

## THE TOTAL ANTIOXIDANT STATUS IN CIGARETTE SMOKING INDIVIDUALS

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### ABSTRACT

Active smokers are exposed to reactive free radicals that are present in cigarette smoke. Oxygen free radicals, including superoxide, hydroxyl radicals, peroxy radical hydrogen peroxide and singlet oxygen, are highly reactive species that can cause a wide spectrum of cell damage including enzyme inactivation, lipid peroxidation, protein and lipoprotein oxidation, and DNA damage. Free radicals are believed to be involved in the pathogenesis of cardiovascular diseases and cancer. The purpose of the present study was to measure the total antioxidant status (TAS) in active cigarette smoking individuals in Mosul city. Twenty active smokers and twenty nonsmokers participated in the study. Blood sample was taken from each individual and the TAS had been measured in the sera using the Cayman chemical antioxidant assay kit (USA). The results obtained from the study revealed a significant ( $P < 0.001$ ) reduction of the TAS in the smoker's group as compared with the non-smoker's group. In conclusion, smokers possess low TAS than non smokers which may be due to the presence of high amounts of free radicals in cigarette smoke that generate an oxidative stress in the smoker's body that may cause exhaustion of antioxidants of the body.

### INTRODUCTION

Active smokers are exposed to reactive free radicals that are present in cigarette smoke<sup>[1]</sup>. Oxygen free radicals and other oxygen-derived species including superoxide, hydroxyl radicals, peroxy radical hydrogen peroxide and singlet oxygen<sup>[2]</sup> are highly reactive species that's cause a wide spectrum of cell damage including enzyme inactivation, lipid peroxidation, protein and lipoprotein oxidation<sup>[3]</sup> and DNA damage<sup>[4]</sup>. Free radicals are believed to be involved in the pathogenesis of cardiovascular diseases and cancer<sup>[5,6]</sup>. Cigarette smoke contains high amounts of free radicals and other oxygen-derived species<sup>[7,8]</sup>. It contains more than 1014 free radicals/oxidants per puff and is a complex mixture of over 4700 chemical compounds<sup>[1]</sup>. Short lived free radicals such as superoxide and nitric oxide which are found in cigarette gas phase can react chemically to form highly reactive free radical peroxy nitrite<sup>[9]</sup>. In addition superoxide can react with hydrogen peroxide to form the more active hydroxyl free radical<sup>[10]</sup>. Cigarette smoking alone has been implicated as a major risk factor for several chronic diseases, including cardiovascular disease, pulmonary disease, and cancer<sup>[11]</sup>. Free radicals may be the most critical factors triggering plasma antioxidant depletion, lipid peroxidation, and protein modification<sup>[12-14]</sup>. Products arising from lipid peroxidation and protein modification can in turn react with cigarette smoke constituents, creating additional toxic products. The toxic products resulting from both the direct and

secondary reactions with cigarette smoke are thought to activate inflammatory immune responses, which may themselves be altered by cigarette smoke constituents and play an influential role in smoking-related oxidative tissue damage<sup>[8]</sup>. The subsequent metabolic and molecular changes are thought to induce the onset of cigarette smoking-related chronic diseases<sup>[15-17]</sup>. Free radicals in cigarette smoke deplete some plasma antioxidants in vitro<sup>[3]</sup> and several studies found lower plasma antioxidant concentrations in smokers in vivo<sup>[18-21]</sup>. Because of the dearth of such studies in Iraq, the present study was designed to measure the total antioxidant status in a number of cigarette smoking individuals in Mosul city compared with the total antioxidant status obtained from other non smoker individuals (controls).

### SUBJECTS AND METHODS

Twenty control non-smoker subjects (age range 19-45 years) and 20 chronic active smokers (age range 20-45 years) were studied. Chronic smokers were included if they had a history of smoking of not less than 15 cigarette per day for at least the past consecutive 5 years. No participant had a history of hyperlipidaemia, cardiovascular diseases, kidney disease, diabetes mellitus or any other systemic disease. Further exclusion criteria were current use of antioxidants and none of them had received medication during the study period and for 2 months before the study period. All participants gave consent form, and the study protocol was

