

## Original Article



# Dietary Antioxidant Capacity and Its Association with Preeclampsia

Mahdijeh Sheikhi,<sup>1</sup> Elham Sharifi-Zahabi,<sup>2</sup> Zamzam Paknahad<sup>2</sup>

<sup>1</sup>Department of Food and Nutrition Policy, Health Deputy, Zahedan University of Medical Sciences, Zahedan 98167-43175, Iran

<sup>2</sup>Department of Clinical Nutrition, School of Nutrition and Food Sciences, Isfahan University of Medical Sciences, Isfahan 81746-73461, Iran



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### Correspondence to

**Zamzam Paknahad**

Department of Clinical Nutrition, School of Nutrition and Food Sciences, Isfahan University of Medical Sciences, Hezar Jerib Avenue, P.O.Box 313, Isfahan 81746-73461, Iran.  
Tel: +98-311-7922784  
Fax: +98-311-6682509  
E-mail: paknahad@hlth.mui.ac.ir

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### ORCID

Elham Sharifi-Zahabi  
<http://orcid.org/0000-0001-6182-6357>  
Zamzam Paknahad  
<http://orcid.org/0000-0002-1864-2576>

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### Conflict of Interest

The authors declare that they have no competing interests.

## ABSTRACT

Preeclampsia (PE) is one of the major disorders in pregnancy leading to many adverse maternal outcomes. Although the etiology of PE is not fully understood, recent studies suggest that an imbalance between free radicals production and the antioxidant defense system might have key role. Our aim of the current study was to evaluate the association between dietary total antioxidant capacity (TAC), serum TAC and risk of PE in women with preeclampsia and normal pregnancy. This case-control study conducted on 55 women with preeclampsia and 93 with normal pregnancy. Dietary intakes were obtained by a semi-quantitative food frequency questionnaire (FFQ) with 168 items. Dietary TAC was assessed according to United States Department of Agriculture (USDA) Database for oxygen radical absorbance capacity (ORAC), Release 2. Serum TAC was measured by a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA). After adjusting for energy, pre-pregnant body mass index (BMI) and history of PE, no relationship was found between intake of hydrophilic-ORAC (H-ORAC), lipophilic-ORAC (L-ORAC), total phenolics (TP), total-ORAC (T-ORAC), and PE risk. However, serum TAC had a significant positive relationship with the risk of PE after adjusting for energy (odds ratio [OR], 0.07; 95% confidence interval [CI], 0.16–0.35), BMI and history of PE (OR, 0.04; 95% CI, 0.01–0.32). Findings of this study indicate that serum TAC is positively associated with the risk of PE but no association was found between intake of antioxidant indices and PE risk.

**Keywords:** Dietary total antioxidant capacity; Nutrient intake; Antioxidant status; Serum total antioxidant capacity; Preeclampsia

## INTRODUCTION

Preeclampsia (PE) is a major complication in pregnancy, and defined as new onset of maternal hypertension and proteinuria in the second half of pregnancy. PE occurs in about 3%–10% of pregnancies [1-3]. It leads to a multi systemic syndrome characterized by vasoconstriction, metabolic changes, endothelial dysfunction, increased inflammatory response, and reduced organ perfusion [4,5]. Furthermore, children born to mothers with PE are at higher risk for a variety of disorders, such as endocrine, metabolic, and nutritional disorders during adolescence [6].

Several studies have investigated the association between maternal oxidative stress and the risk of PE. It has been indicated that an imbalance between free radicals production and the antioxidant defense system, may play an important role in the pathogenesis of PE [7-9]. Reduced both enzymatic and non-enzymatic antioxidants activity was also observed in pregnant women with PE [10,11]. Epidemiological and experimental studies have reported that a high intake of dietary antioxidants (e.g., vitamin C, vitamin E, selenium, copper, etc.) may have protective effects on oxidative stress [12-15]. The association between serum total antioxidant capacity (TAC) and preeclampsia was also investigated in some studies and recent data suggest that decreased serum TAC is associated with higher percentage of maternal complication and PE [16]. It has been reported that serum antioxidant status correlates with dietary antioxidant capacity [17]. Therefore, measurement of dietary TAC can also be helpful in detecting the relationship between dietary antioxidants and the risk of diseases. Most studies in field of dietary antioxidants and PE assessed mainly the association between PE and a single antioxidant. Therefore, it is thought that analysis of dietary TAC can give more balanced description of data compared to analysis of a single antioxidant [18].

