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Serum total antioxidant status in cardiovascular patients and in healthy individuals

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Abstract: The previous studies suggest that low serum total antioxidant status (TAS) might be related to oxidative stress. To further examine the TAS, we investigated its relation in patients suffering from cardiovascular diseases. Total 39 samples of males and females were collected; age ranged from 18 to 86 years and divided them into two groups. Group 1 included 30 samples of cardiovascular patients; group 2 included 9 healthy individuals as control group. In group 1, patients suffering from Myocardial infarction (MI) showed low TAS values compared to the patients suffering from angina. The rest of the patients in group 1 showed TAS value within the reference range (1.3- 1.77mmol/L). The present study concluded that the TAS value in MI patients is lower than patients suffering from angina pectoris, further it was also concluded that aging and smoking both leads to the decrease in the level of TAS.

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INTRODUCTION

The term antioxidant is frequently used in the scientific literature but is rarely defined with a strong implication that these are chemicals with chain breaking properties. These oxidative damages are caused by free radicals[1]. Antioxidants prevent diseases against free radical by anticipating the formation of new free radicals. Antioxidants like vitamin E, C, A, uric acid, bilirubin and albumin, superoxide dismutase (SOD) and glutathione peroxidase (GPx) trap the free radicals and prevent the chain reactions. Some of the antioxidants like methionine sulphoxide reductase repair the biomolecules that are damaged by the free radicals[1].

A free radical is any chemical species capable of independent existence and containing one or more unpaired electrons. Free radicals include superoxide (O^{2-}), hydroxyl ion (OH^{-}), nitric oxide (NO^{-}) and nitrogen oxide (NO_2^{-}) etc. These free radicals are extremely reactive molecules and cause cell injury and even death. The body constantly produces these free radicals by the reactions. a). Mostly by biochemical redox reactions, involving oxygen which occur as a part of normal cell metabolism. b). By phagocytes, as a part of a controlled inflammatory reactions. c). In response to exposure to ionizing radiation, UV lights, environmental pollution, cigarette smoking, hyperoxia, excess exercise and ischemia[1].

These free radicals damage the typical cell components including polyunsaturated fatty acids in cell membrane, proteins such as enzymes and membrane ion transporters and DNA. All these free radicals have been implicated over 100 human and animal diseases, ranging from rheumatoid arthritis, cataract, renal failure, cardiovascular diseases and AIDS etc[1].

Recent experimental studies have shown that antioxidants play an important defensive role at cellular level. Supporting the role of antioxidants in the oxidative stress mechanism in previous studies showed that patients suffering from stable angina have diminished activity of superoxide dismutase (SOD) [2]. Moreover, the role of antioxidants (vitamin C, E, carotenoids and selenium) was investigated in the prevention of cardiovascular disease and was found that there was a substantial increase in the risk of ischemic heart disease (IHD) and stroke in individuals with low intake of antioxidants in diet[3]. The scientific evidence for a possible role of antioxidants in the prevention of coronary heart disease (CHD) was studied and it showed that free radical damage and antioxidants plays defense role in the development of CHD[4]. Antioxidants neutralize the O_2^{-} free radicals and inhibit the low-density lipoprotein oxidation and atherosclerosis[5].

Further, serum antioxidant status in CHD was studied and concluded that there was a 40% reduction in coronary artery disease (CAD) in individuals who were supplemented by the vitamin E. Moreover, the change in antioxidants defense system with IHD was also studied in 68 patients and 20 controls. There was a decrease level of antioxidant with a disease aggravation and the conventional therapy improved the defense system[6].

In present study, an attempt has been made to examine the antioxidant status in patients suffering from cardiovascular diseases. We estimated the TAS level in patients and in healthy individuals of both genders and of different age groups.

MATERIALS AND METHODS

This study was conducted at Institute of Biochemistry and Biotechnology, University of the Punjab, Lahore, Pakistan. The blood samples were collected from Sheikh Zayed Hospital Lahore, Pakistan. This is a 750 bedded, tertiary care teaching hospital. The patients reviewed were suffering from cardiovascular diseases (myocardial infarction (MI), unstable angina (USA), ischemia, congestive cardiac failure and left and right ventricular failure). These patients were already admitted in the Cardiac Care Unit (CCU) of the hospital. The history proforma was filled for the evaluation of other disease conditions, smoking or non-smoking and supplement status of the patient. The inclusion criteria for healthy individual were age, having no disease and smoking/non-smoking status. A total of 39 blood samples were collected; 30 of cardiovascular disease patients and 9 of healthy individuals respectively. Three cc blood was drawn by sterile 5 cc BD syringe from each individual and further it was centrifuged at 5000 rpm for 15 minutes to collect the serum. The serum was stored at −20°C for further analysis.

