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Original article

Validation of a food frequency questionnaire for assessing total antioxidant status☆

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SUMMARY

Objective: To evaluate the validity of a food frequency questionnaire (FFQ) developed as a tool for assessing antioxidant intake and to investigate whether dietary total antioxidant status predicted plasma antioxidant status.

Material and methods: This research was conducted at Sağlık Bilimleri University Faculty of Medicine Immunology. Dietary intake of total antioxidants was assessed using an FFQ (82 food items), which was adopted from Satia et al. and translated into Turkish. Total antioxidant status (TAS) in plasma was assessed using enzyme-linked immunosorbent assay (ELISA) in overnight-fasting blood. The validation of the questionnaire against plasma TAS was examined using Spearman's correlation test, Bland–Altman plots, and kappa statistics.

Results: The mean age of the 45 study participants (19 women and 26 men) was 45.9 ± 11.0 years. The mean plasma TAS level was 13.8 ± 6.1 mg/L, and the mean intake of total antioxidants was 114.0 ± 134.6 mg/day. There was a positive correlation between plasma TAS and the intake of total antioxidants calculated using the FFQ ($r = 0.73$; $p < 0.001$). Cronbach's alpha coefficient of the questionnaire was 0.949.

Conclusion: The FFQ adapted to the Turkish population and tested here was a good predictor for dietary intake of total antioxidant status.

1. Introduction

Antioxidants are defined as molecules that reduce or prevent physiologic damage caused by oxidative stress and free radicals [1]. Antioxidants have an inhibitory effect on free radical formation and prevent lipid oxidation. They act as reducing agents for neutralizing peroxides. Enzymatic antioxidants work synergistically with non-enzymatic antioxidants to increase synthesis [2].

The concept of total antioxidant status (TAS) was introduced to consider the cumulative antioxidant capacity of all the antioxidants present in foods or body fluids [3]. The main advantage of TAS testing is that it provides information about the total antioxidant status in biologic samples. Also, it allows the investigation of individual antioxidants [4]. It is stated in the literature that studies on plasma TAS levels should be supported by diet TAS levels [5,6]. The determination of dietary TAS levels is performed through surveys of the frequency of food consumption and 24-h recalls. In some studies, only the relationship between food consumption frequency and plasma TAS levels was investigated [7–10], whereas, in other studies, the relationship between both food consumption frequency and food consumption records and plasma TAS levels was investigated [11–13]. It has been stated that food consumption records reflect plasma TAS levels more accurately because food consumption frequency surveys do not generally cover all foods [14,15].

There are several ways to assess dietary intake. Food frequency questionnaire (FFQ) are a method for assessing the frequency of consumption of specific food items, reflecting eating habits over at least 1 month [16]. They are used most commonly in epidemiologic studies because they are inexpensive, do not require trained personnel, have a lesser burden for respondents, and are easier to

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use. FFQs can be designed both for general food consumption or for assessing the intake of specific foods and nutrients [17]. Validation of FFQs is generally performed through comparisons with the standard methods such as biochemical parameters or other methods by assessing food consumption [18].

The present study aimed to assess the capacity of a previously reported FFQ by Satia et al. [19], which was adapted to the Turkish population, for assessing the total antioxidant intake in a cohort of healthy adults from Turkey.

2. Materials and method

This study sought to characterize the validity and reliability of the Food Frequency Questionnaire for Total Antioxidant Intake, which was developed by Satia et al. [19], in the Turkish population. Permission was obtained from the relevant author via e-mail, and a research protocol was established.

The cross-sectional study was conducted at Sağlık Bilimleri University Faculty of Medicine Allergy and Immunology Department, with the participation of healthy subjects who worked in the hospital. Ethical approval was obtained from Marmara University School of Medicine Clinical Research Ethics Committee (protocol number: 09.2018.680). All participants were informed about the study and each was asked to sign an Informed Voluntary Consent Form. The study was conducted in accordance with the Helsinki Declaration Principles.

