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Dietary Total Antioxidant Capacity and Gastric Cancer: A Case-Control Study

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ABSTRACT

Background: Gastric cancer (GC) is the most common cancer among Iranian men. A diet rich in antioxidant compounds has been offered as an appropriate strategy for reducing the risk of GC. This study aimed to determine the possible association between dietary total antioxidant capacity (dTAC) and the Iranian population's GC risk. **Methods**: In total, 178 newly diagnosed GC patients and 238 healthy controls were recruited for this hospital-based case-control study. Dietary intakes were collected using a validated 146-item diet history questionnaire (DHQ), and dTAC was analyzed using Ferric reducing antioxidant potential (FRAP) assay. **Results**: The mean value of dTAC was 10.42±5.42 for cases and 11.42±5.86 for controls. Black tea, fruits, and cereals consumption were the main contributors to dTAC in both groups. DTAC was associated with a reduction in the risk of GC (OR 0.54; 95%CI: 0.31–0.92) (second vs. lowest tertile). This association did not change after adjustment for body mass index, education, energy intake, smoking, and *Helicobacter pylori* infection (0.55; 95% CI: 0.31-0.99). There was no significant association between fruits, vegetables, nuts, and legumes TAC with GC. **Conclusion**: The whole intake of dTAC from different food sources was not high in the population; however, this amount showed a preventive effect against GC.

Introduction

Based on the Global Cancer Observatory, Gastric cancer (GC) is the fifth most common cancer globally. The number of new cases was 1089, 103 (5.6%), and about 77,000 (8%) of cancer deaths were due to GC in 2020 (International Agency for Research on Cancer, 2020a, b). GC is the most common cause of cancer death in Iran, and 12,994 GC patients died in 2020. It is predicted that GC with breast and colorectal cancers will be the leading cancers nationally in 2025 (Roshandel *et al.*, 2021). Genetic alterations, *Helicobacter pylori (H. pylori)* infection, alcohol consumption, tobacco use, obesity, and dietary factors are the main risk factors for GC (McLean and El-Omar, 2014, Rawla and Barsouk, 2019). Based on a systematic

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review in Iran, *H. pylori*, smoking, poor level of economic situation, and food insecurity increased the risk of GC. Also, there was a direct relationship between the consumption of processed red meat, dairy products, smoked and salty fish, drinking hot tea, and salt consumption with the incidence of GC. An inverse relationship was seen between citrus, fresh fruit, and garlic consumption and risk of GC (Farmanfarma *et al.*, 2020).

The predictive effect of fruit and vegetable consumption against GC has been attributed to several antioxidants that act against oxidative stress and prevent cell damage (Metere and Giacomelli, 2017, Serafini *et al.*, 2002). Previous investigations have examined the preventive role of individual antioxidants concerning GC cell proliferation and differentiation (Chen *et al.*, 2002, Yang *et al.*, 2019). Dietary total antioxidant capacity (dTAC) is a valuable tool in nutritional epidemiological studies. Individual antioxidant components could not reflect the dTAC of the whole diet. This score considers antioxidants interactions or synergetic effects better than individual antioxidants (Nascimento-Souza *et al.*, 2018). Although the contribution of unique antioxidants to the risk of GC has previously been reported, limited data are available associating dTAC to the risk of GC (Praud *et al.*, 2015, Serafini *et al.*, 2012). Serafini *et al.*, in a prospective study conducted in 10 European countries, reported that consumption of dietary antioxidants from various sources of plant foods is associated with a reduction in the risk of GC (Serafini *et al.*, 2012). A case-control study by Praud in Italy has shown that non-enzymatic antioxidant capacity was inversely associated with GC (Praud *et al.*, 2015).

To the best of the authors' knowledge, the data in this field are limited to European countries. Dietary habits have unique characteristics in Asian countries, and environmental factors are different from those in other countries. This study examined the association between dTAC and the odds of GC in a case-control study in the Iranian population.

Materials and Methods

Participants and study design

This hospital-based case-control study was conducted at the Cancer Institute of Iran between 2010 and 2012. There were 178 histopathologically confirmed GC patients admitted to the Cancer Institute of Iran, from all parts of Iran. In order to reduce recall bias, incidence cases diagnosed with GC were selected less than one year before the recruitment without a previous diagnosis of any malignancies. Control samples were 238 apparently healthy caregivers or visitors of patients admitted to the same hospitals. Those participants who had a previous cancer diagnosis were excluded. The control group was selected based on the convenience sampling method from those who did not have long-term dietary restrictions. They were frequency-matched with cases by age (5-year categories), sex, and residential place (living in Tehran or referring from other cities). Twenty-four participants with unusual food intake (those with energy intake below the 3rd or above the 97th percentile) were excluded from the analysis. Ultimately, 166 cases and 226 controls were analyzed.

