Antioxidant Capacity of Plasma after Pomegranate Intake in Human Volunteers

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Abstract- Dietary antioxidants including phenolic compounds are believed to be effective nutrients in the prevention of oxidative stress related disease. Pomegranate has been used for centuries in ancient cultures for its medicinal purpose and is widely acknowledged for antioxidant properties. The present study was designed to assess the effect of pomegranate fresh fruit consumption on the plasma antioxidant capacity. Thirty healthy volunteers were recruited for the study. Volunteers were randomly divided into three groups (pomegranate, vitamin E and water consumption). Blood samples were collected, after at least 12 hours overnight fast, the day before beginning supplementation period and the day after supplementation had finished. Total antioxidant capacity measurement by FRAP method and clinical laboratory test were performed for all volunteers in two selected times. The obtained data revealed that consumption of 100 grams pomegranate and vitamin E per day for ten days resulted in a significant rise (14.05%, 8.28%) plasma antioxidant capacity respectively, but this difference was not significant for water group.

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Key words: Pomegranate, antioxidant, plasma; healthy volunteers

Introduction

Clinical trials and epidemiological studies have established an inverse correlation between intake of fruits and vegetables and the occurrence of disease such as inflammation, cardiovascular disease, cancer and aging-related disorders (1-5). Dietary antioxidants including phenolic compounds, vitamin E and C, carotinoids, are believed to be effective nutrients in the prevention of oxidative stress related disease (6,7). Antioxidants have become a topic of increasing interest recently. A literature search revealed that the number of publication antioxidants and oxidative stress has nearly quadrupled in the past decade (8). It is of great interest to the general public, medical and nutritional expert, and health and food science researchers to know the antioxidant capacity and constituents in the foods we consume. Dietary antioxidant often broadly includes radical chain inhibitors, metal chelators, oxidative enzyme inhibitors, and antioxidant enzyme cofactors (9). Many studied have reported increased in antioxidant capacity of plasma following consumption of antioxidant rich fruits, vegetables, tea, wines and grape juices (10-12). This effect has been ascribed to the low molecular weight phenolics in many plants based food which can act as antioxidants because their extensive conjugation π-electron system allow ready donation of electrons or hydrogen from the hydroxyl moieties to free radical (13). The complete evaluation of antioxidant activity of given food or beverage needs to consider its influence on certain biomarkers before and after intake. Plasma antioxidant capacity has been proposed as a good measure of antioxidant status and encompasses the action of antioxidant compounds from diet and other endogenous compounds that scavenge radicals in the living system. Pomegranate tree (Punica granatum L) originated in the Middle East and India and has been used for centuries in ancient cultures for its medicinal purpose. There are reports of the use of pomegranate as antiviral (14), antimicrobial (15) and anticancer agent (16,17), but it is widely acknowledged for antioxidant properties, which are higher than most other fruit- related food items that were originally thought to contain the highest amounts of antioxidants (18-21). This means that this incredible fruit can be used by patients who wish for a safe, but no literature was found reporting the antioxidant activity of plasma after

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consuming pomegranate fresh fruit in healthy volunteers. In the present study, the ferric reducing\antioxidant power assay (FRAP assay) was employed (22) and the effect of pomegranate fresh fruit consumption on the plasma antioxidant capacity of healthy volunteers was assessed.

Patients and Methods

Materials

TPTZ (2, 4, 6 tripyridyl S triazine) was provided from Fluka and all other solvents/ chemicals used were of analytical grade and obtained from Merck Company (Darmstadt, Germany). Double-distilled deionized water was used for the preparation of aqueous solutions.

Sample collection

Thirty healthy volunteers male and non-pregnant / non-lactating female (BMI= 24.35±3.74, age= 35.27± 9.95) were recruited for study. The subjects had healthy life habits; they were nonsmokers and nonalcoholic drinkers. They had not to consume drugs or antibiotics known to interfere with intestinal absorption or the P_{450} Enzymatic system. Blood samples were collected, after at least 12 hour overnight fast, the day before beginning supplementation period and the day after supplementation had finished. Clinical laboratory test have done for all volunteers (CBC, ESR, FBS, Urea, Creatinin, Uric acid, Cholestrol, Triglycerids, HDL, LDL, VLDL, Cholestrol/HDL, Cholestrol/LDL, LDL/HDL, Total bilirubin, Direct bilirubin, Alkalin Phosphatas, ALT, AST). Ethical approval for the study was obtained from Tehran University of Medical Sciense (TUMS). All subjects were informed and singed a written consent prior taking part in the study. Subjects were instructed to avoid consumption of dietary antioxidants three days before the study according to a detailed list they were given. Volunteers were randomly divided into three groups.