The G*Power 3.1.9.2 software program revealed that 42 participants were required to observe significant correlations ($p < 0.05$) for medium effect size (0.30) to achieve a statistical power of 0.80. The study was conducted with 45 healthy subjects without any chronic diseases, alcohol or cigarette use, supplement use, and they were neither pregnant nor breastfeeding mothers.

2.1. Assessment of dietary intake of total antioxidant from FFQ

For assessing dietary TAS value, an FFQ was adopted from Satia et al. and translated into Turkish [19]. The FFQ comprised frequency of food consumption (never, once per month, 2–3 times per month, 1–2 times per week, 3–4 times per week, 5–6 times per week, once per day, ≥2 times per day) and amount (small serving, medium serving, large serving) of 82 food items containing different types of antioxidants that were natural sources of carotenoids, vitamins A, C, and E (e.g. fruits and vegetables). Food that contains several vitamins such as fruits and vegetables is grown and eaten by people in Turkey, and these are good sources of antioxidants. To determine the exact serving size, the Food Atlas for Turkey was used [20].

The questionnaire was adopted and translated from English to Turkish by two independent translators with skill and fluency in speaking both English and Turkish. The final form of the questionnaire on which a consensus was reached was translated back into the Turkish language by the two translators simultaneously, regardless of the source of the original questionnaire. Finally, in an attempt to determine whether the final version of the questionnaire was appropriate and easily understandable, a pilot study was performed with 10 adults living in different districts of Istanbul, and foods that were never consumed in the last year were removed from the list. This questionnaire was administered through face-to-face interviews to 45 participants by the dietitian in the research group.

2.2. Determination of dietary TAS

The amounts of antioxidants of certain foods were derived from the BeBis Software and the database TAS values of foods from the study of Carlsen et al. [21]. Each participant's food consumption from the FFQ was entered into the BeBis Software [Ebispro for Windows, Stuttgart, Germany; Turkish Version (BeBiS 8.2)] and the software calculated the antioxidant content based on the software's database (Bundeslebensmittelschluessel; German Food Code and Nutrient Database; Version 3.01B [http://www.bfr.bund.de/cd/801]). Dietary antioxidant content also was calculated by multiplying the amount of a food item by its corresponding TAS value of unit weight from a food TAS database from the study of Carlsen et al. [21]. This dietary TAS database included multiple food items that were directly analyzed for TAS using an analytical method called the ferric reducing ability of plasma (FRAP). In this database, including more than 3100 foods, TAS values are represented for 100 g of individual foods taken from different countries (including Turkey). Both the BeBis software and the dietary TAS database interpreted nearly the same results.

2.3. Determination of TAS level in plasma

The validity of this questionnaire was evaluated by comparing the data obtained from the questionnaire with the levels of total antioxidant status in plasma. To determine TAS in the plasma of the participants, after 8 h of overnight fasting, venous blood collected from the antecubital fossa using 10 mL vacuum tubes containing lithium heparin, was centrifuged at 4400 rpm for 10 min in a cooled centrifuge and plasma fractions were separated into Eppendorf tubes. The Eppendorf tubes were stored in a −80 °C freezer until required for analysis. The TAS kit (SunRed Biological Technology-Human TAS ELISA Kit, Catalogue No: 201-12-7412, Shangai China) was investigated in the laboratory using a non-competitive (sandwich) enzyme-linked immunosorbent assay (ELISA) method.

2.4. Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) 20.0 (SPSS Inc., Chicago, IL, USA) software and the Medcalc Software 12.4 (Medcalc Software Corp., Brunswick, ME, USA). The Shapiro–Wilk test was used to evaluate the normality of data distribution. For descriptive statistics, mean, standard deviation, median, the 25th percentile and 75th percentile of plasma TAS, and FFQ TAS levels were shown. Spearman correlation was performed to determine the relationship between plasma TAS and FFQ TAS levels. The internal consistency of the scale was measured using Cronbach's alpha test. A Bland–Altman plot was obtained using the Medcalc Statistical Program. The level of statistical significance was accepted as $p < 0.05$. The classification into the same, adjacent, and opposite quartiles between plasma TAS and intakes of antioxidants from food calculated using the FFQ was evaluated with weighted kappa statistics.