Assessment of dietary intake

Trained nutritionists interviewed participants about their food habits and intake. The Persian version of the Diet History Questionnaire (DHQ), validated previously, was used in this study (Toorang *et al.*, 2019). It includes 146 questions related to the consumption of food and Iranian mixed dishes. Control subjects were requested to report the frequency of food consumption during the past year, and case subjects were asked to recall their intake in the year preceding diagnosis.

The daily intake of all consumed food was calculated. Total energy intake was computed by summing up the energy content of all food. Energy intake was extracted using a Food Composition Table. Since the Iranian Food Composition Table includes only raw food and limited nutrients, The USDA food composition table was used to calculate the daily intakes of

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energy, macronutrients, and micronutrients (United States Department of Agriculture, 2018) and supplemented with the Iranian ones for some special Iranian foods (Azar M and E., 1980). This is a universal food composition table.

Assessment of dTAC

Based on the published papers, plant-based foods are the main contributors of TAC, specifically herbal and traditional plant medicine, spices, berries and their products, and nuts and seeds (Carlsen *et al.*, 2010). Dietary antioxidant capacity was calculated based on the effect of food intakes on ferric reducing antioxidant potential (FRAP). The FRAP measures the power of dietary antioxidants to reduce ferric ions to ferrous ions; it is suggested by an index to obtain the total effect of antioxidants of dietary components (Benzie and Strain, 1996). The FRAP value was considered for each food item using published data (Carlsen *et al.*, 2010). For food items whose TAC values were not found, the value of similar food was assumed as their FRAP value. TAC was computed for each person considering daily food intake multiplied by the corresponding FRAP value (in mmol/100g).

Covariate Assessment

Due to weight changes resulting from GC, it is not sensible to directly measure patients' weight during the interview. As an alternative, self-reported weight data from ten years before the interview was used for the analyses. For consistency, we applied the same method for the control group. Participants' body mass index (BMI) was calculated by dividing weight in kilograms by height in meters squared.

Sociodemographic, general information, and risk factors were collected through a structured questionnaire via a face-to-face interview. Self-reported smoking status was collected and classified into ever and never smoking. Venous blood (10 ml) was collected from all participants and *H. pylori* infection status was measured based on IgG antibody in serum samples.

Ethical consideration

All participants signed written informed consent and the Ethical Committee of Tehran University of Medical Sciences approved this study (Code: 17198). All methods were performed according to the Helenski guidelines.

Data analysis

Data analysis was conducted using Statistical Package for Social Sciences Software (SPSS) version 24 (SPSS Inc., Chicago, IL, USA). dTAC scores were adjusted for total energy intake using the residual method (Willett *et al.*, 1997). Then participants were categorized based on tertiles of dTAC. One-way ANOVA or t-test and $\chi 2$ test were used to compare continuous and categorical variables, across tertiles of dTAC. To examine the association between dTAC and the odds of GC, multivariable logistic regression analysis was used, in which several covariates were controlled. The initial model was adjusted for age and sex. Subsequent adjustments included BMI, educational level, smoking status, and energy intake in the second model. The trend of odds ratios (ORs) was assessed by treating the tertiles of each pattern as a continuous variable in logistic regression models. Statistical significance was defined as P-values < 0.05.

Results

The sociodemographic, anthropometric, and lifestyle-related characteristics of participants in case and control subjects are presented in **Table 1**. Patients with GC were slightly older and less educated. **Table 2** shows participants' general characteristics across tertiles of dTAC. Participants in the highest quartile of dTAC were younger. No other significant differences were seen in other variables across categories of dTAC. The mean value of dTAC was 10.42±5.42 for case subjects and 11.42±5.86 for control group. It should be noted that the interaction between smoking and dTAC was assessed; however, there was no significant interaction effect. The contribution of food groups to overall FRAP intake in each group is shown in **Table 3**. The majority of dTAC in both groups originates

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from beverages. Black tea was the most popular beverage in the present study, and the amount of green tea and coffee was low. Therefore, black tea had the highest contribution to dTAC, followed by fruits, cereals, and vegetables. Nobody reported consumption of alcoholic beverages.

Table 1. Characteristics of stomach cancer patients and visiting controls participated in the case-control study conducted in the Cancer Institute of Iran in 2010-2012s.