For the first group (group A) the pomegranate fresh fruit (100gr/day for ten days) was added to their diet. The subjects in the second group (group B) consumed vitamin E (400units/days). For the third group (group C), the subjects have not to intake vitamin E and pomegranate and other antioxidant compounds for ten days. This group consumed tap water. The pomegranate consumed was from the same cultivar and selected between ten types of pomegranate according to its highest antioxidant activity (23).

Blood sample were collected into evacuated tubes containing heparin as an anticoagulant agent and were immediately centrifuged at 4500 g for 10 minutes, avoiding unnecessary exposure to light. Then the plasma samples were frozen in the liquid nitrogen and stored at -70°c until analysis

FRAP assay

The procedure described by Benzie and Strain was followed (22). The principle of this method is based on the reduction of a ferric-tripyridyl triazine complex to it's ferrous colored form in the presence of antioxidants. Briefly, the FRAP reagent contained 5 ml of a (10 mmol /L) TPTZ (2, 4, 6- tripyridyl- S- triazine) solution in 40 mmol /L HCl plus 5 ml of (20 mmol/L) $FeCl_3$ and 50 ml of (0.3 mol/L) Acetate buffer, PH 3.6 and was prepared freshly and warmed at 37˚C. Aliquots of 50 µl sample supernatant were mixed with 1.5 ml FRAP reagent and the absorbance of reaction mixture at 593 nm was measured spectrophotometrically after incubation at 37˚C for 10 min. Standard solution of 0.25 mM ascorbic acid were prepared. For construction of calibration curve five concentrations of $FeSO_4$ $7H_2O$ (1000, 750, 500, 250, 125 µmol /L) were used and the absorbencies were measured as sample solution. The values were expressed as the concentration of antioxidants having a ferric reducing ability equivalent to that of 1 mmol/L $FeSO_4$. Adequate dilution was needed if the FRAP value measured was over the linear range of standard curve.

Imprecision

The within-run coefficient of variation (C.V.%) was estimated by assaying the FRAP value of each $FeSO_4$ standard and plasma samples three times in the same analytical run.

The between run C.V. was obtained by estimating three replicates of each FRAP value in a further analytical run. The results (2.15% within-day and 5.18% for between-days) indicate a good reproducibility in the method.

Statistical analysis

Results are expressed as mean ± SEM. Data obtained before and after supplementation were compared by using Student's t test. Repeated measures analysis of the variance (ANOVA) was used to evaluate changes in plasma antioxidant parameters after consumption of pomegranate, vitamin E or water. When a significant (P<0.05) difference was detected, means were compared using the post-hoc Newman-kevls' test.

The relationships between variables were assessed by linear regression analyses (P<0.005). These statistical comparisons were performed using the SPSS version 11.5 program.

Results

Methodological consideration

Different analytical methods have been applied to assess antioxidant capacity of biological fluids (24). This study was designed to determine the effect of pomegranate consumption on antioxidant capacity of plasma. For this purpose the total antioxidant capacity of the plasma (TAOC) was selected because this method can be used for evaluating the effect of different treatments on plasma redox status when the results are expressed as change with respect to basal value. The advantage and limitation of this method are described in critical review (25). In this study the FRAP method was selected to assess TOAC, because it treats the antioxidant contained in the sample as reductants in a redox-linked colorimetric reaction and the value reflects the reducing power of the antioxidants. The procedure is relatively simple and easy to standardize, thus, it has been used frequently in the assessment of antioxidant activity of various fruits and vegetables and some biological samples (22).

Some authors use plasma (24), while others use serum (26) but values obtained for antioxidant capacity of plasma are usually higher than those determined in serum. Serum is obtained after collating blood at room temperature. During aggregation, platelets release radical oxygen species (ROS) and this could be a possible explanation of the lower values of serum with regard to plasma (25), therefore plasma samples are usually employed for antioxidant assay in normal clinical practice. Moreover, in our study, endogenous antioxidants such as uric acid and bilirubin (total and direct) have been determined.

Anthropometric and clinical characteristics of volunteers in each group are shown in Table 1 and 2 respectively. Analysis of variance (ANOVA) revealed that there is no significant difference in the anthropometric and clinical laboratory test of the volunteers in each group before supplementation.

