International Journal of Medical and Health Research
ISSN: 2454-9142
Received: 20-08-2018; Accepted: 24-09-2018
www.medicalsciencejournal.com
Volume 4; Issue 10; October 2018; Page No. 168-172

Association between plant-based dietary antioxidant intake and the oxidative stress of healthy adults in the health unit area of Bope-Poddala, Sri Lanka

DASM Nishadhi[1], AP Attanayake[2*], CJ Wijesinghe[3]
[1] Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences, University of Ruhuna, Galle, Sri Lanka
[2] Department of Biochemistry, Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka
[3] Department of Community Medicine, Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka

Abstract
Background: Regular consumption of antioxidant rich diet is associated with a myriad of health benefits. At present there are no consistent perspectives about plant-based antioxidant rich diet being associated with a change of serum antioxidants status/oxidative stress in Sri Lankan healthy adults.
Objective: The present study aimed to assess the association between plant-based dietary antioxidant intake and the oxidative stress in a group of healthy adults (n=80) in Bope-Poddala health unit area, Galle, Sri Lanka.
Method: The concentration of serum malondialdehyde (MDA) was estimated as a marker of lipid peroxidation and oxidative stress. The information on the plant based antioxidant rich food intake of the participants of the study were obtained using a pre tested food frequency questionnaire. Using the questionnaire, amount of each food consumed over a month was determined based on intake of individual food types. The consumption of vitamin E, vitamin C and carotene levels were calculated using food composition tables. The average daily intake of vitamin C, vitamin E and carotene content in selected food were considered in assessing dietary antioxidant intake.
Results: The serum malondialdehyde concentration was in the range of 0.012-0.360 nmol/mg in the study sample. There was a weak, non-significant negative correlation (-0.156) between daily antioxidant intake and malondialdehyde concentration/lipid peroxidation (p=0.166).
Conclusion: The results failed to reveal an association between plant-based dietary antioxidant intake and the oxidative stress in healthy adults in health unit area of Bope-Poddala, Sri Lanka.

Keywords: antioxidant status, malondialdehyde, plants based dietary antioxidant intake

1. Introduction
A common character in the occurrence and progression of most of the chronic diseases is the involvement of oxidative stress, caused by free radical species [1, 2, 3]. In addition to the diverse roles of free radicals at the cellular level, the increased reactive free radical species damage cellular structures and alter biochemical reactions. In response to such damage, a complex antioxidant defense is established and antioxidants comprise an important role in this defense [1].
The bottom-line importance of the antioxidant system is to neutralize the action of reactive substances. Antioxidants are defined as 'any substance that prevents or significantly constricts the oxidation of an oxidizable substrate, such as proteins, lipids, carbohydrates and deoxyribonucleic acid' [4, 5, 6]. The defense system is constituted of all manner of enzymatic and non-enzymatic components. The enzymatic molecules include glutathione peroxidase (EC 1.11.1.9), glutathione reductase (EC 1.8.1.7), catalase (EC 1.11.1.6) and superoxide dismutase (EC 1.15.1.1) etc. Non-enzymatic substances include endogenous antioxidants and exogenous antioxidants. Reduced glutathione, ubiquinone, uric acid, alpha lipoic acid, metallothionein, albumin, transferrin and ceruloplasmin act as endogenous antioxidants and exogenous antioxidants are mostly those ingested from food such as vitamin E (especially ∞-tocopherol), carotenoids, vitamin C, flavonoids, mannitol, aminoguanidine and pyridoxine. Thus, diet is an origin of nutrients that function as exogenous sources of antioxidants [7, 8].
Whole meal flours of barley, common millet and oats contain antioxidants in dispersion though the cereals. White flour from corn maize is composed most among the white flours, followed by barley, oats and common millet. Roots and tubers are variable in their content of antioxidants. Ginger, sweet potatoes and red beets contain a very high concentration of antioxidants. Furthermore, there is a large variation in antioxidant content of vegetables as red cabbage, pepper and spinach. The analysis of fruits demonstrated that pomegranate contains a very high concentration of antioxidants. Other fruits with high antioxidant content included grapes, oranges, plum, pineapple, lemon and grapefruits [9].
Sri Lanka is an agricultural country with a tropical climate, blessed with plenty of plant food such as fresh fruits, vegetables and green leaves. Exotic fruits, vegetables and edible green leaves leading to thrive on long-standing healthy food habits are rich in the land. Throughout years of colonization and influence from other countries, Sri Lanka has

adapted its native food culture into a blend of different curry concoctions and tasty dishes enriched with vegetables and spices. Generally typical Sri Lankan diet is rich with cereals, legumes, vegetables, fruits and spices. In addition, a diverse plethora of traditional beverages such as tea, coffee, herbal extracts etc. are widely used by Sri Lankans since antiquity. Doubtlessly, Sri Lankan diet accommodates high level of antioxidants rich plant based food and beverages. Although traditional Sri Lankan diet includes plenty of vegetables and rice, dietary behaviors of Sri Lankans have been altered drastically during the last few decades as a result of epidemiological, socio-cultural, demographic and economic transitions occurred in the country throughout the period. Studies also have shown that gender influences habits and their beliefs about food and health among young generations [10].