Variable	**Case** (n=166)	**Control** (n=226)	**P-value**[a]
Age (years)	61.27±11.93[b]	51.05±11.50	<0.0001
Body mass index (kg/m^2)	26.25±4.45	25.48±5.31	0.135
Gender (male)	122 (46.6)[c]	140 (53.4)	0.011
Education (illiterate)	106 (63.5)	61 (36.5)	<0.0001
Smoking (yes)	72 (50.3)	71 (49.7)	0.025
Helicobacter pylori (positive)	63 (32.8)	129 (67.2)	<0.0001

[a]: For quantitative variables result from the independent t-test and for qualitative variables result from the Chi-square test; [b]: Mean±SD; [c]: n (%).

Table 2. General characteristic of participants across tertiles of dTAC in a case-control study of GC in Iran.

Variable	**Tertiles of dTAC**			**P-value**[a]
	T1 (n= 128) (<8.46)	**T2 (n= 129) (8.46-11.53)**	**T3 (n= 128) (>11.53)**	
Age (years)	57.41±13.19[b]	55.19±12.03	53.54±12.70	0.048
Body mass index (kg/m^2)	26.09±5.68	25.48±4.54	25.85±4.66	0.617
Gender (male)	91 (34.7)[c]	91 (34.7)	80 (30.5)	0.227
Education (illiterate)	61 (36.5)	56 (33.5)	50 (29.9)	0.891
Smoking (yes)	49 (34.3)	45 (31.5)	49 (34.3)	0.958
Helicobacter pylori (positive)	61 (31.8)	62 (32.3)	69 (35.9)	0.766

*[a]: For quantitative variables result from the independent t-test and for qualitative variables result from the Chi-square test; [b]: Mean±SD; [c]: n (%; **dTAC**: dietary total antioxidant capacity.*

Table 3. Contribution of food groups to overall FRAP intake (%) in all participants stratified by group.

Group	**Fruits**	**Vegetables**	**Nuts**	**Legumes**	**Oils**	**Dairy**	**Grains and Cereals**	**Meats**	**Beverages**[a]
Case n=166	29.1	7.2	1.3	1.2	0.9	5.6	11.6	0.4	43.3
Control n=226	30.1	6.5	1.3	1.1	0.7	4.5	9.0	0.4	46.4

*[a]: Include black tea, green tea, cola, coffee; **FRAP**: Ferric reducing antioxidant potential..*

ORs and 95% CIs for GC across tertiles of dTAC are presented in **Table 4**. dTAC was significantly and inversely associated with GC risk in the unadjusted model. This association remained significant when several potential confounders were further controlled and individuals in the second tertile of dTAC were 0.45 times less likely to have GC than those in the lowest tertile (OR: 0.55; 95% CI: 0·31-0·99). No significant association was seen between scores of vegetable dTAC, nuts dTAC, and odds of GC. Participants in the highest tertile of fruit dTAC had a lower risk of GC; however, it was not statistically significant.

Discussion

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The present study reported that total dietary antioxidant intake is associated with a lower risk of GC. Scores of dTAC from fruits, vegetables, and nuts were not associated with the risk of GC in the adjusted models; however, it was near to significant for fruits. Beverages were the main contributor to dTAC in the diet.

dTAC score considers the antioxidant capacity of the whole diet and is a new approach in the study of diet and chronic diseases. Reactive oxygen species (ROS) production induced oxidative stress which could cause DNA damage, gene mutation, and consequently carcinogenesis (Zamani *et al.*, 2019). Dietary antioxidants can detoxify ROS (Halliwell *et al.*, 2000), and reduce the risk of DNA damage. Therefore, they protect against different cancers, including GC (Foksinski *et al.*, 2007). Based on this fact, several studies have investigated the association between antioxidant-rich food intake and the risk of cancers.

Table 4. Odds Ratio and 95% confidence interval of GC across tertiles of dTAC in a case-control study in Iran.

Variables	T1 N=128	T2 N=129	T3 N=128	P-trend
dTAC				
Score	(<8.46)	(8.46-11.53)	(>11.53)	
Model A	reference	0.51 (0.31-0.83)	0.52 (0.32-0.86)	0.011
Model B	reference	0.55 (0.31-0.99)	0.59 (0.33-1.07)	0.081
Vegetable TAC				
Score	(<0.49)	(0.49-0.73)	(>0.73)	
Model A	reference	0.84 (0.51-1.38)	1.08 (0.66-1.76)	0.74
Model B	reference	0.93 (0.51-1.72)	1.09 (0.60-2.01)	0.76
Fruit TAC				
Score	(<2.24)	(2.24-3.58)	(>3.58)	
Model A	reference	0.61 (0.37-1.01)	0.57 (0.35-0.94)	0.03
Model B	reference	0.67 (0.35-1.28)	0.55 (0.30-1.03)	0.06
Nuts TAC				
Score	(<0.05)	(0.05-0.12)	(>0.12)	
Model A	reference	0.71 (0.43-1.17)	0.84 (0.51-1.37)	0.49
Model B	reference	0.87 (0.44-1.72)	0.97 (0.51-1.81)	0.96
Coffee tea TAC				
Score				
Model A	reference	1.00 (0.60-1.65)	0.73 (0.44-1.22)	0.24
Model B	reference	0.79 (0.42-1.47)	0.74 (0.39-1.38)	0.34

