The Relationship Between Dietary Total Antioxidant Capacity with Serum Antioxidant and Oxidant Parameters in Hemodialysis Patients

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ABSTRACT

Objective: The dietary total antioxidant capacity (dTAC) is the cumulative total of all antioxidant compounds contained in food and is associated with dietary food consumption. Therefore, it can be used as an indicator of dietary quality. The aim of the study is to assess the relationship between dTAC values with serum antioxidant (total antioxidant capacity (TAC); paraoxonase1 (PON1), and arylesterase (ARES)) and oxidant (total oxidant status (TOS); Oxidative Stress Index (OSI), and malondialdehyde (MDA)) parameters in hemodialysis patients.

Methods: This experimental randomized controlled human study was conducted in 2 dialysis centers in Bingol, Turkey. In this study, 46 participants were included in each of the 2 groups of hemodialysis and control. The dietary intake was assessed with a 7-day food diary, and the dTAC values were calculated from databases of oxygen radical absorbance capacity (ORAC), ferric reducing antioxidant power (FRAP), total radical-trapping antioxidant parameter (TRAP), trolox equivalent antioxidant capacity (TEAC), and the equation of vitamin C equivalent antioxidant capacity (VCEAC).

Results: The values of dTAC were lower in the hemodialysis patients than in the control group ($P < .01$). There was a positive correlation between the values of TEAC, TRAP, FRAP, and serum PON1, whereas there was a significant negative correlation between C-reactive protein (CRP) values ($P < .05$).

Conclusion: The dietary TAC values were lower in the hemodialysis patients, and they were related with serum PON1 and CRP. Increasing the dTAC content is thought to have positive effects on patients, and it should be increased by providing healthy nutrition.

Keywords: Arylesterase, dietary total antioxidant capacity, hemodialysis patients, malondialdehyde, oxidative stress index, paraoxonase

INTRODUCTION

Oxidative stress (OS) is defined as the breakdown of the balance between the pro-oxidants and antioxidants, by the pro-oxidants, causing cell damage.1 OS is responsible for the pathogenesis of many diseases and the formation and progression of chronic renal failure. It has been shown that OS might cause morbidity and mortality, especially in end-stage renal disease (ESRD) patients receiving renal replacement therapy.2 In order to reduce OS, increasing the intake of dietary antioxidants and balancing pro-oxidant–antioxidant levels by supporting the antioxidant defense system might have positive effects in these patients. It is also suggested that it is more appropriate to evaluate the dietary total antioxidant capacity (dTAC), instead of adding and/or evaluating a single antioxidant component to the diet.

The dTAC, the cumulative sum of antioxidant components in all foods consumed during the day, has been used for diet quality assessment in recent times.
Previous studies have shown that there is a significant relationship between dTAC and the plasma total antioxidant capacity (plasma TAC). Thus, the dTAC is an important parameter in kidney disease and it should be further evaluated. This study aimed to: i) calculate the dTAC value of hemodialysis patients with 2 different methods (via database and formula), and examine whether the dTAC value of hemodialysis patients differs from healthy individuals; ii) evaluate and compare both serum antioxidant parameters (paraoxonase1 (PON1) and arylesterase (ARES) enzyme activities, serum TAC, and oxidant parameters (malondialdehyde (MDA), Oxidative Stress Index (OSI) and serum total oxidant status (TOS)) in hemodialysis patients; and (iii) examine the relationship between patients’ dTAC values of patients and both the serum antioxidant and oxidant parameters.

METHODS

Study Design
This randomized controlled human study was carried out in Bingöl, Turkey. It was conducted in accordance with the Declaration of Helsinki, and the study protocol was approved by the Hacettepe University Non-Interventional Clinical Researches Ethics Board (Ref Code: GO 16/459). Informed consent was obtained from all participants.

Study Population
In this study, the sample patient group consisted of 19-64 year-old volunteers in 2 hemodialysis units, who met the study criteria. The control group consisted of healthy volunteers who were matched in terms of age and gender with the patient group.

Patients who had been on hemodialysis for at least 6 months, had a stable clinical condition, and had dialysis at least 2 days a week were included in the hemodialysis group. The control group consisted of individuals who applied to the hospital for check-up and accepted to participate in the study.

