levels decreased in IDA groups when compared with controls. These results were in line to previous reports, which also found decreased vitamin A in iron deficiency anemia [33].

On the basis of the results of the present study, it may be concluded that iron deficiency anemia is associated with free radical generation; abnormalities and peroxidation of vital body molecules which implies increased risk for pregnant women as well as for fetus. However, further in-depth studies are needed to assess the status of antioxidant minerals and molecules in pregnancy related anemia.

Table 5 Inter-correlation of biochemical parameters of all pregnant anemic women ($n = 180$)

Variables	Severity	Hb	Hct	MCV	MCH	RBC	Fe	Ferritin	CAT	SOD	GPx	GR	GSH	GSSG	Vit. C	Vit. E	Vit. A	LPO	PC	CD
Severity	1.00																			
Hb	**−0.96**	1.00																		
Hct	**−0.87**	**0.86**	1.00																	
MCV	**−0.47**	**0.45**	**0.38**	1.00																
MCH	**−0.48**	**0.49**	**0.44**	**0.34**	1.00															
RBC	**−0.77**	**0.76**	**0.67**	**0.30**	**0.40**	1.00														
Fe	**−0.86**	**0.83**	**0.75**	**0.34**	**0.39**	**0.67**	1.00													
Ferritin	**−0.80**	**0.78**	**0.68**	**0.44**	**0.36**	**0.61**	**0.68**	1.00												
CAT	**−0.82**	**0.80**	**0.72**	**0.39**	**0.42**	**0.59**	**0.73**	**0.67**	1.00											
SOD	**−0.60**	**0.57**	**0.49**	**0.31**	**0.29**	**0.45**	**0.47**	**0.46**	**0.49**	1.00										
GPx	**−0.68**	**0.66**	**0.63**	**0.36**	0.22	**0.57**	**0.60**	**0.54**	**0.51**	**0.45**	1.00									
GR	**−0.39**	**0.37**	**0.41**	*0.16*	0.22	**0.26**	**0.38**	**0.28**	**0.32**	0.22	**0.31**	1.00								
GSH	**−0.77**	**0.75**	**0.70**	**0.41**	**0.38**	**0.61**	**0.64**	**0.65**	**0.66**	**0.47**	**0.55**	**0.28**	1.00							
GSSG	**0.83**	**−0.79**	**−0.73**	**−0.37**	**−0.38**	**−0.63**	**−0.73**	**−0.61**	**−0.69**	**−0.54**	**−0.61**	**−0.35**	**−0.62**	1.00						
Vit. C	**−0.78**	**0.73**	**0.66**	**0.28**	**0.38**	**0.57**	**0.65**	**0.62**	**0.64**	**0.45**	**0.52**	**0.26**	**0.62**	**−0.66**	1.00					
Vit. E	**−0.59**	**0.57**	**0.49**	0.24	**0.32**	**0.41**	**0.50**	**0.42**	**0.39**	**0.40**	**0.39**	**0.36**	**0.45**	**−0.51**	**0.49**	1.00				
Vit. A	**−0.26**	0.25	*0.19*	*0.17*	*0.09*	*0.12*	*0.18*	0.22	*0.17*	*0.07*	*0.11*	*0.10*	0.21	−0.25	0.22	0.25	1.00			
LPO	**0.77**	**−0.73**	**−0.73**	**−0.38**	**−0.40**	**−0.54**	**−0.68**	**−0.64**	**−0.68**	**−0.49**	**−0.56**	**−0.37**	**−0.61**	**0.67**	**−0.55**	**−0.39**	*−0.18*	1.00		
PC	**0.56**	**−0.56**	**−0.47**	**−0.31**	**−0.29**	**−0.43**	**−0.51**	**−0.42**	**−0.41**	**−0.39**	**−0.46**	−0.27	**−0.40**	**0.47**	**−0.40**	**−0.38**	*0.10*	**0.45**	1.00	
CD	0.22	*−0.19*	−0.20	*−0.16*	−0.21	*−0.17*	*−0.16*	*−0.15*	*−0.11*	−0.23	*−0.10*	*−0.08*	*−0.04*	*0.19*	*−0.12*	**−0.27**	*−0.15*	0.23	*0.17*	1.00

The values are correlation coefficient; values in bold font are significant at $P < 0.01$; values in light font are significant at $P < 0.05$; values in italics are insignificant ($P > 0.05$)

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