***Model A**: Adjusted for age and sex; **Model B**: Adjusted for age, sex, body mass index, education, energy intake, smoking, Helicobacter pylori; **TAC**: Total antioxidant capacity.*

The results are compatible with Serafini (Serafini *et al.*, 2012) and Praud (Praud *et al.*, 2015), who reported the predictive effect of dTAC based on FRAP against GC. Serafini considered only TAC from plant food (Serafini *et al.*, 2012). However, the present study considered all food groups, since the consumption of fruits and vegetables in Iran is not high, and other food groups like grains, cereals, and pulses are more consumed. Iranian people use cereals more than populations investigated in Serafini (Serafini *et al.*, 2012) and Praud (Praud *et al.*, 2015) studies. Moreover, the contribution of vegetables in dTAC was more minor than these two studies. The low amount of dTAC in each group might not be strong to have a significant effect on the cancer risk and further studies with larger sample sizes are required. Black tea is reported as the most common beverage in Iran (Wikipedia, 2016) and in line with the study by Serafini, tea was the most significant contributor to dTAC in the present study population. However, the consumption of coffee and green tea was rare. It

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should be considered that the temperature of tea is important. Some studies have shown the negative effect of high temperature of tea on GC (Deandrea *et al.*, 2010, Wang *et al.*, 2015); so, people should drink low-temperature tea.

Although there is a strong association between *H. pylori* infection, and GC, the prevalence of *H. pylori* infection was lower in GC patients compared to the control group. Such findings have been previously reported in other case-control studies (Helicobacter Cancer Collaborative Group, 2001). This could be attributed to different misclassifications in such study designs. *H. pylori* infection has been assessed by IgG antibody, which might be eradicated during gastric atrophy and the development of GC (Ekström *et al.*, 2001). Also, patients with GC might have received *anti H. pylori* treatments, and their infection had been removed before the diagnosis of carcinoma (Ekström *et al.*, 2001).

There are several strengths in the current study. The study used a valid dish-based questionnaire. Although a hospital-based study was used, healthy visitors were recruited as control participants. Since the patients in the control group may change dietary habits due to their disease condition, healthy visitors appropriately represented the exposure distribution of the reference populations that generated the GC cases. Moreover, this study considered the dTAC of the whole diet rather than single antioxidants and controlled for a wide range of confounders.

Our study had some limitations. Selection and recall bias are inherent in case-control studies, which can deter casual interpretation. Moreover, recent dietary intakes might affect the diet reports in the previous year. Patients were asked to report their dietary intake prior to being diagnosed with cancer. The frequency matching method was used to match the case and control groups for age; however, the mean age was lower in the control group compared to the patients. Younger patients were more selected for the control group; as a result, there was a significant difference between the two groups. Age was included in all regression models to decrease concerns about confounding effects of this variable. The consumption of dietary supplements which may contribute to the overall FRAP score was asked; however, the consumption was low, so it was not considered in the analysis. In the DHQ, the frequency of spices consumption was not measured, so in the calculation of dTAC, the FRAP of spices was not considered. FRAP assay was applied to compute dTAC, while this method might underestimate the accurate antioxidant capacity of the diet due to not considering lipophilic antioxidants. In addition, FRAP measures in vitro antioxidant activity, and it might not truly represent in vivo antioxidant activity, since the bioavailability of antioxidants is highly variable (Benzie and Strain, 1996). Some food items have not published FRAP values, so we used similar ones to compute the TAC score, which might affect the findings. Finally, we did not have data about *H. pylori* treatment drugs.

Conclusion

The whole intake of dTAC from different food sources is not high in the Iranian population; however, this amount showed a preventive effect against GC. Further studies assessing biomarkers of dTAC and oxidative stress in biological fluids can provide better interpretation. Encouraging people to consume fruits, vegetables, and tea at moderate temperatures is recommended.

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Authors' contributions

S. Narmcheshm and F. Toorang designed the study. S. Narmcheshm analyzed the data under the supervision of F. Toorang and wrote the paper. A. Dorosty-Motlagh, F. Toorang, and B. Sasanfar contributed to data analysis. M Hadji contributed to the design of the study and data collection. K. Zendehdel and A. Dorosty-Motlagh supervised the project. All authors contributed to the interpretation of results and approved the final version of the manuscript.

Conflict of interests

Authors have no potential conflicts of interest.

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