Regular consumption of antioxidant rich diet is associated with a myriad of health benefits. At present there are no consistent perspectives about plant-based antioxidant rich diet being associated with a change of serum antioxidants status/oxidative stress in Sri Lankan healthy adults. Considering the health benefits of intake of antioxidants rich food for a healthy life and for wellbeing with an aim of prevention/suppression of the risk of occurrence of chronic diseases, it is worth to assess the association between antioxidant rich food intake and serum oxidative stress level in healthy adults in a selected health unit area in Sri Lanka.

2. Materials and methods

2.1 Study design

The study was a descriptive cross sectional study.

2.2 Study setting

The study was carried out in Bope-Poddala health unit area, Galle district which is semi urban area in the southern province, Sri Lanka.

2.3 Study participants and sample size

The subjects included 80 healthy volunteers of either sex, aged between 20 – 40 years. Individuals with a history of any chronic disorder such as renal disease, diabetes mellitus, hepatic disease, hypertension, dyslipidemia, inflammatory disease, infection, malnutrition were excluded. Since smoking and alcohol consumption can influence the body's antioxidant levels, the smokers and alcoholics were also excluded from the study.

2.4 Study instruments and Data collection

The information on the plant-based antioxidant rich food intake of participants of the study were obtained using a pre-tested food frequency questionnaire. The amount of each food consumed over a month was determined based on intake of individual food types assessed using the questionnaire. The consumption of vitamin E, vitamin C and carotene levels were calculated using food composition table [12]. The average intake per day was calculated.

After eight hours fasting, venous blood samples were collected from the subjects by a trained research personnel or a trained nursing officer into a plain tube. The sample was allowed to clot at room temperature (27 ºC) without disturbance for 15 to 30 minutes. The sample was centrifuged for 5 -10 minutes at 2500 rpm. Then serum (supernatant) was separated by using a pasture pipette. The serum was used for the estimation of serum malondialdehyde concentration.

2.5 Chemicals and reagents

All chemicals and solvents used in bioassays were of analytical grade and used without any purification. UV-visible spectrophotometer (UV-1800 shimadzu, USA) was used in quantitative estimation of serum malondialdehyde and total protein in samples.

2.6 Estimation of serum malondialdehyde concentration

The serum malondialdehyde concentration was estimated based on the reaction of malondialdehyde (MDA) with thiobarbituric acid (TBA) forming a MDA-TBA2 product that absorbs strongly at 532 nm [13].

2.6.1 Assay procedure

A volume of 0.2 mL of serum was mixed with ice-cold 1.15% potassium chloride (1.8 mL) in a ratio of 1:9. Then distilled water (2.0 mL) was added to the tube. Subsequently, the mixture was incubated at 37±2 ℃ for 30 minutes and 0.375% thiobarbituric acid (2.0 mL) was added. The mixture was heated at 95℃ for 30 minutes avoiding the evaporation of the sample. After cooling to 27 ℃, the resultant solution was centrifuged at 4000 rpm for 10 minutes and absorbance of the resultant supernatant was measured spectrophotometrically at 532 nm against distilled water blank. The serum concentration of malondialdehyde (by product of decomposition of polyunsaturated fatty acids) estimated using the following equation.

$$A = \varepsilon Cl$$

A = Absorbance; ε = Molar extinction coefficient 1.56×M-1cm-1,

C = Concentration of the sample (mol/L); l = the path length (cm)

2.7 Estimation of serum concentration of total protein

Method of Lowery *et al*. was used [14]. The serum total protein concentration of samples was estimated spectrophotometrically using a total protein assay kit (Stanbio, USA). A volume of 0.01 mL serum was mixed with 1.0 mL reagent and 0.01 mL of standard mixed with 1.0 mL reagent. The solutions were mixed well and the tubes were allowed to keep for five minutes at room temperature (27℃). After that, the absorbance was measured spectrophotometrically at 550 nm against reagent blank. The total protein concentration was estimated.

The serum MDA concentration was expressed as nmol/mg protein.

2.8 Calculation of body mass index (BMI)

The height, weight of the participants was measured by a one trained person. The data were obtained using the same calibrated equipment in order to minimize variability, under standard condition according to the International Society for Advancement of Kinanthropometry (ISAK) protocol [15]. The BMI was calculated.