Plasma antioxidant capacity

Figure 1 (a, b, c) includes mean value and standard deviation of the triplicate measurements for every sample in each group. The mean plasma antioxidant capacities of each group with FRAP assay before and after supplementation was also shown in Fig 2. Plasma samples were collected at two different times (time 0 or base line value and time 1 or ten days after supplementation). Mean values for the whole volunteers in each group taking part in the study were calculated. Pomegranate was chosen between a varied of fruits according to its highest value for antioxidant activity (27). All pomegranate consumed by group A volunteers were from same cultivar and the mean FRAP value was 1.439±0.049 (23). FRAP value was increased 8.28% in group B who consumed vitamin E and 14.05% in group A which consumed pomegranate. For the evaluation of vitamin E and pomegranate consumption on plasma antioxidant capacity the paired sample t- test was applied in each group before and after supplementation and the analysis of variance (ANOVA) was also used for comparison between three groups.

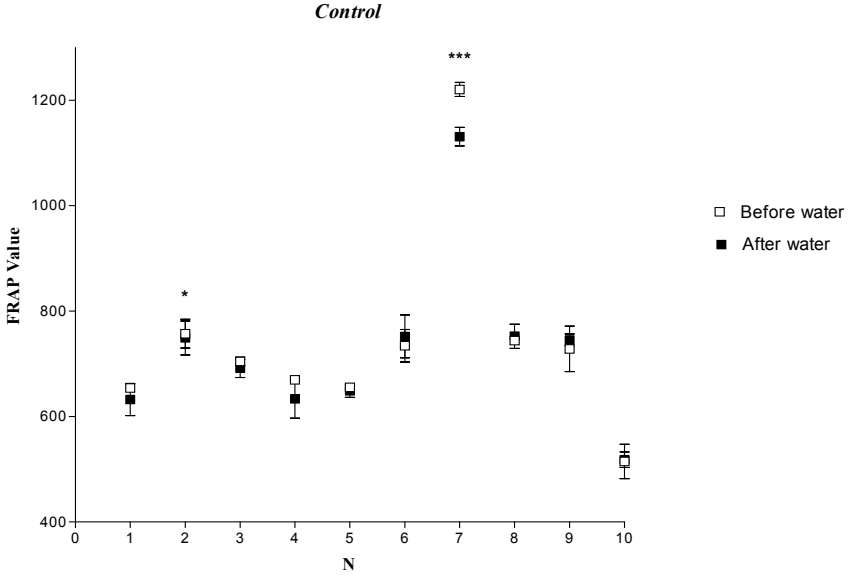

Figure 1a. Comparison of mean FRAP value in each volunteers consuming water

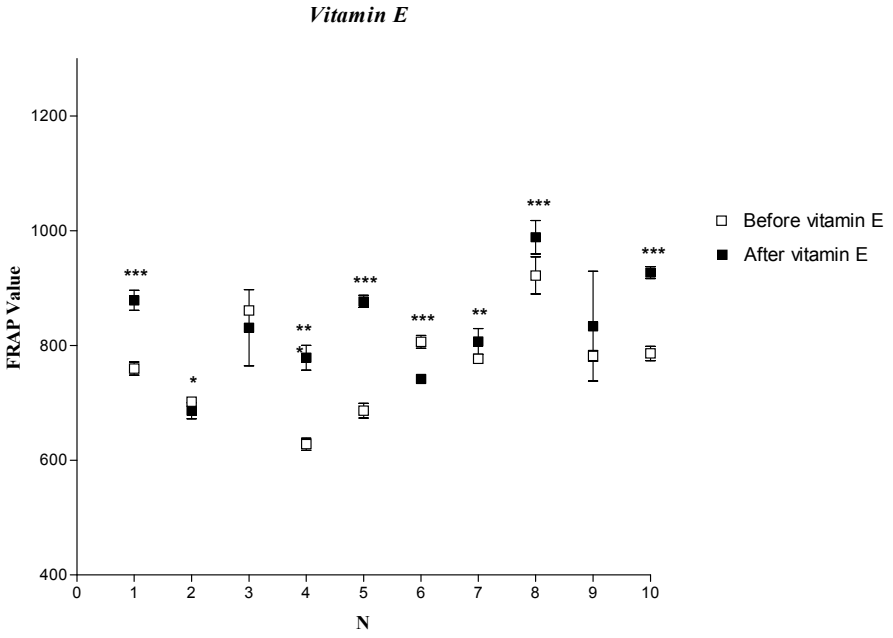

Figure 1b. Comparison of mean FRAP value in each volunteers consuming vitamin E

Paired sample t-test revealed that antioxidant value determined before vitamin E and pomegranate intake was statistically different from those determined after ingestion ($P<0.05$), but this difference was not significant in group C who consumed water.