Exclusion Criteria
The criteria for exclusion from the study were: smoking and alcohol consumption, patients having diabetes and related complications, chronic inflammation such as active hepatitis, HIV (+), liver and endocrine disease (such as thyroid, adrenal gland), heart disease, or symptomatic benign prostatic hyper trophy, having neurological and psychiatric diseases, having had a kidney transplant or being scheduled for one, the use of aspirin, beta-blockers and others such as vitamins other than folic acid, fish oil, and antioxidants in the last 3 months, receiving lipid-lowering therapy, pregnancy, and lactation.

In addition to all of the exclusion criteria in the patient group, the exclusion criterion of the control group was having renal insufficiency (GFR <90 mL/min/1.73 m²; proteinuria).

A questionnaire was applied to these subjects in order to gather information about their disease and general characteristics such as age, gender, and physical activity level. Moreover, the Subjective Global Assessment (SGD) form was used to determine malnutrition risk, which is an important indicator of morbidity and mortality in dialysis patients. The evaluation was divided into 3 categories: well-fed, risk of malnutrition or moderate malnutrition, and severe malnutrition.

Determining of dTAC
The food intake was assessed by a 7-day food diary (3 dialysis days and 4 non-dialysis days for hemodialysis patients) using a photographic atlas of food portion sizes according to institutional standard recipes, and the dTAC was calculated from these portions.

Two different methods were used for determining the dTAC. First, the formula created for the theoretical dTAC calculation using the values of the National Food Composition Databases (NFCD) determined by the United States Department of Agriculture (USDA) for each nutrient [Theoretical dTAC = \( \sum (\text{Antioxidant Content (mg/100 g)}) \times \text{(Antioxidant Capacity (mg VCE/100 g))} \)] was used. After determining the individual antioxidant content, the total amount of antioxidants taken per day was calculated for each food. The averages of the 7-day dTAC values were expressed as Vitamin C equivalent mg/day (VCE mg/day).

Second, due to the lack of a country-specific database containing the antioxidant values of foods, the dTAC database was created using the values determined for 100 g of foods in the databases of international studies. The following methods were used to determine the TAC of foods in this database: oxygen radical absorbance capacity (ORAC), ferric reducing antioxidant potential (FRAP), trolox equivalent antioxidant capacity (TEAC), and total radical-trapping antioxidant parameters (TRAP). The antioxidant contents of similar foods were used for foods not included in any of the relevant databases.

Routine Biochemical and Antioxidant–Oxidant Parameters
The samples required for the study-specific analysis were collected from the remaining serum samples taken for routine analysis. Serums were stored at −80°C until the analysis.

Main Points

- Hemodialysis patients’ dietary total antioxidant intake and serum antioxidant parameters were lower and their oxidant parameters were higher, compared to healthy individuals.
- In hemodialysis patients, the dietary total antioxidant capacity (dTAC) was associated with the antioxidant paraoxonase1 (PON1) enzyme and CRP, which is an inflammatory marker.
- The dietary total antioxidant capacity (dTAC) is a useful parameter in the assessment of diet quality.
Routine Biochemical Checks
Values of urea, creatinine, albumin, high-density lipoprotein (HDL), low-density lipoprotein (LDL), C-reactive protein (CRP), and total cholesterol, triglycerides, and glucose were obtained from the patient files.

Additional Laboratory Analysis for the Study: Antioxidant parameters such as TAC, PON1 and ARES enzyme activity and oxidant status parameters such as TOS, OSI, and MDA levels of were examined. Serum TAC and TOS,20 PON1 and ARES,21 and MDA22 analyses were performed with local commercial kits (Rel Assay® Diagnostics), and OSI20 was calculated from the ratio of TAC and TOS levels. All analyses were duplicated in an automated microplate reader with spectrophotometry at the Central Research Laboratory of Bingöl University.

Data Evaluation and Statistical Analysis
The SPSS 22.0 statistical package program23 was used for statistical analysis. The numerical data were expressed as arithmetic mean (x), standard deviation (S), and minimum-maximum values. Additionally, the categorical data were presented as numbers and percentages, and compared with the chi-square test. The parametric distribution of all the quantitative parameters such as dTAC, serum TAC, and TOS was checked with the Kolmogorov–Smirnov test. Because of the non-parametric distribution, the Mann–Whitney U-test was used for comparison between 2 groups, and the Wilcoxon test was used to compare the change in antioxidant and oxidant parameters between before and after dialysis. Spearman correlation analysis was performed to show the relationship between the parameters. A P value of < .05 was considered statistically significant.