2.9 Statistical analysis

The database was created and analyzed using SPSS (Version 20) statistical software. The association between plant based antioxidant intake and the oxidative stress of subjects was tested by assessing correlation coefficient. The Shapiro-Wilk test for normality revealed that both the total plant based dietary antioxidant intake and the serum antioxidant concentration as determined by the MDA concentration showed skewed distribution. To assess the correlation between total antioxidant intake of the participants and their serum malondialdehyde concentration, Spearman's rank-order correlation was used. Level of significance was set at a probability level of 0.05. Correlation coefficient of 0.3 or above was considered as a moderate or higher level of correlation.

2.10 Ethical considerations

Ethical clearance was obtained from the Ethical Review Committee, Faculty of Allied Health Sciences, University of Ruhuna, Galle, Sri Lanka (Reference No.14.02.2018:010).
Informed written consent was obtained from all participants prior to enrollment for the study. Fresh fasting blood samples were collected from the participants under strict aseptic conditions. Privacy and confidentiality of the participants were strictly protected.

3. Results & Discussion

The study sample was consisted of eighty adults who were residents in Bope-Poddala area. The mean age (±SD) of the sample was 29 (±6.28) years. All participants were within the normal body mass index range and mean body mass index (±SD) was 58 (±7.41) kg/m^2. The socio demographic data of study participants are included in Table 1.

Table 1: Socio-demographic characteristics of the study participants

Characteristic	Frequency(n)	Percentage (%)
Gender		
Female	39	43.8
Male	41	46.1
Age (years)		
20 – 30	46	51.6
31 – 40	34	48.4
Educational levels		
Not schooled	1	1.1
Post primary education	23	25.8
Secondary education	19	21.3
Tertiary education	37	41.6
Monthly income (LKR.)		
<5000.00	49	61.3
5000.00 – 30000.00	7	8.8
>30000.00	24	30.0

There were 41 males and 39 females who participated in the study. Majority (41.6%) of the sample had undergraduate education or above, while 61.3% earned a monthly income of less than Rs.5000.00.
When considering the daily intake of antioxidants, males had a higher intake than in females. In a similar manner, males had a higher malondialdehyde concentration than in females. The antioxidant intake with respect to the selected food groups; namely, cereals and starchy foods, vegetables, fruits, beverages, spices and legumes are shown in Table 2.

Table 2: Antioxidant intake from selected plant based food among study participants

Food groups	Antioxidant intake(g)		
	Mean (±SD)	Median	Inter Quartile Range
Cereals and starchy foods	0.012 ± 0.028	0.005	0.004-0.009
Vegetables	0.058 ± 0.095	0.031	0.019-0.064
Fruits	0.360 ± 0.331	0.244	0.171-0.351
Beverages	0.031 ± 0.027	0.026	0.011-0.039
Spices	0.017 ± 0.002	0.016	0.016-0.017
Legumes	0.151 ± 6.983	0.000	0.000-0.000
Total antioxidant intake	1.562 ± 6.961	0.343	0.260-0.657

According to the information in Table 2, the mean antioxidant intake from selected food groups was in the range of 0.012± 0.028 to 0.360± 0.331g. Fruits were the main antioxidant source in habitual diet with a daily antioxidant intake of 0.360 ± 0.331g. Cereals and starchy foods contained the lowest amount of antioxidants with a value of 0.012± 0.028g.
The antioxidant intake and serum malondialdehyde (MDA) concentration was assessed according to gender, age, education level and monthly income of the participants and the results are shown in Table 3 and Table 4 respectively. A high antioxidant intake was found in males (1.668±7.295g) than in females (0.311 ±0.015nmol/mg) (Table 3). In addition, participants who had an undergraduate education or above had a higher mean malondialdehyde concentration of 0.030±0.015 nmol/mg and a high antioxidant intake of 0.363±0.365g. A higher mean antioxidant intake of 2.406±9.542g was obtained for participants who had a monthly income of more than Rs.30000.00 and a higher mean MDA concentration of 0.027±0.012 nmol/g was observed in participants who had monthly income between Rs. 5000.00- 30000.00.