Dietary TAC has been developed in order to assess the beneficial effects of dietary antioxidants [19]. It has been reported that TAC in the food groups including vegetables, fruits, legumes, and nuts is higher than the other groups. Therefore, a healthy diet rich in antioxidants with high TAC content may improve serum antioxidant status [20]. Some studies evaluated the association between dietary total antioxidant and several diseases. However, to our knowledge, no study has investigated the association between dietary TAC and the risk of PE.

Therefore, our study aimed to evaluate the association between dietary TAC, serum TAC and the risk of PE using data from standardized antioxidant databases in a population based case-control study conducted in Isfahan, Iran.

## MATERIALS AND METHODS

### Study design

We conducted a population based case-control study in Obstetrics and Gynecology Department of the Shahid Beheshti Hospital at 2014, from April 2014 to July 2014 in Isfahan. This hospital is a referral center for high risk pregnancies and women from different social classes were referred there. The protocol of the study was approved by the Research and Ethics Committee of Isfahan University of Medical Sciences, and written informed consent was obtained from each participant before the study.

A sample size of 43 subjects in each group was considered based on a power of 80% at the 5% significance level. A total of 63 women with preeclampsia and 105 with normal pregnancy with ages 18–35 years and gestational age of 34 to 42 weeks volunteered to participate in the study. PE was diagnosed by a systolic blood pressure of  $\geq 140$  mmHg and/or diastolic blood pressure of  $\geq 90$  mmHg on 2 occasions more than 4 hours apart and proteinuria  $\geq 300$  mg protein in 24-hour urine collection after 20 weeks [2].

Women were excluded if: they did not give consent, were of gestational age under 18 years, have a history of pre-existing health condition including type 1 or type 2 diabetes, gestational diabetes, hypertension disorders, renal disease, heart disease, or other conditions that

require special diets. We also excluded patients who reported a total energy intake outside the range of 800–4,200 kcal/day [21].

Finally based on the exclusion criteria 55 subjects in PE group and 93 subjects in normal pregnancy group met the inclusion criteria and enrolled in the study. Those with > 70 blank item on the food frequency questionnaire (FFQ) (PE,  $n = 5$ ; normotensive,  $n = 8$ ) and those who reported a total daily energy intake outside the range of 800–4,200 kcal (PE,  $n = 3$ ; normotensive,  $n = 4$ ) were excluded from the study. A team of trained gynecologist and dietitians conducted the study under standard protocols. Women also were interviewed about demographic data.

### Dietary assessment and dietary TAC estimation

Dietary intakes of participants were assessed by a validated 168-items of semi-quantitative FFQ developed in Iran [22]. The FFQ was a Willet format questionnaire modified for Iranian food items [23]. This questionnaire was used to determine the frequency of food consumption during pregnancy and asks about the usual dietary intake in the past 6 months. Information on the frequency of intake and portion size was converted to the amount of each food item in grams consumed on average per day. Dietary data was extracted by Nutritionist-4 software (First Databank Inc., Hearst Corp., San Bruno, CA, USA) and a trained dietitian.

To calculate dietary TAC for each participant, we used the United States Department of Agriculture (USDA) Database for oxygen radical absorbance capacity (ORAC), Release 2. Total daily intake of food items known to be high in antioxidant content from the FFQ, including over 50 food items—such as fruits, vegetables, juices, grains was computed first. Daily consumption was derived based on frequency and portion size, and food values were converted from their unit listed in the FFQ to grams per day. Antioxidant values were attributed to each food item based on data in the antioxidant databases. Dietary TAC describes the ability of food antioxidants to scavenge free radicals [20]. The ORAC values were utilized to develop individual indices for TAC; total-ORAC (T-ORAC), hydrophilic-ORAC (H-ORAC), lipophilic-ORAC (L-ORAC), and total phenolics (TP). H-ORAC, L-ORAC, and T-ORAC are reported in  $\mu\text{mol}$  of Trolox equivalents per 100 grams ( $\mu\text{molTE}/100\text{ g}$ ), while TP is reported in mg gallic acid equivalents per 100 grams (mgGAE/100 g).