2.5. Findings

There were 45 participants (19 males and 26 females) with a mean age of 45.9 ± 11.0 years. The majority of the participants (71.2%) had a higher education level (Table 1). The mean plasma TAS level was 13.8 ± 6.1 mg/L, and the mean intake of total antioxidants was 114.0 ± 134.6 mg/day (Table 2). There was a positive correlation between plasma TAS and intake of total antioxidants calculated using the FFQ ($r = 0.73$; $p < 0.001$) (Fig. 1).

The values of plasma TAS and intake of total antioxidants calculated using the FFQ were divided into quartiles; the weighted kappa value was calculated for evaluating compatibility between the two methods. The quartiles are shown in Table 3. The rate of classifying into the same or adjacent quartile was 98.7%. For the reliability analysis, Cronbach's alpha coefficient for the Turkish

Table 1
Demographic characteristics of participants (n = 45).

Characteristics	
Gender, n (%)	
Male	19 (42,2%)
Female	26 (57,8%)
Education Status, n (%)	
Below high school	13 (28,8%)
High school or above high school	32 (71,2%)
Marital Status, n(%)	
Married	29 (64,5%)
Single	16 (35,6%)

version of the FFQ for this sample was 0.949. The Bland–Altman plot was used to evaluate the compatibility between plasma TAS and the intake of total antioxidants calculated using the FFQ, indicating the regression line between the two methods of measurement; the correlation coefficient between the two methods was r = 0.73 (95% confidence interval), which can be evaluated as good agreement (Fig. 2).

3. Discussion

The study aimed to develop a biochemically validated dietary questionnaire to assess dietary antioxidant intake and its relationship to total antioxidant status in healthy subjects. There was a statistically significant correlation between dietary antioxidant intake and plasma TAS. This positive correlation shows that the FFQ can be evaluated as a biochemically validated questionnaire.

Plasma TAS levels provide information about the balance between pro-oxidative and anti-oxidative molecules. Especially in animal homeostasis, TAS values and malondialdehyde (MDA), which is the main product for lipid peroxidation, are good indicators for assessing antioxidant-oxidant balance [22]. TAS is generally low in patients with chronic diseases. It is an important marker in migraine [23], coronary artery diseases [24], hematologic diseases [25], irritable bowel disease [26], and asthma, and low levels are associated with oxidative stress and inflammation [27]. Thus, low values can either be the cause or the result of certain diseases. In particular, in autoimmune diseases (e.g. rheumatoid

Table 2
Descriptive Statistics for plasma TAS and antioxidant amount from FFQ.

	X ± SS n = 45	Median	25th percentile (Q1)	75th percentile (Q3)
Antioxidant amount from FFQ	13,8 ± 6,1	12,7	9,8	17,4
Plasma Total Antioxidant Status	114,0 ± 134,6	97,8	6,1	187,7

FFQ; Food Frequency Questionnaire.

