

# The Nutritional Habits and Relationship Between The Antioxidant Activity and Iron Deficiency Anemia During Pregnancy

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## ABSTRACT

The aim of this study was to investigate the changes in nutritional habits, and also relationship among oxidative stress, iron deficiency anemia and some antioxidant enzyme activities during pregnancy.

Twenty pregnant women in the first trimester, 20 pregnant women in the last trimester and 20 non-pregnant women (control group) were included in the study. The study groups were similar in terms of age and body mass index. None of the participants had any systemic disease or smoked. After having filled out the questionnaire about nutritional habits, the blood samples were collected, and the malondialdehyde (MDA) level and activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) enzymes were measured together with hemoglobin (Hgb), hematocrit (Hct), iron, ferritin and the mean erythrocyte volume (MCV) levels.

The most frequent meat consumption was on every other day in controls, but once a week in the first and last trimester groups. In addition, the most frequent egg consumption was on every other day and once a week in controls, but rare in the first trimester group, and once a week in the last trimester group. Although iron and vitamin use was 10% and 30% in the first trimester group, it was 50% and 65% respectively in the last trimester group. The MDA level was higher, and the SOD, CAT and GSH-Px enzyme activities were lower in both pregnant groups than controls. The Hgb and Hct values were lower in the last trimester group compared to the controls and the first trimester group. The first trimester group and the controls were similar in terms of Hgb and Hct levels. There was no significant difference among groups in terms of ferritin, iron or MCV values.

It was concluded that; a) the egg and meat consumption might decrease with the progress of pregnancy, b) the oxidative stress increased in pregnancy, c) Hgb and Hct values might be lower in the last trimester group despite increased intake of oral iron and vitamin supplements, and normal serum iron levels due to decreased CAT activity. Therefore, more studies are needed to demonstrate the relationship among oxidative stress, anemia and CAT enzyme activity in pregnancy.

**Keywords:** Antioxidants, nutrition, iron deficiency anemia, pregnancy

## Introduction

Health problems resulting from vitamin and mineral deficiency are observed in two billion people all around the world. The most serious of them are iron deficiency anemia, iodine deficiency diseases and vitamin A deficiency (1). Iron is an essential element in our body for some metabolic events such as the synthesis of hemoglobin and many other proteins, oxygen transport, DNA synthesis, and electron transport (2).

According to the World Health Organization (WHO), it is estimated that approximately 30% of the world population and more than half of

pregnant women suffer from anemia (3). For a woman to become pregnant, complete this process in a healthy way, and breastfeed her baby after birth, she must be healthy and receive a balanced nutrition before she becomes pregnant. Inadequate and unbalanced nutrition before gestation affects the maternal and fetal health negatively (4).

The WHO suggests that anemia may play a role in 20% of maternal mortalities (3). Although maternal anemia causes fetal complications such as intrauterine growth retardation, pre-term delivery and low birth weight, and is associated with maternal complications such as preeclampsia

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and eclampsia; it is not associated with poor perinatal outcomes (5,6). These findings draw attention to anemia, which needs to be addressed in terms of women and pregnant health.

Several studies have recently been conducted on antioxidants. In case of any deficiency in the antioxidant system, tissues cannot be protected from the harmful effects of free radicals that emerge during cellular metabolism for a long duration. In pregnant women, free radicals are produced in response to excessive lipid peroxide secretion from the placenta and as a result, the plasma levels of antioxidants increase. This is important for demonstrating the benefit of antioxidants for a normal pregnancy (7). Although there are studies on the antioxidant levels and oxidative stress in anemic pregnant women (8), and on anemia and antioxidant enzyme activity in women with preeclampsia (9); in our knowledge, there is no study that investigates the relationship among oxidative stress, iron deficiency anemia and antioxidant enzyme activity during pregnancy. Therefore, this study was designed to investigate the changes in nutritional habits, and also relationship among oxidative stress, iron deficiency anemia and some antioxidant enzyme activities during pregnancy.

## Materials and Methods

**Study Groups:** All participants signed an informed consent form. A total of 60 women including 20 pregnant women in the first trimester (first trimester group), 20 pregnant women in the last trimester (last trimester group) and 20 non-pregnant women (control group) were included in the study. The control group consisted of married but non-pregnant women. All women participating in the study were between 18-49 years of age. Smokers, patients exceeding the normal body mass index and those with any systemic disease were excluded from the study. The three study groups were similar in terms of age, educational level, spouses' educational level, occupation, spouses' working status, family type, and income level. A questionnaire consisting of 40 questions was applied to each participant to determine the relationship between the subjects' nutritional habits and anemia.