A total antioxidant status (TAS) kit manufactured by Randox Laboratories (Crumlin, United Kingdom) was used for the estimation of TAS. The standard, reagents were prepared according to the manufacturer's instructions provided with the kit. The principle of the assay states that ABTS® (2, 2'-azino-di-[3-ethylbenzthiazoline sulphonate]) incubated with the peroxidase (metmyoglobin) and H_2O_2 (hydrogen peroxide) produces radical cation ABTS®$^+$. It gives a relatively stable blue-green color and is measured at 600nm. When a sample containing an antioxidant is added, it causes the suppression of the color to a degree proportional to the concentration of antioxidants. The absorbance was measured on Spectronic 21 UV Spectrophotometer at 37ºC against air. Cuvettes for blank, standard and samples were taken. The initial absorbance (A_1) and that after the addition of substrate, second absorbance (A_2) was read. Total antioxidant status was then calculated by applying the following formula:

TAS mmol/L = (ΔA Blank−ΔA Sample) × factor

Where,

ΔA of Blank/Sample/Standard = A_2–A_1

A_1 = Initial absorbance prior to the addition of substrate

A_2 = Absorbance taken 3 min after the addition of substrate

Factor = Concentration of the Standard / (ΔA Blank−ΔA Std.)

Reference range of the Total Antioxidant Status is 1.3-1.77 mmol/L in the serum, according to the literature provided along with the Randox kit. Linearity of the assay was 2.5mmol/L, and samples having value higher than this was diluted with 0.9% NaCl and was re-assayed[7].

RESULTS AND DISCUSSION

Total antioxidant status in cardiovascular patients (Group 1)

Antioxidant system plays an important role against various diseases like AIDS, cataract, cardiovascular diseases, renal failure, and rheumatoid arthritis[1]. In the current study we have estimated the TAS in patients suffering from cardiovascular diseases. Regarding cardiovascular diseases, literature review has shown that males are more prone to have these diseases than females[8]. In this study, maximum patients were above 45 years of age and most of them were males. Antioxidant status of total 30 cardiovascular patients was estimated (Table 1). Out of these, 16 patients showed TAS value above the normal reference range. These patients were on antioxidant (vitamins and minerals) supplements, which might be a contributory factor for the normal TAS. Seven of the patients (sample no. 13, 22, 23, 24, 25, 27 and 30) had TAS value less than 1.00 mmol/L.

These patients were smokers and had myocardial infarction. While the other 7 patients (sample no. 4, 5, 8, 14, 26, 28 and 29) had TAS value greater than 1.00 mmol/L but less than the reference range. They were suffering from angina pectoris, as in angina there is a decrease in blood flow to the heart muscle, but in myocardial infarction, a portion of myocardium is dead. This might explain the extremely low TAS value in the patients of MI as compared to those of angina, without any tissue damage. This is evident that patients suffering from MI have more production of free radicals and hence showed a decrease in TAS value. It has also been shown that smokers have high oxidative stress and thus show low level of TAS[9].

Smoking causes increased production of free radicals and hence very low TAS value was seen in seven of the patients (sample no. 13, 22, 23, 24, 25, 27 and 30). Two of the patients (sample no. 4 and 5) had CRF, in addition to USA. Thus it may be suggested that CRF patients are more prone to have cardiovascular diseases[10]. This can provide evidence to the low value of TAS of these patients. The current study estimated the TAS with no specificity to the type of antioxidant. Previous research has revealed that it is the level of vitamin E, C and β-

carotene, which is more related to cardiovascular diseases than other antioxidants[11,12]. The p-value of the cardiovascular patients was highly significant and percentage analysis of TAS of cardiovascular patients (Figure 1) showing 43.3 percent having less TAS value than the reference range. While 33.3 percent of the patients TAS value lie within the reference range and 23.3 percent TAS value is above the reference range i.e. 1.3-1.77 mmol/l.