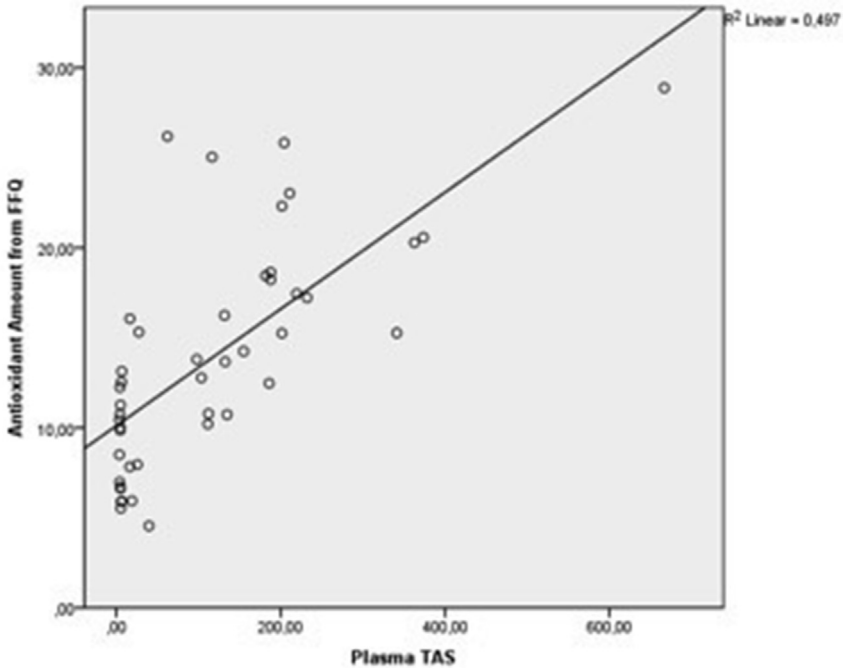

Fig. 1. Relationship between antioxidant amount from FFQ and plasma TAS. *, p < 0,05; †, Spearman correlation test; FFQ, Food Frequency Questionnaire; TAS, Total antioxidant status (r = 0.73).

Table 3
Average values of plasma total antioxidant status and intakes of total antioxidants calculated by FFQ were divided into quartiles, and weighted kappa values.

	Same quarter n (%)	Adjacent quarter n (%)	Opposite quarter n (%)	Weighted Kappa[a]	p
Antioxidant amount from FFQ – Plasma TAS	20 (44,44)	24 (53,33)	1 (2,22)	0,259	**0003***

*, p < 0,05.
[a] Weighted Kappa Analysis; FFQ, Food Frequency Questionnaire; TAS, Total antioxidant status.

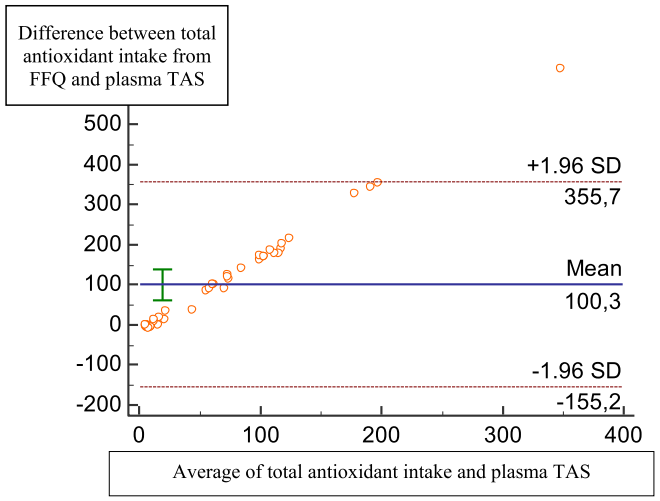

Fig. 2. Bland Altman plots comparing the difference and means of total antioxidant intakes and plasma TAS.

arthritis, multiple sclerosis, type I diabetes mellitus), low TAS levels with high oxidative stress and inflammation are a cause of dysfunction of the inflammatory cascade, which causes disease progression. There is a negative correlation between TAS and inflammation and disease severity; as TAS levels decrease, inflammation and disease severity increase [28]. On the other hand, in non-communicable diseases (e.g. type II diabetes mellitus, obesity, metabolic syndrome, hypertension, and coronary artery disease), TAS levels decrease as a result of increased glucose and lipid biomarkers [29,30].