approved by the local Ethics Committee of the University of Mosul. The total dietary intakes of fruits and vegetables were not significantly different between the study groups, thereby enabling isolation of the effect of smoking. The study was carried out during fasting time in the morning. Blood was withdrawn by syringe without stasis from an antecubital vein in each subject and immediately centrifuged and the sera were analyzed within few hours of sample collections. The total antioxidant status levels (TAS) was measured using the Cayman chemical antioxidant assay kit (USA); the assay relies on the ability of the antioxidants in the sample to inhibit the oxidation of ABTS® (2,2 1- azinodi- [3-ethylbenzthiazoline suiphonate]) to ABTS<sup>+</sup> by metmyoglobin. The amount of ABTS<sup>+</sup> produced can be monitored by reading the absorbance at 750 nm, under the reaction condition used, the antioxidants in the sample cause suppression of the absorbance at 750 nm to a degree which was proportional to their concentration. The capacity of the antioxidants in the sample to prevent ABTS® oxidation was compared with that of Trolox, water soluble tocopherol analogue and was quantified as millimolar Trolox equivalents. The plate reader used was Bio-Tek instrument plate reader ELX800.

## RESULTS

The results obtained from the study revealed a low mean value of total antioxidant status in the smoker group (1.504 nmol/L) compared with the non-smokers group (1.944 mmol/L) (Table-1) and (Figure-1). A highly significant differences were obtained ( $P < 0.001$ ), with 95% confidence interval lies between 0.311-0.569. The TAS levels of the subjects are normally distributed (Figure 1). Figure 3 shows changes in TAS of the control and smoker groups.

**Table 1. Values of TAS concentrations in nmol/L in smokers and non- smokers ( $P < 0.001$ ).**

Subject number	Smokers	Non-smokers
1	1.86	1.92
2	1.42	1.80
3	1.64	1.96
4	1.58	1.87
5	1.76	1.82
6	0.96	1.90
7	1.38	1.90
8	1.64	1.98
9	1.34	1.82
10	1.05	1.74
11	1.50	2.02
12	1.40	2.06
13	1.10	1.92
14	1.51	2.18
15	1.65	1.90
16	1.65	2.26
17	1.74	1.96
18	1.46	2.02
19	1.86	2.04
20	1.59	1.82
Mean	1.504	1.944
SD	0.251	0.128
SE mean	0.056	0.029