Table 1:Total Antioxidant Status (TAS) in cardiovascular patients from age 18 - 75 years (Group 1)

Sample No.	Age (years)	Disease and Smoking status	TAS (mmol/L)
1	18	Lateral wall ischemia	1.5
2	28	CCF	1.5
3	38	Acute LVF	2.42
4*	40	USA, CRF	1.08
5*	51	USA, CRF	1.16
6	42	USA	1.5
7	47	USA	1.5
8*	60	USA	1.17
9	60	USA	2.01
10	70	USA	1.42
11	72	USA, LVF	2.51
12	70	USA	1.42
13*	65	MI, Smoker	0.77
14*	70	USA	1.04
15	60	IHD, CCF	1.5
16	65	IHD, CCF	2.34
17	55	IHD	2.11
18	50	IHD, CCF	1.5
19	49	IHD, CCF	1.9
20	39	IHD	2.21
21	63	IHD, CCF	1.4
22*	50	MI, Smoker	0.41
23*	65	MI, Smoker	0.92
24*	70	MI, Smoker	0.92
25*	60	MI, Smoker	0.83
26*	59	USA	1.2
27*	66	MI, Smoker	0.98
28*	44	USA	1.07
29*	61	USA	1.16
30*	63	MI, Smoker	0.77

*Low TAS value as compared to the normal reference range (1.3 - 1.77 mmol/L); IHD: Ischemic heart disease; CCF: Congestive cardiac failure; LVF: left ventricular failure; USA: Unstable angina; CRF: Chronic renal failure; MI: Myocardial infarction

Total antioxidant status in healthy individuals (Control group).

In the control group total 9 healthy individuals were studied for the estimation of TAS (Table 2). Out of these, 6 controls showed normal TAS value within the reference range and three of the individuals showed low TAS than the normal reference range. Sample 9, 86 years of age had low TAS value. This low value might be explained on the basis of age factor. Literature has shown that aging is associated with impaired function of the immune system, which might cause the high production of free radicals and hence decreased level of antioxidant[12].

Table 2: Total Antioxidant Status (TAS) in healthy individuals from age 18 - 86 years (Control Group)

Sample No.	Age (years)	Smoking/ Non-smoking status	TAS (mmol/L)
1	18	Non-smoker	1.35
2	30	Non-smoker	1.46
3	32	Non-smoker	1.3
4	37	Non-smoker	1.43
5	40	Non-smoker	1.4
6	49	Non-smoker	1.67
7*	54	Smoker	0.58
8*	58	Smoker	0.5
9*	86	Non-smoker	0.32

* Low TAS value as compared to the normal reference range (1.3 - 1.77 mmol/L)

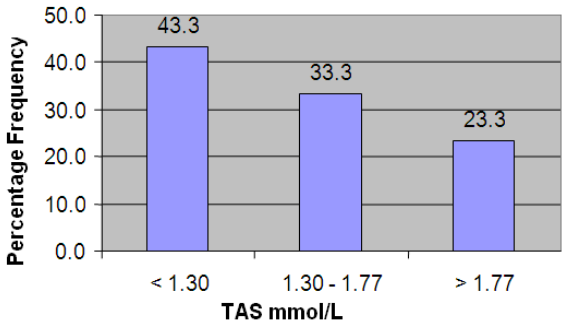

Figure 1: Percentage analysis of TAS in cardiovascular patients.

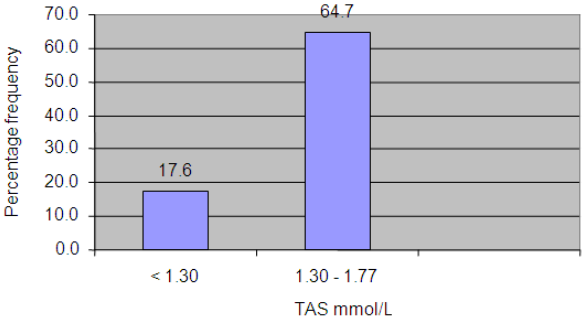

Figure 2: Percentage analysis of TAS in healthy individuals.

The other sample no. 7 also showed very low TAS value. The individual was a smoker. This could be due to the high production of oxidative stress in smokers, which thus lowers the level of TAS as compared to non-smokers [9]. In sample no. 8 the TAS value was found to be very low than the reference range. The individual was smoker and gardener by occupation. The low TAS level might be due to smoking and socioeconomic factor, because of their

low income, which leads to less nutritional diet uptake or an unbalanced diet (deficiency in vitamins and minerals).

The p-value of the control group showed highly significant value. The percentage analysis of TAS of control group (Figure 2) showed 17.6 percent of the individuals TAS value less than 1.3 mmol/L. Among this control group 64.7 percent showed TAS value within the reference range (1.3-1.77 mmol/L).

CONCLUSION

Findings of present study are in agreement with the previous reports that TAS value becomes decreased in smokers and also with the increase in age. Among cardiovascular diseases, patients suffering from MI had low TAS as compared to those who are suffering from angina pectoris. By giving the antioxidant supplements to the patients of cardiovascular diseases the severity of the disease can be delayed. Moreover, the antioxidant supplementation (vitamin E) given to the healthy individuals might provide a defense against cardiovascular diseases[13,14].

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