RESULTS
A total of 92 people (38 men and 54 women) participated in the study. The main characteristics of the participants are given in Table 1. The weight and body mass index (BMI) values of the hemodialysis patients were significantly lower than the corresponding values in the control group (P < .05). According to the SGA, the hemodialysis patients had moderate or severe malnutrition levels, and their physical activity levels were low (P < .05). The age at first diagnosis in hemodialysis patients was 41.2 ± 12.6 years, and the duration of dialysis exposure was 9.9 ± 8.1 years.

The total dTAC values of the individuals are given in Table 2. The values of T-ORAC and H-ORAC, TEAC, TRAP, FRAP-1, FRAP-2,
mon renal replacement therapy is hemodialysis.25 In hemodialysis patients due to anorexia has been shown in previous studies.

In hemodialysis patients, nutrition is crucial to reduce the complications of the disease as well as to prevent and control comorbidity. In this study, although hemodialysis patients had normal BMI values, they had moderate or severe malnutrition, according to the SGA, and the dTAC value was significantly lower than in the healthy individuals. A decrease in the food intake of hemodialysis patients due to anorexia has been shown in previous studies.26 In hemodialysis patients, it is important to provide an adequately-balanced diet and food diversity, by eliminating the factors that reduce the nutritional intake. In this way, it would be possible to increase the antioxidant intake with the diet, and this would have a protective effect against oxidative damage and its complications in patients. Studies have also stated that dTAC content is protective against oxidative damage and the associated metabolic complications,27 and the determination of dTAC content is a new and usable method for investigating the effects of dietary antioxidants.4, 12, 27

FRAP-3, FRAP-4, and VCEAC were lower in the hemodialysis patients than in the control group (P < .01). Moreover, the serum antioxidant parameters (TAC, PON1, AREs) and levels of albumin, LDL and HDL of hemodialysis patients were lower than in the control group, while the oxidant parameters (TOS and OSI) and CRP, triglyceride, and phosphorus levels were higher than in the control group (P < .01; Table 3). The correlation among TAC values of the diet, serum antioxidant–oxidant parameters, and biochemical results are given in Table 4. The level of PON1 had a positive correlation with TEAC, TRAP, FRAP3, and FRAP4 in hemodialysis patients (P < .05), and the PON1 levels showed an increase corresponding to the increase in dietary antioxidants (Table 4). There was a significant inverse relationship between TEAC, TRAP, FRAP3 values and CRP, both in the hemodialysis and the control groups (P < .05).

DISCUSSION
Chronic kidney failure is an important public health problem in Turkey, as well as all over the world.24 According to the 2018 data of the Turkish Nephrology Association, it has been stated that the prevalence and incidence of ESRD were 988.4 and 149.2 per million population, respectively. In Turkey, the most common renal replacement therapy is hemodialysis.25 In hemodialysis patients, nutrition is crucial to reduce the complications of the disease as well as to prevent and control comorbidity. In this study, although hemodialysis patients had normal BMI values, they had moderate or severe malnutrition, according to the SGA, and the dTAC value was significantly lower than in the healthy individuals. A decrease in the food intake of hemodialysis patients due to anorexia has been shown in previous studies.26 In hemodialysis patients, it is important to provide an adequately-balanced diet and food diversity, by eliminating the factors that reduce the nutritional intake. In this way, it would be possible to increase the antioxidant intake with the diet, and this would have a protective effect against oxidative damage and its complications in patients. Studies have also stated that dTAC content is protective against oxidative damage and the associated metabolic complications,27 and the determination of dTAC content is a new and usable method for investigating the effects of dietary antioxidants.4, 12, 27

In hemodialysis patients, the serum antioxidant parameters were lower and serum oxidant parameters were higher than in healthy individuals (Table 3). In this study, no significant relationship was shown between dTAC values and serum antioxidant–oxidant parameters, except for the PON1 antioxidant enzyme (Table 4). The direct effects of dietary antioxidants in plasma samples cannot be determined, due to many factors, particularly individual metabolic differences and insufficient nutrient intake. In the present study, the correlation between dTAC values and the PON1 enzyme might be due to the antioxidant relationship between the PON1 enzyme and lipoproteins, as the HDL-associated PON1 enzyme has protective effects on lipid peroxidation.28 Furthermore, when the correlation is considered in terms of dTAC, some foods contain antioxidants with lipophilic properties. Therefore, the FRAP, TEAC, and TRAP assays used in dTAC calculation might detect lipophilic antioxidant components in foods more accurately. In conclusion, the relationship between dTAC values and the PON1 enzyme might have been established due to the lipophilic antioxidants and antioxidant properties of PON1. In other words, the increase of lipophilic antioxidants in dTAC content.