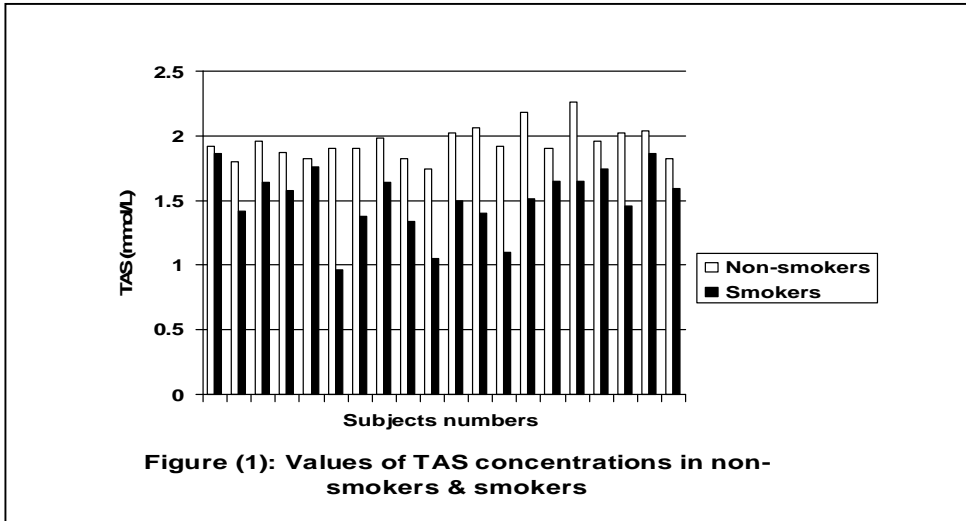


Fig 1. Values of TAS concentrations in non-smokers & smokers

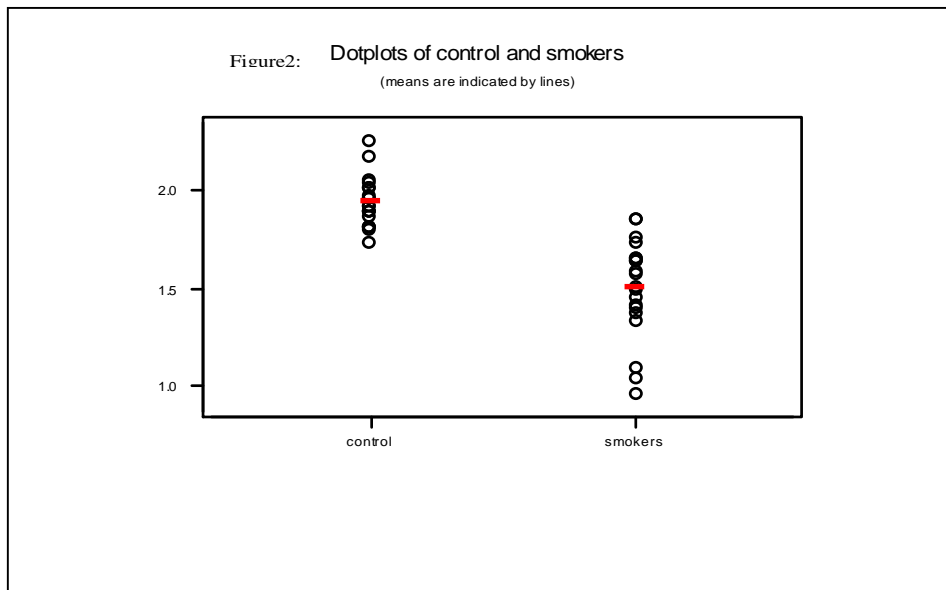


Fig 2. Dotplots of control and smokers (means are indicated by lines).

## DISCUSSION

The data obtained from the present study revealed a significant low total antioxidant values in smokers compared with normal values in the control group. Measuring total antioxidant activity is better than measuring the individual antioxidant activity because: the measurement of all known antioxidants in biological fluid is time consuming, many antioxidants may be as yet undiscovered; and the total activity may be greater than the sum of the individual antioxidants because of cooperative interaction<sup>[22]</sup>. The results obtained in the present study which indicated that the total antioxidant status was low in smoker individuals may confirm the results obtained from previous studies which measured the antioxidant parameters separately. Several researchers have observed changes in various elements of the antioxidant defense system in smokers. Many studies reported lower plasma ascorbic acid concentrations in smokers than in non-smokers<sup>[23-27]</sup>. Dietrich et al<sup>[28]</sup> found that both ascorbic acid and n-carotene concentrations were lower/in the smokers than did non-smokers. However, a further study did show a decrease in glutathione concentrations and the activities of glutathione peroxidase and glutathione S-transferases<sup>[29]</sup>. Smokers had significantly lower concentrations of 13-carotene<sup>[30]</sup>. A low antioxidant capacity in plasma suggests an increased oxidant burden in the blood. Many researchers have reported increased levels of superoxide anion release from circulating neutrophils<sup>[31,32]</sup> and increased lipid peroxidation products in the plasma<sup>[32,33]</sup> of smokers, supporting the concept of systematic oxidative stress in these individuals. It is unclear, however, whether the oxidative stress that occurs in smokers is a direct effect of the oxidants/free radicals present in cigarette smoker or whether the endogenous inflammatory response is primarily responsible<sup>[34]</sup>. Antioxidants are of particular importance to smokers because antioxidants are believed to scavenge free radicals, which are found in large quantities in tobacco smoke<sup>[1]</sup> and have been implicated in the development of coronary heart disease, cancer, and other diseases<sup>[35]</sup>. Studies have reported that an increased intake of a natural complex source of antioxidants from fruit and vegetables had a

protective effect on the susceptibility of LDL to oxidation, which offers protection against oxidative diseases<sup>[8,36]</sup>. Some trials reported that the oxidant effect of cigarette smoke may be due to the fact that, the nutrient intake of smokers differs from those of non-smokers. Some of these differences may exacerbate the deleterious effects of smoke components on cancer and coronary heart diseases risk<sup>[37,38]</sup>. Other previous studies found lower concentration of antioxidants even in non-smokers (passive smokers) who were exposed to cigarette smoke<sup>[28,39-41]</sup>.

*In the present study* both the studied groups have identical fruit and vegetable nutrient intake, so we reduced the effect of low fruit and vegetable intake on the results. Accordingly, the results suggest that the low TAS obtained in the present study is mostly due to cigarette smoking.

*In conclusion*, smokers possess low antioxidant values than non-smokers which may be due to the presence of high amounts of free radicals in cigarette smoke that generate an oxidative stress in the smoker bodies causing an exhaustion of the antioxidants of the body.

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