### Measurement of serum TAC levels

TAC of serum was determined from the sera that were frozen at  $-70^{\circ}\text{C}$ . The human T-AOC kit (MyBioSource, San Diego, CA, USA) was used to assay the serum TAC in samples. The kit used the double antibody sandwich enzyme-linked immunosorbent assay (ELISA) in samples. The principle of test was 1) adding T-AOC to monoclonal antibody enzyme well which was pre-coated with human TAC monoclonal antibody, incubation; 2) adding TAC antibodies labeled with biotin, and combining with Streptavidin-HRP (Vector Laboratories, Burlingame, CA, USA) to form immune complex; and 3) chromogen solution A, B (Novateinbio, Woburn, MA, USA) was added and the color of the liquid changed into blue, at the effect of acid, the color finally became yellow. The chroma of color and the concentration of the human substance TAC of sample were positively correlate.

### Statistical analysis

Data analysis was performed using Statistical Package for the Social Sciences (SPSS) version 20.0 (SPSS Inc., Chicago, IL, USA) software and a  $p$  value  $< 0.05$  was considered

as significant. The data were expressed as mean  $\pm$  standard deviation (SD) or percentages. The relationship between PE and the TAC indices was assessed by evaluating the indices as categorical variables. Medians of each TAC index were created based on the distribution of each index among cases and controls. Energy adjusted analysis of covariance (ANCOVA) and cross-tabulations were conducted to assess any association between the TAC indices and case-control status. Binary logistic regression models were developed to estimate odds ratios (ORs) and 95% confidence intervals (CIs) while controlling for potential confounders including body mass index (BMI), total energy intake, age, and history of PE was considered.

## RESULTS

Data regarding socio-demographic and lifestyle variables are presented in **Table 1**. The mean of pre-pregnancy BMI and number of pregnancy in PE women were higher ( $p < 0.001$ ) compared to the normal pregnancy group. The percentage of women with history of PE was higher in the PE group compared with normal pregnancy group (25.5% vs. 1.1%,  $p < 0.001$ ).

Based on the results obtained from the FFQ, the mean of energy intake was  $2,157.7 \pm 670.6$  and  $2,577.7 \pm 730.4$  kcal/day in PE and normal pregnancy groups respectively and was significantly higher ( $p < 0.001$ ) in PE women. Women with PE had significantly lower intakes of vegetables ( $p < 0.001$ ) and grains ( $p < 0.001$ ) compared to the normal pregnancy group (**Table 2**). All women did not consume any supplements except iron and folic acid supplementation that is routinely used during pregnancy.

**Table 3** shows the energy-adjusted mean TAC intake based on antioxidant indices in PE and normal pregnancy groups. Women with PE had significantly lower intakes of H-ORAC, L-ORAC, and TP. However, no significant differences were observed between PE and normal pregnancy groups for dietary intake of L-ORAC. Since there are no cut-off values for dietary

**Table 1.** General characteristics of PE and normal pregnancy groups

Characteristics	Preeclamptic (n = 55)	Normal pregnancy (n = 93)	p value
Age, yr	29.3 $\pm$ 5.5	26.6 $\pm$ 6.0	0.009
Education, yr	9.3 $\pm$ 4.5	7.9 $\pm$ 5.2	0.095
Gestational age, wk	34.1 $\pm$ 2.7	35.7 $\pm$ 2.7	0.125
Pre-pregnancy BMI, kg/m <sup>2</sup>	25.7 $\pm$ 5.5	22.8 $\pm$ 4.7	0.001
No. of pregnancy	1.9 $\pm$ 1.2	2.5 $\pm$ 2.1	0.049
Primiparous	10.9	5.4	0.049
History of preeclampsia	25.5	1.1	< 0.001
History of abortion	21.8	18.3	0.603
History of stillbirth	7.3	12.9	0.290
History of twin birth	5.5	7.5	0.630

Values are presented as mean  $\pm$  SD or percentage; p value < 0.05 was considered significant.

