

High blood lead levels compromise the total antioxidant status of normal healthy adults in a population in Karachi

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ABSTRACT

Objective: To find out the total antioxidant status (TAS) and its relationship to blood lead (Pb) in apparently healthy adults in a population in Karachi.

Methodology: In a cross-sectional study, a cohort of 132 healthy adults (67 having blood Pb levels below 7µg/dl and 65 having blood Pb levels above 11µg/dl) were randomly selected from 872 healthy adults (age 18-60 years) recruited from a low-income population in Karachi. TAS in serum was analyzed using spectrophotometric assay based kit, while blood Pb levels were determined using Atomic Absorption Spectrometric method.

Results: Mean serum TAS levels were significantly less in subjects with high blood Pb concentration compared to those with low blood Pb concentration (1.05±0.27 mmol/l vs 1.16±0.24 mmol/l; $P=0.01$). In a correlation analysis adjusted for age and gender, TAS levels were found to be modestly associated with blood Pb [Pearson's $r=-0.2$; $P=0.02$].

Conclusion: Mean TAS levels were significantly decreased in apparently healthy adults with high blood Pb in a general population in Karachi. Exposure to Pb pollution could be compromising antioxidant ability of individuals in the study population.

KEY WORDS: Blood lead, Lead pollution, Oxidative stress, Pakistani adults, Total antioxidant status.

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INTRODUCTION

Lead (Pb) has been known to interact with superoxide radical to generate very reactive free radicals such as reactive oxygen species (ROS) which can compro-

mise the antioxidant status of individuals leading to various oxidative stress related diseases such as cardiovascular disease and cancer.^{1,2} Pakistan, like most developing countries, has an epidemic of cardiovascular disease (CVD)³ and oxidative stress is considered to be one of the major factors for this increased risk of CVD in this population.⁴

There have been a few reports on the effect of Pb pollution on oxidative stress in general population. In a previous communication, we have reported that approximately, 59% of subjects in a low-income population in Karachi had levels of blood Pb greater than 10µg/dl-a level no more considered to be acceptable.^{5,6} Since Pb is known to disrupt the prooxidant/antioxidant balance in the body,⁷ it is imperative that total antioxidant status (TAS) of subjects and its relationship to blood Pb must be investigated in population of a developing country like Pakistan where Pb pollution is one of the major environmental health problems.

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METHODOLOGY

A cohort of 132 apparently healthy subjects (67 having blood Pb levels below 7µg/dl and 65 having blood Pb concentrations above 11µg/dl) were randomly selected from 872 healthy individuals of age 18-60 years recruited from a low-income urban population in Karachi. The details of recruitment in the larger study and methods for blood collection and analysis of blood Pb have been described in previous communications.^{5,8}

TAS in serum was determined using spectrophotometric assay based kit (RANDOX Laboratories Ltd, UK) following Manufacturer's instructions. Briefly, 20 µl of serum sample (or standard, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was incubated with 1 ml of chromogen (consisting of metmyoglobin and ABTS®) at 37°C in a cuvette in temperature-controlled spectrophotometer. After noting down the initial absorption (A₁) at 600 nm, 200 µl of substrate (hydrogen peroxide, 250 mmol/l) was added. After gentle mixing, the timer was started and the absorbance was again noted at exactly 3 minutes (A₂). A reagent blank was also run in which 20 µl of distilled water (instead of standard or serum sample) was incubated with 1 ml of chromogen followed by 200 µl of substrate. TAS concentration in mmol/l was determined by using the formula:

$$A_2 - A_1 = \Delta A \text{ of sample/standard/blank}$$

$$\text{Factor} = \frac{\text{concentration of standard}}{(\Delta A \text{ blank} - \Delta A \text{ standard})}$$

$$\text{TAS (mmol/l)} = \text{Factor} \times (\Delta A \text{ blank} - \Delta A \text{ sample})$$

The study had been approved by the Ethics Review Committee of the Aga Khan University. Statistical analysis was carried out using Statistical Package for Social Sciences® (SPSS) software version 13 for Windows®. Continuous variables such as blood Pb and TAS were expressed as mean±standard deviation (SD). Independent sample t-test was used to compare mean values in two groups. Analysis of variance (ANOVA) was used to compare the above mentioned continuous variables across occupation, followed by Bonferroni test for multiple pair-wise comparisons. Multiple linear regression was employed to study the relationship between age and TAS and influence of

plasma homocysteine and smoking on blood Pb and TAS. Pearson's correlation test was used to study the relationship between levels of blood Pb and TAS. P-value less than 0.05 was considered significant.