Table 2. Participants’ Dietary Total Antioxidant Capacity (n = 92)

<table>
<thead>
<tr>
<th>Total dTAC</th>
<th>Hemodialysis (n = 46)</th>
<th>Control (n = 46)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD (Min-Max)</td>
<td>Mean ± SD (Min-Max)</td>
<td></td>
</tr>
<tr>
<td>T-ORAC (µmol TE)</td>
<td>12535.0 ± 4298.0 (4196.6-25274.6)</td>
<td>18232.6 ± 5866.4 (7443.7-30782.3)</td>
<td>.001*</td>
</tr>
<tr>
<td>L-ORAC (µmol TE)</td>
<td>1197.3 ± 628.4 (246.3-2605.0)</td>
<td>1425.5 ± 685.1 (267.0-3257.5)</td>
<td>.064</td>
</tr>
<tr>
<td>H-ORAC (µmol TE)</td>
<td>11338.6 ± 4004.9 (3950.3-23127.8)</td>
<td>16798.1 ± 5364.6 (7176.1-27872.9)</td>
<td>.001*</td>
</tr>
<tr>
<td>TEAC (µmol TE)</td>
<td>3.15 ± 2.43 (1.31-18.14)</td>
<td>6.24 ± 3.04 (2.20-18.88)</td>
<td>.001*</td>
</tr>
<tr>
<td>TRAP (µmol TE)</td>
<td>3.14 ± 1.15 (1.16-6.60)</td>
<td>7.30 ± 4.30 (2.73-25.16)</td>
<td>.001*</td>
</tr>
<tr>
<td>FRAP</td>
<td>2.29 ± 0.89 (0.85-4.82)</td>
<td>3.47 ± 1.62 (1.25-8.56)</td>
<td>.001*</td>
</tr>
<tr>
<td>FRAP2 (mmol)</td>
<td>2.19 ± 2.29 (0.77-16.42)</td>
<td>3.30 ± 1.56 (1.20-8.94)</td>
<td>.001*</td>
</tr>
<tr>
<td>FRAP3 (mmol)</td>
<td>8.12 ± 7.88 (3.30-58.05)</td>
<td>16.85 ± 9.69 (6.09-60.72)</td>
<td>.001*</td>
</tr>
<tr>
<td>FRAP4 (mmol)</td>
<td>5.38 ± 9.16 (1.97-65.22)</td>
<td>9.29 ± 4.86 (3.80-30.31)</td>
<td>.001*</td>
</tr>
<tr>
<td>VCEAC Total (mg VCE)</td>
<td>839.62 ± 331.88 (360.87-1633.12)</td>
<td>1506.54 ± 588.92 (465.98-3298.49)</td>
<td>.001*</td>
</tr>
</tbody>
</table>

Mann-Whitney U-test was performed.

*P < .05.

ORAC, oxygen radical absorbance capacity; H-ORAC, hydrophilic; L-ORAC, lipophilic; T-ORAC, total; TEAC, trolox equivalent antioxidant capacity; TRAP, total radical-trapping antioxidant parameters; FRAP, ferric reducing antioxidant potential (FRAP1; Carlsen et al. 2010; FRAP2, Halvorsen et al. 2006; FRAP3, Pellegrini et al. 2003, 2006; FRAP4, Zujko et al. 2008, 2014, 2015). TE, trolox equivalent; VCE, vitamin C equivalent.
might increase the protection of lipoproteins against oxidation by the PON1 enzyme, and thus, the increase can reduce oxidative damage. Nevertheless, the dTAC values in each group had a significant negative relationship with CRP, which is one of the inflammation parameters. Similar to the results of this study, a study conducted with Japanese women found that there was a significant correlation between the CRP value, which is one of the inflammation indicators, and the dTAC values calculated using FRAP, TEAC and TRAP. In addition, there was an association between high CRP levels and low dTAC content.\(^{29}\)

It was also shown that the risk of developing abdominal obesity, hypertension, and metabolic syndrome decreased as the dTAC content increased.\(^{29}\) In addition, it has been stated that consuming diets with high TAC values might provide protection from cardiovascular diseases.\(^{31}\) The TAC content of the diet in hemodialysis patients is an important parameter for reducing the risk of OS in diseases and inflammation.