In addition in this study some of the clinical parameters were investigated in whole subjects in each group (Table 2). The results revealed that drinking water for ten day period caused some risk factors of cardiovascular disease decrease.

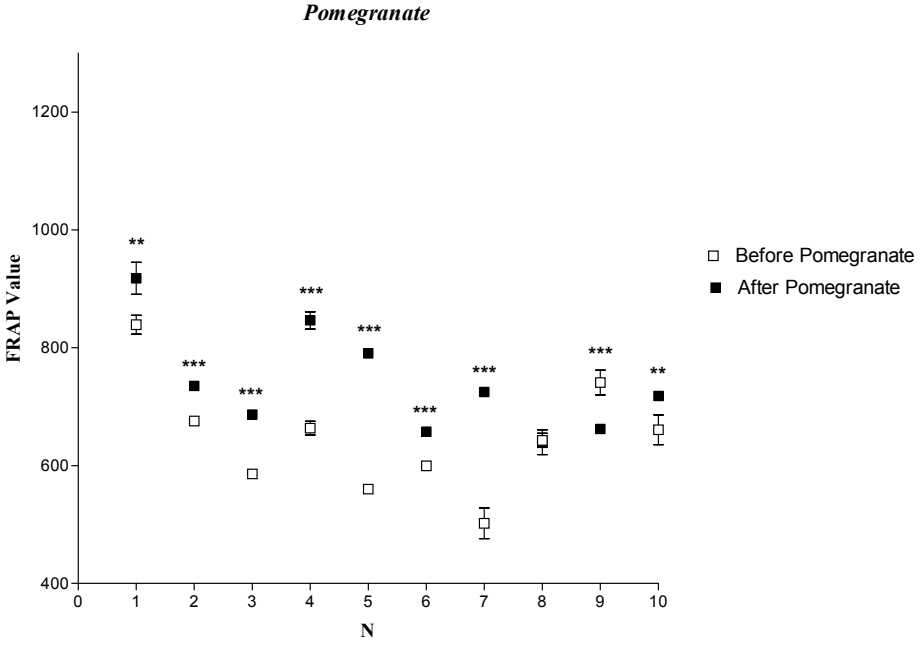

Figure 1c. Comparison of mean FRAP value in each volunteers consuming pomegranate

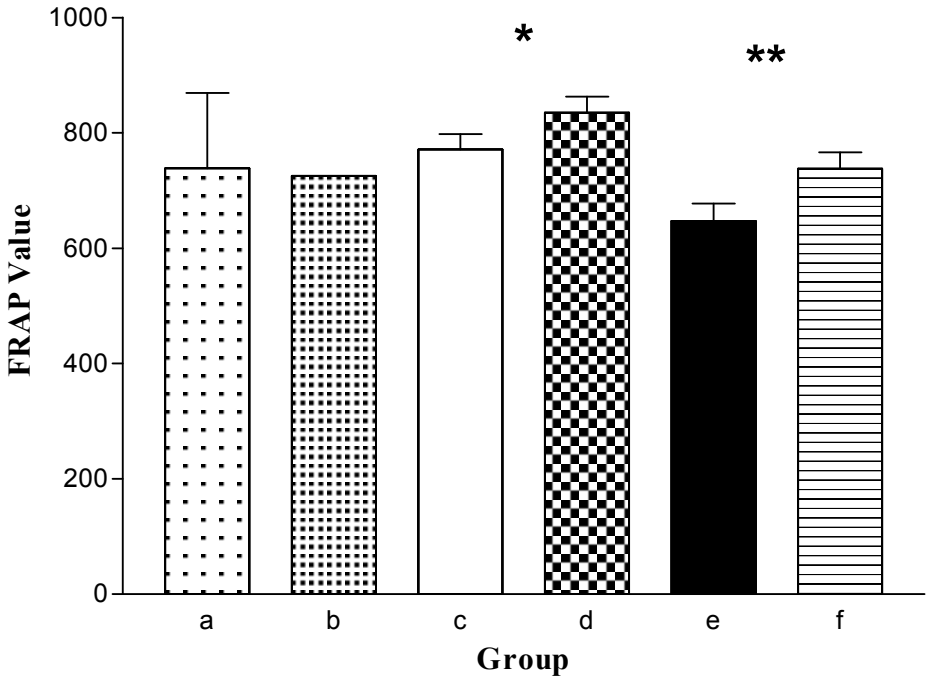

Figure 2. Comparison of FRAP value in each group before and after supplementation
a: Before water consumption
b: After water consumption
c: Before vitamin E consumption
d: After vitamin E consumption
e: Before pomegranate consumption
f: After pomegranate consumption
* $P<0.05$ ** $P<0.01$