RESULTS

While males had significantly high mean levels of blood Pb compared to females ($P < 0.001$), the mean concentrations of TAS were not significantly different between men and women (Table-I). Table-II shows the relationship between blood Pb and serum TAS versus occupation. Individuals working in open environment, such as laborers, street vendors, construction workers, vehicle drivers, bus/wagon conductors etc., had significantly increased mean concentration of blood Pb compared to those working in closed environment, such as office workers, factory workers and house-wives, perhaps because of greater exposure to environmental pollution ($P = 0.019$). Mean levels of TAS among these groups were not significantly different. Mean serum TAS levels were found to be significantly decreased in the group of individuals having blood Pb levels $> 11 \mu\text{g/dl}$ (Table-III; $P = 0.01$) compared to those with low blood Pb concentration ($< 7 \mu\text{g/dl}$). In a correlation analysis adjusted for age and gender, serum TAS levels were found to be modestly associated with blood Pb (Pearson's $r = -0.20$; $P < 0.02$). However, no significant association was observed for serum TAS with age (across gender), plasma homocysteine and smoking (data not shown).

DISCUSSION

Oxidative stress plays a major role in the pathogenesis of atherosclerosis. Initial studies focused on levels of antioxidant enzymes and/or antioxidants as determinants of oxidative stress in various diseases/disorders,¹ however, the results were inconsistent because all the components of antioxidant defence system had not been taken into account. Assessment of integrated antioxidant system, therefore, has an advantage because it provides the net antioxidant status taking into account all the biological components – oxidants as well as antioxidants. In the present study we have focused on total antioxidant status (TAS) to study the role of Pb pollution on it in a general population in Karachi.

Table-I: Mean blood Pb and serum total antioxidant status (TAS) in study population. Mean±SD

	Total n=132(Range)	Males n=48	Females n=84	P-value*
Blood Pb(µg/dl)	13.15±8.94(1.61-39.1)	17.69±9.07	10.56±7.81	< 0.001
TAS (mmol/l)	1.11±0.26(0.24-1.7)	1.13±0.21	1.09±0.28	0.4

*P-value is obtained by comparing mean±SD values among gender using independent sample t-test

Table-II: Mean blood Pb concentration and serum total antioxidant status (TAS) versus occupations. Mean±SD

	Pb($\mu\text{g}/\text{dl}$)	P-value*	TAS (mmol/l)	P-value*
Occupation				
Housewives	10.49±7.71		1.1±0.27	
Closed environment	10.85±7.17	<0.001	1.1±0.22	0.6
Open environment	19.59±9.40		1.14±0.19	

*P-value is obtained from analysis of variance (ANOVA) by comparing mean±SD values among occupations of individuals.

Our observation that a significant decrease in TAS ($P=0.01$) of subjects with high levels of blood Pb supports the notion that Pb is one of the major risk factors in increasing the oxidative stress leading to compromised antioxidant status. Similar findings have been reported by Bijoor et al in Indian healthy subjects in Bangalore.⁹ In their study they have shown that blood Pb levels around $14\mu\text{g}/\text{dl}$ significantly decrease TAS. Mean TAS values in the study population were not significantly different from those reported earlier in Pakistani healthy subjects (0.785 ± 0.042 mmol/l).¹⁰ However, the sample size in that study was very small ($n=21$) and not representative of the general population. Therefore, the population in the present study is not only representative of the general population in an urban community but also involves a relatively large sample size ($n=132$).