**Biochemical Analysis:** After the questionnaire was applied to the women in the experimental and control groups, venous blood was drawn from all participants for hematological and biochemical analyses. Hemoglobin (Hgb), hematocrit (Hct), the

mean erythrocyte volume (MCV), the serum iron and ferritin levels were measured from fresh venous blood using an automated blood counting device (Nihon Kohden Celltac F Mek-8222, Japan). The serum samples were obtained by centrifugation of blood at 3,500 rpm for 15 min, and stored in Eppendorf tubes at -20 °C for the days of measurements of malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT). The serum MDA levels were measured by spectrophotometric (Ati Unicam uv/vis-uv2-100, UK) measurement of thiobarbutyric acid (TBA), and the pink colored MDA-TBA compound formed by MDA (10). The serum SOD activity was determined by spectrophotometric (Ati Unicam uv/vis-uv2-100, UK) measurement of the enzyme using commercial kits (Randox: 55 Diamond Road, Crumlin County antrim, United Kingdom) (11). The serum CAT activity was measured in U/ml unit by the method developed by Aebi (12). The serum GSH-Px level was measured by spectrophotometric (Ati unicam uv/vis-uv2-100, UK) measurement of the enzyme (13).

**Statistical Analysis:** The sample size of this study; Calculated using the G\*Power statistical program. According to this; A total of 60 subjects, 20 in each group, were included in the sample; Taking Effect size 0.5 and Type-1 error ( $\alpha$ ) 0.05, Power of test was found to be 93% for 60 observations.

Kolmogorov-Smirnov ( $n > 50$ ) and Skewness-Kurtosis tests were used to check whether the continuous measurements in the study were normally distributed, and because the measurements were normally distributed, Parametric tests were applied. Descriptive statistics for continuous variables in research; mean, standard deviation; were expressed as numbers ( $n$ ) and percentage (%) for categorical variables. The one-way ANOVA was used to compare the measurements according to the groups. Duncan test was used to identify different groups following analysis of variance. Pearson correlation coefficients were calculated to determine the relationships between measurements. Chi-square test was used to determine the relationships between groups and categorical variables. The statistical significance level ( $\alpha$ ) was taken as 5% in the calculations and the SPSS (IBM SPSS for Windows, ver.13) statistical package program was used for analysis.

**Table 1.** Nutritional Habits of The Study Groups

Variables	Controls		First Trimester		Last Trimester		p	$\chi^2$
	n	%	n	%	n	%		
Beverages for breakfast								
Tea	19	95	17	85	19	95	0.95	2.645
Milk	1	5	2	10	1	5		
Fruit juice	0	0	0	0	0	0		
Coke	0	0	0	0	0	0		
Others	0	0	1	5	0	0		
Tea consumption after meals								
I don't drink	1	5	4	20	4	20	0.77	3.254
I drink just after	5	25	3	15	4	20		
I drink 1 hour later	9	45	7	35	6	30		
I drink 2 hours later	5	25	6	30	6	30		
Other	0	0	0	0	0	0		
Skipping meals								
Yes	6	30	13	65	12	60	0.06	5.740
No	14	70	7	35	8	40		
Inter-meal drinks and nuts habits								
Tea	17	85	10	50	11	55	0.39	10.638
Milk	0	0	2	10	2	10		
Fruit juice	0	0	0	0	2	10		
Coke	0	0	0	0	0	0		
Nuts	0	0	0	0	0	0		
Other	3	15	8	40	5	25		
Kinds of pots used for cooking								
Steel	18	90	19	95	19	95	0.76	0.536
Teflon	2	10	1	5	1	5		
Aluminum	0	0	0	0	0	0		

## Results

There was no difference ( $P>0.05$ ) among groups in terms of nutritional habits such as beverages for breakfast, consumption of tea after meals, skipping meals, beverages between meals, nuts consumption and the characteristics of the pot used for cooking (Table 1). There was no statistical difference ( $P>0.05$ ) between the groups in terms of legumes, vegetables, fruit, and molasses consumption, whereas the study groups were different ( $P<0.05$ ) with regard to egg and meat consumption. The most frequent meat consumption was on every other day (55%) in controls, but once a week in the first (50%) and last (40%) trimester groups (Table 2). In addition, the most frequent egg consumption was on every other day (40%) and once a week (40%) in controls, but rare (55%) in the first trimester group, and once a week (40%) in the last trimester

group (Table 2). There was a significant difference ( $P<0.05$ ) between the women in the first trimester and the last trimester groups regarding iron and vitamin use. Although iron and vitamin use was 10% and 30% in the first trimester group, it was 50% and 65% respectively in the last trimester group (Table 3).