These factors include Cholesterol (P< 0.01), LDL (P<0.05), Cholesterol/HDL (P<0.05) and LDL/HDL (P<0.001). The significant differences in clinical laboratory parameters in group B were not observed but, the differences in bilirubin were significant in group C (P<0.01).

The obtained data revealed that consumption of 100 grams pomegranate per day for ten days resulted in a significant rise (14.05%) plasma antioxidant capacity in vivo. Previous study demonstrated a maximum peak increase (31.6%) 30 minutes after consumption of pomegranate extract in plasma antioxidant activity (28). In this paper subjects were allowed to consume pomegranate for ten days and the TAOC was determined after an overnight fast in tenth day, therefore, result indicate an average effect of pomegranate consumption instead of maximum peak. Several recent studies have assessed the relationship between different fruits or vegetables consumption and oxidative stress (29-35).

Table 1. Anthropometric characteristics of the volunteers

Variable	Group A	Group B	Group C
Age	38.8±6.67	39.1±6.22	26.6±4.42
Weight	65.3±11.74	66.9±9.17	62.1±9.10
BMI	24.420±3.50	25.68±2.79	23.88±3.51
High	163.2±7.43	161.4±9.27	161.4±9.85

Table 2. Comparison of clinical parameters in each group before and after supplementation

Clinical	Group A		Group B		Group C	
parameters	Before	After	Before	After	Before	After
ESR	8.0±4.3	13.4±10.4	12.3±5.35	12.1±7.5	17.5±14.09	15.6±11.31
FBS	82.0±7.85	82.8±8.25	89.0±10.56	87.5±13.9	90.50±7.56	90.0±8.04
Urea	22.2±4.89	21.4±6.43	22.9±6.33	20.3±5.88	20.1±7.38	17.0±7.41
Uric acid	0.81±0.72	0.79±0.71	4.06±0.61	3.82±1.00	4.23±1.78	4.07±1.28
Creatinin	4.02±0.05	3.83±0.11	0.82±0.11	0.80±0.11	0.82±0.20	0.83±0.20
Bilirubin	0.82±0.21	0.63±0.25	0.86±0.41	0.75±0.28	0.59±0.12	0.78±0.27
TG	94.8±28.56	111.5±40.38	119.0±59.86	109.1±39.52	116.4±40.71	118.4±59.58
Cholestrol	155.8±57.25	186.3±28.73	202.1±30.38	195.7±34.48	216.2±58.87	202.5±57.95
LDL	90.7±14.75	96.0±20.22	103.7±18.31	108.0±24.51	122.6±39.67	113.4±40.09
HDL	48.50±10.33	49.0±11.96	52.50±9.30	50.2±7.87	50.6±15.71	47.0±7.48
Chol/ HDL	3.75±0.72	3.93±0.83	3.92±0.75	3.95±0.71	4.56±1.10	4.24±1.00
LDL/HDL	1.92±0.50	2.02±0.50	2.02±0.50	2.18±0.53	2.57±0.74	2.39±0.71

Data are expressed as mean ± SD

Among them the Tyssandier work (35) was only the paper which evaluates the mean plasma effect of tomato consumption instead of maximum peak. The results showed that the tomato puree supplementation did not significantly affect the mean plasma TAOC. Only a limited number of studies have looked at in vivo antioxidant activity of pomegranate consumption in human (29) and the results showed that a more comprehensive study including a placebo group is required to confine the antioxidant effects of pomegranate. In the present study to find more acceptable result the two other groups were included and the obtained data clearly demonstrate that consumption of pomegranate can induce a significant rise in plasma antioxidant activity comparison with water and vitamin E. We did not find any correlation between uric acid and bilirubin content of plasma samples with TAOC. Further work was needed to identify which of the antioxidant micro constituents present in pomegranate juice are mainly involved in this effect.

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