Diet has been shown to have a significant effect on TAS and vegetarians and those consuming increased amounts of fruit juices are known to have improved TAS.^{11,12} The results of present study also show that the mean value of TAS in this population which is comprising of all non-vegetarians and less frequent consumers of fruit juices (data not shown) was relatively lower than the mean values reported for strictly vegetarian Indian population (1.1 ± 0.27 mmol/l vs 1.5 ± 0.76 mmol/l).⁹ In a recent communication, we have shown that daily consumption of fruit juices significantly reduces blood Pb levels.⁵ It is conceivable that low blood Pb levels in such individuals could be partly contributing to their improved TAS. High blood Pb levels found in those individuals working in open environment compared to those working in closed environment point towards the potential role of environmental pollution on blood Pb and TAS. Other than environmental pollution, smoking and plasma

homocysteine have been reported to be the confounding factors for increased blood Pb levels^{5,13}.

In a regression model, we analyzed the relationship between blood Pb and TAS while adjusting for plasma homocysteine. Our results indicate a significant association between plasma homocysteine and blood Pb ($P=0.02$). However, there was no significant effect on TAS.

Smoking is known to increase not only blood Pb and oxidative stress but also decrease total antioxidant capacity.^{13,14} This is because of the fact that a single puff contains at least 10^{14} free radicals which can not only decrease the level of antioxidants but also stimulate inflammatory processes leading to greater oxidative stress.¹⁵ In the present study, however, we did not find any significant difference in mean levels of TAS among smokers and nonsmokers. This could be due to small number of smokers ($n=11$) among the study subjects. Similar findings have been reported in a study by Risal et al on Nepalese adults.¹⁶

Apart from the nutritional and environmental effects on TAS levels, the genetic contributions to variations in plasma TAS must also be taken into account.¹⁷ In a study carried out on Mexican Americans, TAS levels were found to be significantly lower in women compared to men [1.68 ± 0.004 vs 1.81 ± 0.005 mmol/l; $P<0.001$], and there was significant decrease in levels of TAS with age in men.¹⁷ In the present study on a Karachi population, we did not find any significant difference between males and females in terms of their mean TAS levels [$P=0.4$], and age, too, did not seem to have any noticeable effect on TAS.

Our results must be viewed within the context of certain limitations of the study. In spite of the fact that we had 132 subjects, the number of smokers in them were only 11. Therefore, the expected variation in TAS

Table-III: Concentrations of serum total antioxidant status (TAS) and blood Pb in two groups of subjects with low and high blood Pb. Mean±SD

	Group with blood Pb< $7\mu\text{g}/\text{dl}$ ($n=68$)	Group with blood Pb> $11\mu\text{g}/\text{dl}$ ($n=64$)	P-value*
Blood Pb ($\mu\text{g}/\text{dl}$)	5.16±1.01	21.55±5.06	<0.001
TAS (mmol/l)	1.16±0.24	1.05±0.27	0.01

*P-value is obtained by comparing two mean values in a row using independent sample t-test

concentration due to smoking as reported in other studies was not observed due to this small number. Analysis of the levels of oxidized low density lipoprotein (ox-LDL) would have been useful in assessing the impact of oxidative stress on the study subjects in terms of oxidation of LDL, an essential step in the pathogenesis of atherosclerosis.¹⁸ This could have been of help in ascertaining the effects of Pb pollution towards increasing the risk of atherosclerosis. This, however, remains an interesting but still unexplored study question for Pakistani population.

Results of this study indicate that Pb pollution which has been reported to be alarmingly high in Karachi appears to be compromising the antioxidant status of adults.^{5,19} Adequate control of Pb pollution along with education of masses towards adoption of healthy food habits such as increased consumption of vegetables and fruit juices would be required to improve antioxidant status of individuals.

CONCLUSION

Mean TAS levels were significantly decreased in apparently healthy adults with high levels of blood Pb compared to those with low blood Pb indicating that exposure to Pb pollution appears to compromise antioxidant ability of individuals in a Pakistani urban population.

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Authors Contribution:

MPI and MY were involved in planning and design of the study, analysis and interpretation of data and write-up of the paper.