Comparisons of the study groups regarding the descriptive statistics results are shown in Table 4. The MDA level was higher ( $P<0.05$ ), and the SOD, CAT and GSH-Px enzyme activities were lower ( $P<0.05$ ) in both pregnant groups than controls. The Hgb and Hct values were lower ( $P<0.05$ ) in the last trimester group compared to the controls and the first trimester group. The first trimester group and the controls were similar ( $P>0.05$ ) in terms of Hgb and Hct levels. There was no significant difference ( $P>0.05$ ) among groups in terms of ferritin, iron or MCV values.

**Table 2.** General Feeding Habits of The Subjects

Variables	Controls		First Trimester		Last Trimester		p	$\chi^2$
	n	%	n	%	n	%		
Meat								
Everyday	1	5	3	15	4	20	0.05	12.439
Every other day	11	55	3	15	3	15		
Once a week	7	35	10	50	8	40		
Rare	1	5	4	20	5	25		
Egg								
Everyday	8	40	4	20	3	15	0.05	12.874
Every other day	1	5	1	5	4	20		
Once a week	8	40	4	20	8	40		
Rare	3	15	11	55	5	25		
Legumes								
Everyday	0	0	0	0	2	10	0.11	5.787
Every other day	4	20	6	30	5	25		
Once a week	7	35	8	40	8	40		
Rare	9	45	6	30	5	25		
Vegetables								
Everyday	9	45	14	70	10	50	0.40	6.130
Every other day	3	15	1	5	3	15		
Once a week	7	35	2	10	5	25		
Rare	1	5	3	15	2	10		
Fruits								
Everyday	13	65	18	90	14	70	0.23	5.600
Every other day	3	15	2	10	4	20		
Once a week	4	20	0	0	2	10		
Rare	0	0	0	0	0	0		
Molasses								
Everyday	3	15	2	10	3	15	0.54	4.977
Every other day	0	0	0	0	1	5		
Once a week	1	5	2	10	4	20		
Rare	16	80	16	80	12	60		

The correlation among the numerical parameters of controls is shown in Table 5. The ferritin was negatively correlated ( $P<0.05$ ) with both Hgb and Hct. There was a positive correlation ( $P<0.05$ ) between Hct and Hgb, MCV and Hgb, and MCV and Htc.

The correlation analysis among the numerical parameters of pregnant women in the first trimester group is shown in Table 6. Similar to controls, the ferritin was negatively correlated with both Hgb and Hct, and the Hct was positively correlated ( $P<0.05$ ) with Hgb. Different than controls, there was a positive correlation ( $P<0.05$ ) between CAT and SOD enzyme activities.

The correlation analysis among the numerical parameters of pregnant women in the last trimester group is shown in Table 7. Similar to controls and first trimester group, there was a positive correlation ( $P<0.05$ ) between Hct and Hgb. However different than controls and also first trimester group, there was a positive correlation between Hct and CAT, Hgb and CAT, and iron and MCV.

## Discussion

The aim of this study was to determine the importance of nutritional habits in preventing anemia in pregnant women, and to investigate the

**Table 3.** Distribution of Pregnant Women According To Drug Use

Variables	First Trimester		Last Trimester		P	$\chi^2$
	n	%	n	%		
Iron						
Yes	2	10	10	50	0.006	7.619
No	18	90	10	50		
Vitamin						
Yes	6	30	13	65	0.020	4.912
No	14	70	7	35		