Besides the strengths, the present study has also 2 main limitations, in addition to those generally encountered in human studies. The first limitation is the lack of having any country-specific database of the antioxidant content of foods. Another limitation of the study is the high consumption of animal-based food and the low consumption and variety of vegetables and fruits, due to the prevalence of animal husbandry in the region where the study was conducted. Therefore, further studies with a multicenter approach would be beneficial to gather more detailed data.

**CONCLUSION**

The causes of malnutrition in hemodialysis patients should be investigated and eliminated, and dietary intake of the patients should be increased. An adequately-balanced diet and food variety are critical in order to increase the dietary antioxidant intake. Providing food variety might increase the dTAC thorough the higher intake of functional components. The important point to note is the preservation of the antioxidants already available in antioxidant-rich foods. Explaining the best way of consumption, and care at each stage, such as purchase, preparation, cooking, and storing of foods will minimize the loss of nutrients, which can be achieved by...
<table>
<thead>
<tr>
<th>Total dTACs</th>
<th>Antioxidant Parameters</th>
<th>Oxidant Parameters</th>
<th>Biochemical Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>sTAC</td>
<td>PON1</td>
<td>ARES</td>
</tr>
<tr>
<td>Hemodialysis (N = 46)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t-ORAC-T (µmol TE)</td>
<td>−0.26</td>
<td>−0.26</td>
<td>−0.14</td>
</tr>
<tr>
<td>ORAC-L (µmol TE)</td>
<td>−0.09</td>
<td>−0.28</td>
<td>0.06</td>
</tr>
<tr>
<td>ORAC-H (µmol TE)</td>
<td>−0.24</td>
<td>−0.25</td>
<td>−0.16</td>
</tr>
<tr>
<td>TEAC (mmol TE)</td>
<td>−0.21</td>
<td>0.37*</td>
<td>−0.18</td>
</tr>
<tr>
<td>TRAP (mmol TE)</td>
<td>−0.20</td>
<td>0.36*</td>
<td>−0.18</td>
</tr>
<tr>
<td>FRAP1 (mmol of Fe)</td>
<td>−0.28</td>
<td>−0.15</td>
<td>−0.02</td>
</tr>
<tr>
<td>FRAP2 (mmol of Fe)</td>
<td>−0.25</td>
<td>−0.27</td>
<td>−0.19</td>
</tr>
<tr>
<td>FRAP3 (mmol of Fe)</td>
<td>−0.22</td>
<td>0.35*</td>
<td>−0.20</td>
</tr>
<tr>
<td>FRAP4 (mmol of Fe)</td>
<td>−0.26</td>
<td>0.31*</td>
<td>−0.22</td>
</tr>
<tr>
<td>VCEAC Total (mg VCE)</td>
<td>−0.08</td>
<td>−0.13</td>
<td>−0.14</td>
</tr>
<tr>
<td>CONTROL (N = 46)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ORAC-T (µmol TE)</td>
<td>0.05</td>
<td>0.23</td>
<td>−0.13</td>
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<tr>
<td>ORAC-L (µmol TE)</td>
<td>0.11</td>
<td>0.23</td>
<td>−0.03</td>
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<tr>
<td>ORAC-H (µmol TE)</td>
<td>0.05</td>
<td>0.23</td>
<td>−0.14</td>
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<tr>
<td>TEAC (mmol TE)</td>
<td>0.08</td>
<td>0.24</td>
<td>−0.04</td>
</tr>
<tr>
<td>TRAP (mmol TE)</td>
<td>0.04</td>
<td>0.11</td>
<td>0.05</td>
</tr>
</tbody>
</table>
providing frequent training on nutrition and increasing the awareness in patients. Thus, patients will be protected from OS and inflammation.

**Ethics Committee Approval:** Ethical committee approval was received from the Hacettepe University Non-Interventional Clinical Researches Ethics Board (Ref Code: GO 16/459; Date: July 13, 2016).

**Informed Consent:** Written informed consent was obtained from all participants who participated in this study.

**Peer Review:** Externally peer-reviewed.


**Conflict of Interest:** The authors have no conflict of interest to declare.

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**REFERENCES**