TAS may be useful as an early marker of oxidative stress to monitor and optimize antioxidant therapy as an adjunct to medical therapy. Apart from diseases, assessing TAS is important for healthy individuals to prevent disease progression. Plasma TAS and dietary antioxidant intake reflect diet quality. As is known, better diet quality is related to increased quality of life, as well as decreased mortality. Consuming fruits, vegetables (except potatoes), nuts, legumes, whole grains, and seafood increases diet quality in parallel with TAS levels and decreases the prevalence of chronic diseases based on inflammation and oxidative stress. Thus, TAS may be a valid predictor of the early development and progression of chronic diseases and help prevent their clinical consequences in the healthy population [6,31–33].

In the validation studies of FFQs, the relationship between FFQs and dietary records is generally examined. The correlation coefficient varies between studies. In the validation study of an FFQ developed by Satia et al., the correlation coefficient between dietary antioxidant intake levels from the FFQ and the antioxidant amount from food record values was 0.06–0.56, and the dietary antioxidant intake levels from the FFQ values and plasma TAS values was 0.10–0.33 ($p < 0.05$, for both).

Rautiainen et al. conducted a biochemical validation study of the FFQ that they developed, performing different plasma TAS analyses such as oxygen radical absorption capacity (ORAC), total peroxyl radical trapping antioxidant parameter (TRAP), and FRAP. The Pearson correlation values between the antioxidant intake amount from the FFQ and TAS values from the plasma samples were $r = 0.35$, 0.31, and 0.28, respectively. According to the study, there was a correlation between antioxidant intake from the FFQ and plasma TAS levels as evaluated using different analysis methods [7].

Pellegrini et al. assessed 53 foods using an FFQ, a 3-day food record, and different analyses of plasma TAS levels. Plasma TAS levels were assessed using Trolox equivalent antioxidant capacity (TEAC), TRAP, and FRAP methods. The FFQ was associated with the 3-day food record (quadratic-weighted $\kappa = 0.49$ for TEAC, 0.53 for TRAP, and 0.49 for FRAP; $p < 0.001$). The Spearman correlations of FFQ repeatability were 0.66 for TEAC, 0.70 for TRAP, and 0.68 for FRAP ($p < 0.001$) [34].

Plasma TAS levels are related to the amount of antioxidants taken from food. In a study performed on healthy individuals by Limberaki et al., the pre and post plasma TAS levels of the participants were analyzed. The individuals consumed an antioxidant-rich diet for 30 days. It was observed that TAS values increased significantly in 70% of the 55 participants after 30 days. It was stated that individuals in the 30% segment did not follow their diets from the food consumption records. It was also emphasized that a diet with a combination of different antioxidants was proposed instead of a single antioxidant [12].

FFQs are simple tools for assessing required data. Our biochemically validated FFQ can be used for assessing TAS in healthy subjects. Other studies about the validation of FFQs for antioxidant intake levels also showed the feasibility of FFQs. However, validation studies are only suitable for their specific populations or countries. Accordingly, results can be different between studies due to nutritional habits and lifestyles. There is a need for further large studies for assessing TAS values.

One of the limitations of this study was the low number of participants. However, the number of participants could not be larger because this study was a biochemical validation. Another limitation was that all factors leading to the loss of total antioxidant levels could not be evaluated. Also, the TAS ELISA kit used in the study assessed TAS values from the plasma; however, antioxidants are also located within cells and the inability to measure within cells in this study was a further limitation. As a strength of the study, all nutritional stages of this research were performed by a research group consisting of dietitians to provide a more accurate assessment. To the best of our knowledge, this FFQ is the first questionnaire to be adapted and validated in our country to determine total antioxidant intake.

4. Conclusions

The findings indicate that this FFQ is validated with biochemical analysis from plasma and can be used as a valid tool to determine the total antioxidant intake of healthy individuals in Turkey.

Statement of authorship

Merve Öztağ: study conception and design, data collection, analysis and interpretation of results, draft manuscript preparation, critical review manuscript.

Fatma Esra Güneş: study conception and design, data collection, draft manuscript preparation, critical review manuscript, supervision of the study.

All authors reviewed the results and approved the final version of the manuscript.

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Declaration of competing interest

None of the authors has any conflict of interest to declare related to this manuscript.

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