**Table 4.** Comparison of Groups In Terms of Descriptive Statistics Results

	Controls (n=20) (min-max)		First Trimester(n=20) (min-max)		Last Trimester (n=20) (min-max)	
MDA (nmol/ml)	18.30±1.13b	(15.65-19.65)	44.26±1.66a	(41.54-46.93)	44.23±1.61a	(41.43-46.43)
SOD (EU/ml)	41.97±3.05a	(35.34-46.35)	30.52±0.97b	(28.44-31.99)	29.35±1.10b	(27.43-31.25)
CAT (EU/ml)	16.47±2.57a	(13.45-21.72)	7.66±0.95b	(5.66-8.93)	7.64±0.41b	(7.10-7.99)
GSH-Px (EU/ml)	76.32±2.08a	(71.43-79.93)	35.25±1.34b	(32.54-36.74)	35.15±1.37b	(33.21-36.98)
HGB (g/dl)	12.65±1.10a	(10.30-14.30)	12.83±0.86a	(11.00-14.50)	11.89±1.36b	(8.10-14.00)
HCT (%)	39.06±3.41a	(29.10-44.10)	39.22±2.68a	(34.50-44.20)	36.61±3.95b	(24.50-42.70)
MCV (fl)	83.91±7.39	(63.10-93.60)	88.22±3.31	(81.00-92.90)	85.33±5.93	(69.70-91.70)
Ferritin (ng/ml)	17.59±13.72	(5.76-56.27)	26.71±19.18	(6.47-86.91)	28.86±52.80	(5.22-245.17)
Iron (□ g/dl)	48.40±20.09	(23-98)	61.95±23.42	(28-106)	66.20±32.19	(30-140)

\*Descriptive statistics are expressed as mean  $\pm$  standard deviation or (minimum-maximum)  
Different letters indicate statistical difference (P<0.05)

relationship between iron deficiency anemia due to nutritional habits and antioxidant activity in pregnancy. There was a significant difference among groups in terms of egg or meat consumption. The most frequent meat consumption was on every other day in controls, but once a week in the first and last trimester groups. In addition, the most frequent egg consumption was on every other day and once a week in controls, but rare in the first trimester group, and once a week in the last trimester group. Schnefke et. al, (2009) suggested that the main obstacle to egg consumption during pregnancy was the perception that was caused by nausea and vomiting (14). The same researchers also reported that almost all participants had negative perceptions of eggs, and they actually believed

that high egg consumption during pregnancy would result in a big baby, birth complications and cesarean. Furthermore, participants also believed that high consumptions of meat and eggs during pregnancy might lead to cardiac and respiratory problems, heart diseases and high blood pressure. Therefore, all these beliefs, nausea and vomiting might be the reason of decreased egg and meat consumption as the pregnancy progress.

In this study, we found the MDA level was higher, and the SOD, CAT and GSH-Px enzyme activities were lower in both pregnant groups than controls. Increased superoxides and decreased antioxidants are a threat even in normal pregnancy (15). Many studies have reported that free radicals formed in the placenta during pregnancy cause an increase in serum MDA levels (16). In a study with

**Table 5.** Pearson Correlation Analysis Results Between Numerical Variables In Controls

	MDA	SOD	Ferritin	HGB	CAT	HCT	GSH-Px	MCV	Iron
MDA	1								
SOD	0.164	1							
Ferritin	-0.265	-0.211	1						
HGB	0.07	0.117	-0.524*	1					
CAT	0.017	0.388	-0.071	-0.064	1				
HCT	0.108	0.090	-0.538*	0.911*	-0.208	1			
GSH-PX	-0.284	0.043	-0.317	0.132	-0.154	0.155	1		
MCV	-0.011	0.081	-0.291	0.664*	0.222	0.580*	-0.231	1	
Iron	-0.197	0.080	-0.261	0.228	0.291	0.315	0.165	0.412	1

\* Indicates a significant difference (P<0.05)

**Table 6.** Pearson Correlation Analysis Results Between Numerical Variables In The First Trimester Group

	MDA	SOD	Ferritin	HGB	CAT	HCT	GSH-PX	MCV	Iron
MDA	1								
SOD	-0.304	1							
Ferritin	0.170	-0.049	1						
HGB	0.006	-0.238	-0.630*	1					
CAT	0.035	0.488*	0.018	-0.350	1				
HCT	0.063	-0.338	-0.602*	0.959*	-0.407	1			
GSH-PX	0.141	-0.398	-0.060	-0.066	-0.309	0.023	1		
MCV	-0.217	0.311	-0.316	0.293	0.081	0.172	-0.160	1	
Iron	0.313	-0.204	-0.145	-0.019	-0.057	-0.026	0.336	0.015	1

\*Indicates a significant difference (P<0.05)

post-term pregnant women, the authors proposed that oxidative stress might occur with the rapid development of the placenta in the third trimester (17). In normal pregnancies, increased MDA level is parallel to the increased total serum lipid level, and therefore, the ratio of lipid peroxidation to total lipid levels remains constant (18). In a previous study, the MDA level was found to be higher in pregnant women compared to non-pregnant, and this increase was gradual with the progress of pregnancy (15).

