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Estimation of Total Antioxidants capacity levels in blood plasma by new modified ferric reducing abilility of plasma (FRAP) Assay Method

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Abstract

Aim: The aim of this study was to estimate the TAOC by new modified FRAP assay method.

Material and method: 25 healthy individuals were selected from the OPD in the department of periodontology of CSMSS Dental College and Hospital, Aurangabad. Blood plasma samples were collected from 25 healthy individuals and were analyzed for TAOC by new modified FRAP assay in Biochemistry Department.

Result: TAOC levels of healthy individuals: 1082.36 ± 91.32 µmol/l.

Conclusion: Modification in the method of Benzie and Strain (1996) by increasing the quantity of plasma sample, water and FRAP proportionately fulfilled the quantity required for taking readings. New modified method is economically much cheaper and less time consuming.

Keywords: TAOC, FRAP assay, oxidative stress, antioxidants

Introduction

Oxidative stress is defined as a persistent imbalance between the production of highly reactive molecular species (e.g., reactive oxygen species [ROS], reactive nitrogen species) and anti-oxidant defenses.[1]

Several reactive oxygen species (ROS) and lipid peroxidation products are produced in physiological quantities in the human body, but it has been well established that over-production of ROS occurs at sites of chronic inflammation. The human body does contain an array of antioxidant defense mechanisms (non-enzymatic and enzymatic antioxidants) to remove harmful ROS as soon as they are formed and to prevent their deleterious effects. The enzymatic antioxidants include superoxide dismutase, catalase (CAT), and

glutathione peroxidase, while the nonenzymatic antioxidants include vitamins E and C, and reduced glutathione.[2]

There are a lot of systemic disorders giving oral manifestations based on imbalance between free radicals and antioxidants, with consequential destruction of host tissue as a result of oxidative stress (diabetes mellitus, atherosclerosis, multiple sclerosis, etc). Thereby, recent research is very oriented to determine, not only periodontal biomarkers, but also the risk markers of systemic diseases giving oral manifestations, which reflect disease onset, progression and therapy outcome, with emphasis on available and low invasive procedures.[3]

The antioxidant has been described as a substance that, when, present at low concentrations compared to that of an oxidizable substrate, significantly delays or prevents oxidation of that substrate. Antioxidants exist in all body fluids and tissues and protect against free radicals. Antioxidants in the body protect the cells from harmful oxidants (ROS) by removing the oxidants or repairing the damage caused by ROS in vivo. The human body incorporates a plethora of complex antioxidant systems. Therefore, the total antioxidant capacity (TAOC) assessment method has been developed to reduce the costly and time-consuming task of measuring individual antioxidant species.[4]

Benzie and Strain (1996) described the ferric reducing antioxidant power (FRAP) assay to measure the TAOC of plasma.[5]

Material and Method:

25 healthy individuals aged 20-45 (12 males and 13 females) were selected from the OPD in the department of periodontology of CSMSS Dental College and Hospital, Aurangabad. In this study modification is done to the method given by Benzie and Strain in 1996 as follows.

Venous blood samples were collected for plasma sampling. Working FRAP was prepared by mixing Acetate buffer (PH 3.6), TPTZ (2,4,6-tripyridyl-s-triazine) Ferric chloride solution as described by Benzie and Strain (1996)[5]. The reaction was performed by adding 20 µl of plasma 60 µl water and 600 µl of working FRAP. The optical density (OD) readings were taken at 593 nm on spectrophotometer [Systonic (S-923)] at 2 min, 4 min, 6 min, 8 min. The average of four readings OD was used to calculate the FRAP as the FRAP - Uric acid standard. The results were expressed in µmol/l.

Statistical analysis:

Independent sample t test or mean was conducted to test wether mean TAOC level is equal to the standard value of 1076 µmol/l. (Neha Bansal et al. 2017)[6].

Results:

Comparison of Mean TAOC level with the standard value of 1076 µmol/l.The P value is 0.691 which shows

Taoc level	N	Mean	Std. Deviation	Std. Error Mean
	25	1082.36	91.326	18.265

That the mean TAOC level is not different statistically from the standard level of 1076 µmol/l.

Test Value = 1076					
t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
				Lower	Upper
.403	24	.691	7.360	-30.34	45.06

Discussion

According to Collins,[7] measurement of TAC is a promising tool for the assessment of oxidative stress.

TAC is not a simple aggregate of all known and unknown antioxidants present in the body, but rather an integrated parameter which evidences the complex interactions among all antioxidants and their effect on the redox potential.

The TAOC assessment method has been developed to reduce the costly and time-consuming task of measuring individual antioxidant species. In this study TAOC evaluated by new modified FRAP assay. The modification is done to the method given by Benzie and Strain in (1996)[5] by increasing the plasma sample, water and FRAP quantities proportionately. The dilution of the plasma sample and FRAP was essential as the quantity required for taking readings on spectrophotometer was more.

Neha bansal et al (2017)[6] measured TAOC of plasma with FRAP assay in healthy and periodontitis patients. The values of healthy individuals were 1076.08 ±193.82 µmol/. This value taken as standard value in our study. Dr. Danesh Nair et al (2018)[8] measured TAOC of plasma with FRAP assay in subjects with chronic periodontitis compared to that of periodontally healthy subjects. The values of healthy subjects were 712.93 ± 23.58 mM/l. The difference in results may be due to use of spectrophotometer in our study whereas calorimeter was used by Dr. Danesh Nair et al (2018)[8]

Ali E. Abou Sulaiman et al (2010)[4] measured Plasma TAOC levels by an ABTS assay. The values of healthy individuals were 625 ± 88.7 (497.5 to 876.2) µm Teq.

Kranti Konuganti et al (2012)[9] measured whole blood total antioxidant capacity using a novel nitroblue tetrazolium reduction test in patients with periodontitis and healthy subjects. they showed a mean of antioxidant level 52.40 µg/ml for healthy group. Disparity in results may be due to difference in method of analysis and varied dietary habits.[10,11]

Conclusion

Modification in the method of Benzie and Strain (1996) by increasing the quantity of plasma, sample and FRAP proportionately fulfilled the quantity required for taking readings. we got appropriate results. New modified method is economically much cheaper and less time consuming.

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