PE, preeclampsia; BMI, body mass index; SD, standard deviation.

**Table 2.** Comparison of dietary intakes of some different food groups in PE and normal pregnancy groups

Food group	Preeclamptic (n = 55)	Normal pregnancy (n = 93)	p value
Meat or beans, oz	3.7 $\pm$ 2.1	4.1 $\pm$ 2.9	0.830
Milk, cup	2.4 $\pm$ 1.6	2.4 $\pm$ 1.4	0.920
Grains, oz	10.7 $\pm$ 5.3	15.8 $\pm$ 7.4	< 0.001
Vegetables, cup	1.8 $\pm$ 0.9	3.1 $\pm$ 1.9	< 0.001
Fruit, cup	3.9 $\pm$ 2.0	3.7 $\pm$ 2.6	0.280

p value < 0.05 was considered significant.

**Table 3.** Comparison of dietary TAC in PE and normal pregnancy groups

Dietaries	Mean (SE)		p value
	Preeclamptic	Normal pregnancy	
H-ORAC, $\mu\text{molTE}/100\text{ g}$	14,383.1 (795.0)	17,405.7 (1,204.8)	0.040
L-ORAC, $\mu\text{molTE}/100\text{ g}$	151.0 (9.3)	166.5 (14.1)	0.360
T-ORAC, $\mu\text{molTE}/100\text{ g}$	14,817.9 (1,236.1)	20,284.5 (1,873.3)	0.010
TP, mgGAE/100 g	1,173.1 (78.1)	1,585.3 (118.3)	0.005
Serum TAC, $\mu\text{mol}/\text{L}$	3.9 (0.1)	3.3 (0.2)	0.040

TAC, total antioxidant capacity; SE, standard error; H-ORAC, hydrophilic-oxygen radical absorbance capacity; L-ORAC, lipophilic-oxygen radical absorbance capacity; T-ORAC, total-oxygen radical absorbance capacity; TP, total phenolics;  $\mu\text{molTE}/100\text{ g}$ ,  $\mu\text{mol}$  of Trolox equivalents per 100 grams; mgGAE/100 g, mg gallic acid equivalents per 100 grams.

**Table 4.** TAC intake

ORAC database	Preeclamptic (n = 55)	Normal pregnancy (n = 93)	OR1 (95% CIs)	OR2 (95% CIs)
H-ORAC, $\mu\text{molTE}/100\text{ g}$				
< 13,673	24	49	1.00	1.00
$\geq 13,673$	31	42	0.51 (0.25–1.05)	0.58 (0.25–1.38)
L-ORAC, $\mu\text{molTE}/100\text{ g}$				
< 139.4	27	46	1.00	1.00
$\geq 139.4$	28	46	0.64 (0.30–1.33)	0.87 (0.36–2.13)
T-ORAC, $\mu\text{molTE}/100\text{ g}$				
< 14,356.1	24	48	1.00	1.00
$\geq 14,356.1$	31	41	0.04 (0.23–0.99)	0.16 (0.22–1.29)
TP, mgGAE/100 g				
< 1,183.2	29	44	1.00	1.00
$\geq 1,183.2$	26	47	0.76 (0.36–1.59)	1.09 (0.43–2.73)
Serum TAC, $\mu\text{mol}/\text{L}$				
< 3.7	24	48	1.00	1.00
$\geq 3.7$	31	41	0.07 (0.16–0.35)	0.04 (0.01–0.32)

OR1 means adjusted for energy (continuous); and OR2 means further adjusted for BMI (continuous) and history of preeclampsia.

TAC, total antioxidant capacity; OR, odds ratio; CIs, confidence intervals; ORAC, oxygen radical absorbance capacity; H-ORAC, hydrophilic-oxygen radical absorbance capacity; L-ORAC, lipophilic-oxygen radical absorbance capacity; T-ORAC, total-oxygen radical absorbance capacity; TP, total phenolics;  $\mu\text{molTE}/100\text{ g}$ ,  $\mu\text{mol}$  of Trolox equivalents per 100 grams; mgGAE/100 g, mg gallic acid equivalents per 100 grams.