Antioxidants protect the cells from peroxidant reactions, limit cellular damage, and help the maintenance of membrane integrity (19). Oxidative damage on cells, tissues, and organs occurs if there is an overproduction of oxidants (20). Mechanisms for cleaning free oxygen radicals include SOD, GSH-Px, glutathione reductase (GSH-Rx), and enzymatic antioxidants such as CAT, which limit the cellular concentration of free radicals and prevent excessive oxidative damage (15). Since the antioxidant defense systems are involved in removing free radicals, their concentration or activity decreases due to increased use in cases of increased oxidative

stress. Therefore, because SOD, CAT and GSH-Px enzymes were used to remove the increased free radicals, their activities were found to be lower in both pregnant groups compared to the controls in our study.

CAT is a common enzyme found in the cells of almost all living organisms for catalyzing the decomposition of hydrogen peroxide into water and oxygen (21). Acharya et. al, (1991) found that the CAT enzyme activity had decreased in patients with iron deficiency (22). In a study investigating antioxidant levels in pregnant women with iron deficiency anemia, a significant decrease in the CAT enzyme activity was observed (8). Patil et. al, (2007) found that the CAT enzyme activity was lowest in the third trimester of pregnancy (15). In our study, we observed that the CAT enzyme activity positively correlated with Hgb and Hct. This result shows that the Hgb value increases due to increased CAT enzyme activity or it decreases due to a decrease in the activity of this enzyme. In this respect, our study is the first one to show a direct positive relationship between CAT enzyme activity and Hgb value.

**Table 7.** Pearson Correlation Analysis Results Between Numerical Variables In The Last Trimester Group

Data	MDA	SOD	Ferritin	HGB	CAT	HCT	GSH-PX	MCV	Iron
MDA	1								
SOD	-0.193	1							
Ferritin	-0.227	0.132	1						
HGB	-0.107	0.282	0.047	1					
CAT	-0.120	0.369	0.096	0.527*	1				
HCT	-0.036	0.348	0.035	0.981*	0.541*	1			
GSH-PX	-0.055	0.189	-0.241	-0.093	-0.041	-0.132	1		
MCV	0.216	-0.003	0.156	0.386	-0.052	0.322	0.281	1	
Iron	0.242	-0.177	0.341	0.284	-0.039	0.261	-0.111	0.475*	1

\*Indicates a significant difference (P<0.05)

It has been suggested that the iron requirement and absorption is not high in the first trimester of pregnancy, but increases with the progress of pregnancy (23). In our study, while iron and vitamin use was not high in the first trimester group, their use increased in the last trimester group. Previous studies have shown that using iron preparations reduces the anemia during pregnancy (24,25). In our study, although iron and vitamin uses increased in the last trimester group, Hgb and Hct values in this group were still lower than the controls and also the first trimester group. This may be due to reduced CAT enzyme activity since a reduction in CAT enzyme activity in erythrocytes causes excess H<sub>2</sub>O<sub>2</sub> accumulation in these cells. Accumulation of H<sub>2</sub>O<sub>2</sub> increases the rate of oxidation of hemoglobin to methemoglobin and disrupts the antioxidant mechanisms of erythrocytes, causing oxidative stress and shortening of the life span of erythrocytes (26). In our study, the decreased CAT enzyme activity might be due to the damaging effects of free radicals on the CAT protein since the MDA level was higher in first and last trimester groups than controls. Therefore, increased formation of free radicals with the progress of pregnancy may cause a damage on CAT protein. The decreased blood CAT enzyme activity causes oxidative stress and shortening of the life span of erythrocytes.

It was concluded that; a) the egg and meat consumption might decrease with the progress of pregnancy, b) the oxidative stress increased in pregnancy, c) Hgb and Hct values might be lower in the last trimester group despite increased intake of oral iron and vitamin supplements, and normal serum iron levels due to decreased CAT activity. Therefore, more studies are needed to demonstrate the relationship among oxidative

stress, anemia and CAT enzyme activity in pregnancy.

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