Table 3: The association between the antioxidant intake and selected socio demographic parameters of study participants

Antioxidant intake(g)	Mean (SD)	Median	IQR
Gender			
Female	1.614 ±7.494	1.432	0.248-0.431
Male	1.668 ±7.295	1.443	0.311-0.665
Education level			
Post primary	0.311 ±0.000	0.232	0.311-0.311
Secondary education	0.328 ±9.740	0.301	0.259-0.665
Tertiary education	0.338 ±10.723	0.210	0.291-0.637
Undergraduate education and above	0.363± 0.365	0.259	0.253-0.642
Monthly income (Rs)			
<5000	1.380 ±6.684	1.243	0.253-0.642
5000 - 30000	0.847 ±0.633	0.551	0.338-1.543
>30000	2.406 ±9.542	2.302	0.271-0.635

Table 4: The association between the malondialdehyde concentration and selected socio-demographic parameters of study participants

MDA concentration	Mean(SD)	Median	IQR
Gender			
Female	0.020 ±0.009	0.011	0.012-0.025
Male	0.311 ±0.015	0.213	0.019-0.042
Education level(years)			
Post primary	0.016 ±0.000	0.016	0.016-0.016
Secondary education	0.021 ±0.012	0.020	0.015-0.021
Tertiary education	0.022 ±0.012	0.022	0.012-0.027
Undergraduate education and above	0.030 ±0.015	0.013	0.019-0.040
Monthly income(Rs.)			
<5000	0.016 ±0.000	0.025	0.015-0.030
5000 - 30000	0.027 ±0.012	0.020	0.012-0.041
>30000	0.022 ±0.012	0.018	0.017-0.026

The association between average antioxidant intake and serum malondialdehyde concentration (indicative of serum antioxidant level) was assessed using Spearman's Rank Order correlation coefficient and the results are shown in Table 5.

Table 5: Correlation between malondialdehyde (MDA) concentration and daily antioxidant intake

	Correlation coefficient	Significance (p)
MDA concentration and daily antioxidant intake	-0.156	0.166

The serum malondialdehyde concentration was in the range of 0.012-0.360 nmol/mg in the study sample. There was a weak, non-significant negative correlation (-0.156) between daily antioxidant intake and malondialdehyde concentration/lipid peroxidation (p=0.166). The results failed to reveal an association between plant-based dietary antioxidant intake and the oxidative stress in healthy adults in health unit area of Bope-Poddala, Sri Lanka. Data on health status, alcohol consumption, smoking habits, anthropometric data and biochemical measurements were obtained in 1821 women aged 35-60 y and 1307 men aged 45-60 y, participant to the SU.VI.MAX Study. Data on dietary intake were available on a subsample who reported six 24-h dietary records during the first 18 months of the study. There were optimistic associations of dietary beta carotene, vitamin C and E with their serum concentrations. Age, nutrient and intake of alcohol, serum cholesterol, BMI and smoking status explained 15.2% of the variance of serum beta-carotene in males and 13.9% in females, and 10.8 and 10.0% for serum vitamin C, and 26.3 and 28.6% for serum vitamin E, respectively. All these findings emphasize that the serum antioxidant concentrations are first and foremost influenced by sex, age, obesity, tobacco smoking, alcohol consumption and in particular dietary intake of those antioxidant nutrients [16].

Many researches reveal that there was a negative correlation between malondialdehyde concentration and the antioxidant intake [17, 18, 19]. It means that the amount of antioxidants that have been consumed had a positive relationship to the serum antioxidant status. There was a weak negative correlation between these two variables in the present investigation, too however, it was statistically not significant (p>0.05). Negative correlation could be due to over reporting of antioxidant rich food use among individuals who consume these items less than others.

There were some limitations in the present investigation. Mainly it was focused only on three dietary antioxidants namely, vitamin C, vitamin E and carotene. However, there were number of other dietary antioxidants such as tannins, flavonoids, polyphenols etc. that are beneficial for human health and not accounted for in the present investigation [20, 21]. It is better to consider a wide variety of dietary antioxidants rather than restricting to three selected antioxidants. Sri Lankans generally consume cooked foods and it might destroy some antioxidants in diet. However, the cooking methods and processing methods have a significant effect on the antioxidant status in a wide variety of food [22, 23, 24].To date, a number of biochemical parameters and markers were used in evaluating the antioxidant status in serum samples. Estimation of malondialdehyde concentration is one of them. However, estimation of malondialdehyde concentration in the evaluation of oxidative stress and predicting the antioxidant status based on that has been considered as a primary method compared to presently available methods. However, it is better to use at least three antioxidant assays due to variations in redox status by plant antioxidants [25, 26]. A single assay might not represent all biological redox systems at the cellular level and accordingly overall antioxidant status [27].

5. Conclusions

Fruits are the predominant source of dietary antioxidants consumed by majority of healthy residents in Bope-Poddala health unit area, Galle, Sri Lanka. The study revealed that there was a weak, non-significant negative correlation between average daily antioxidant intake and malondialdehyde concentration in this study sample.

6. Acknowledgment

The authors wish to thank academic and non-academic staff at Department of MLS, Faculty of Allied Health Sciences, University of Ruhuna, Galle, Sri Lanka in providing laboratory facilities and in technical assistance respectively.

7. Conflict of interests

The authors declare no conflict of interest.

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