TAC [19], comparing the dietary TAC between 2 groups were considered for interpreting the results. Mean of serum TAC was  $3.86 \pm 1.19\text{ }\mu\text{mol}/\text{L}$  in PE group and significantly higher than normal pregnancy group ( $p < 0.040$ ).

Correlation between TAC intake based on antioxidant indices and PE risk are shown in **Table 4**. After adjusting for major risk factors such as energy between maternal pre-pregnant BMI, and history of PE, no relationship was found between intake of H-ORAC, L-ORAC, TP, T-ORAC, and PE risk. These factors have previously been shown to influence the risk of PE and they were also associated with the dietary intakes. However, serum TAC had a significantly positive relationship with risk of PE after adjusted for energy (OR, 0.07; 95% CI, 0.16–0.35) and further adjusted for BMI and history of PE (OR, 0.04; 95% CI, 0.01–0.32).

## DISCUSSION

This study evaluated the association between dietary TAC and risk of PE among women with preeclampsia and normal pregnancy. In this work we found little evidence that dietary TAC has an effect on PE risk based on antioxidant content values from the USDA database for ORAC. The serum TAC had a significant positive relationship with the risk of PE, but no association was found between intake of antioxidant indices and PE risk.

Studies regarding dietary TAC are limited, but some studies measured total oxidant status (TOS) and total antioxidant status (TAS) in women with PE. Ozturk et al. [24] in their study on preeclamptic women showed that maternal and cord plasma levels of TAS were significantly correlated with maternal and cord plasma levels of TOS. In a study Mert et al. [25] reported that women whose pregnancies were complicated with PE and intrauterine growth restriction had elevated levels of TOS and TAS in comparison with healthy pregnant women. Another study conducted by Fenzl et al. [26] showed that serum TAC and TOS concentrations were significantly higher in pregnant women with PE. Increased TOS in all pregnant women points to latent oxidative stress in pregnancy while increased TAC in the early steps of preeclampsia might represent a defense mechanism of body against stress.

Some studies suggested that adherence to a dietary pattern characterized by high intake of vegetables, fruits, rice, vegetable oils, and poultry was associated with a reduced risk of PE, whereas adherence to a dietary pattern characterized by high intake of processed foods including sausages, hamburgers, white bread, salty snacks, sugar-sweetened drinks, and sweets increase the risk of PE [15]. A study in the central of Africa reported that women who had frequent intake of vegetables had lower risk of PE than women with infrequent intake of vegetables [27]. Similarly, our results showed that women with PE had significantly lower intakes of grain and vegetables compared to the women with normal pregnancy. However, we could not show any relationship between dietary TAC and the risk of PE. The association between serum antioxidant status and dietary antioxidant has been reported in several studies. In our previous study, we failed to find any correlation between intake of vitamin C,  $\beta$ -carotene, riboflavin, copper, and serum TAC. But concurrently a positive association between vitamin E and serum TAC was observed [28].

The existence of a threshold effect for dietary intake and levels of serum TAC, related to the risk of some disease, suggests a fascinating and challenging hypothesis [18].

This study may be involved by the limitations of case-control studies as our participants were selected from a single referral center, we could not generalize our results to a larger population of pregnant women. So we were faced to selection biases occurring in most case-control studies. The income level of the participants might have influence on maternal complication. Unfortunately, in this work, we did not investigate the income level of our subjects. Finally, as there is no database on dietary TAC for Iranian foods, using the USDA database for ORAC is another limitation of our study.

Our study has also some strength; to our knowledge this is the first study that evaluated the association between dietary antioxidant and PE in pregnant women. Our subjects in this study were carefully interviewed by an expert dietitian and their demographic and dietary data were compared to questionnaires which have been filled by them. The large sample size of our study which measured known potential confounders such as energy, BMI, age, and history of PE, and controlled them in our analyses are the other strengths.

In conclusion, these findings indicated that serum TAC are positively associated with the risk of PE but no association was found between intake of antioxidant indices and PE risk. The use of dietary TAC to describe a combined effect of dietary and serum antioxidants on PE risk in prospective cohort studies needs more exploration. Whether dietary and serum antioxidants decrease PE risk also warrants further investigation.



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