Original Paper

Low-Levels of Blood Lead and Antioxidant Status

Anita Bijoor R*, Rashmi Sinha**, Venkatesh T***

ABSTRACT

Lead is not an essential component of the human body, but it is always present due to extensive exposure and easy absorption in various forms. Lead affects virtually all systems of the body. Several metals can indirectly evoke the generation of Reactive Oxygen Species (ROS) by way of disruption of normal calcium homeostasis. This is true, especially at high levels of blood lead. This study was undertaken to see if lead at low levels causes oxidative stress and damage. The level of total antioxidant status in blood was measured to indirectly indicate the extent of oxidative damage, and this was correlated with the blood lead levels. The results reveal that even at blood lead levels as low as 14.15µg/dl ± 4.8µg/dl, there is a significant decrease in the levels of total antioxidant status. The total antioxidant levels were 0.78±0.25mmol/l in the lead-exposed as against 1.5±0.76 mmol/l in the unexposed.

This study supports the introduction of antioxidants along with chelation, as a regular treatment for lead poisoning cases, as also as a preventive measure against lead poisoning in persons exposed to different sources of lead.

Key Words: Antioxidants, Lead, Reactive oxygen species

Introduction

Lead is a soft, silvery gray metal.[1] It is not an essential component of our body, but it is always present in our body due to extensive exposure and easy absorption in various forms. It is highly resistant to corrosion, and has a very low boiling and melting point which makes it an industrially very useful metal. Some of the sources of lead include paints, vermillion, lead acid battery production, and petrol and gasoline.[2]

The absorption of lead from environmental sources is not dependent solely on the amount of lead presented to the portals of entry, but also on its physical and chemical state.[3] Absorption of lead occurs from the lungs and the respiratory tract; from the gastrointestinal tract through intake of lead in food, beverages and soil or dust, while young children may take in lead from non-food items, via normal hand to mouth activity which may include materials such as soil, ash, paint chips and plaster. Dermal absorption of inorganic lead through unabraded human skin is considered to be minimal.[4] After absorption, lead enters the circulation. The half-life of lead in blood is short and is said to be about 36 days, after which it gets deposited in soft tissues such as the kidney, liver, brain and finally the bone, where it remains for years.[5] Lead in blood is derived from tissue lead by mobilization. It is readily transferred to the growing infant during pregnancy and accumulates in fetal bone.[6]

Lead virtually affects all systems of the body.[7] It affects the haematopoetic system by effecting haem synthesis and erythrocyte formation and function. It also affects the central nervous and cardiovascular systems. Repro-

*Corresponding author: Department of Biochemistry & Biophysics, St. John's National Academy of Health Sciences, Bangalore –560 034. E-mail: anita.bijoor@gmail.com

**Administrative Management College, Bangalore

***Department of Biochemistry & Biophysics, St. John's National Academy of Health Sciences, Bangalore

ductive processes in both men and women are adversely affected.

A free radical is defined as the molecular species capable of independent existence and which contains one or more unpaired electrons. Such molecules are highly reactive. Antioxidants are cytoprotective enzymes or substances that quench an in-vivo free radical generating system. It is a scavenging system operating in close cooperation in the presence of oxidative stress. Chronically elevated calcium levels within the cells can initiate excess ROS (reactive oxygen species) generation by several means. These include activation of phospholipids and arachidonic acid cascade. Calcium can also activate superoxide production by polymorphonuclear lymphocytes which can be present in the post-ischemic neural tissues. By these means, calcium acts as a mediator of oxidative stress; and lead, by mimicking the action of calcium, behaves like a mediator of oxidative stress. Calcium chelators are known to inhibit lipid peroxidation.

Several metals can indirectly evoke the generation of ROS by way of disruption of normal calcium homeostasis. This has been seen at high levels of blood lead. Increased formation of reactive oxygen species and/or decreased antioxidant defense can be defined as oxidative stress, which is widely recognized as an important feature of many diseases.

Materials and Methods

Forty volunteers were taken for this study and they were divided into two groups –

Cases:

Inclusion Criteria: (n=20) Persons with exposure to lead and having low blood lead levels were taken as cases and EDTA samples were collected from them. These persons were employed in lead-based industries for periods varying from 6months to two years.

Exclusion Criteria: Persons working in lead based industries having high blood lead levels

Controls:

Inclusion Criteria (n=20) Individuals not exposed to lead were taken as controls and EDTA samples drawn from them.

Exclusion Criteria: Persons exposed to lead from any source

Estimation of lead using the ESA 3010 B lead analyzer –

Sample Preparation: Lead in blood may be bound to various binding sites on the cells. Bound lead will not be plated on to the electrode and thus will not be detected by the lead analyzer. The Metexchange reagent contains chromium chloride, calcium acetate, mercuric ion, a surfactant to minimize foaming, and acids and buffers for pH control. It is designed to rapidly displace lead from the bound condition so that all lead in the sample is present in the unbound state. Once the blood sample has been mixed with Metexchange reagent, it will be stable at room temperature for about one month. The sample is then analyzed on the ESA 3010 B which uses the principle of Anodic Stripping Voltammetry.

Method for the measurement of antioxidant activity –

A standardized solution of Fe – EDTA complex reacts with H_2O_2 by a Fenton type reaction leading to the formation of hydroxyl radical (OH). These reactive oxygen species degrade benzoate, resulting in the release of TBARS [14-16]. Antioxidants from the added sample of human fluid cause suppression of the production of TBARS. This reaction can be measured spectrophotometrically and the inhibition of colour development defined as the AOA.

Results and Discussion

In this study, we observed that the blood lead levels in the control group was in the range of 2.3 µg/dl – 6.4µg/dl, with a mean of 4.01µg/dl ± 1.5µg/dl (**Fig 1**), while in those with exposure to low lead levels, the blood lead ranged from 9.4µg/dl – 23.6µg/dl, with a mean of 14.15µg/dl ± 4.8µg/dl. The difference between these two groups is highly significant ("p" value < 0.005). The total antioxidant level was 1.5±0.76 mmol/l in the controls, and 0.78±0.25mmol/l in the lead exposed individuals, with a "p" value < 0.001, indicating a significant difference (**Fig 2**).

Quite a number of studies have shown that lead causes oxidative stress. Yiin and Lin demonstrated marked increase in the levels of malondialdehyde (MDA) when linoic, linolenic and arachidonic acids were incubated with lead.9 Increased contents of brain thiobarbituric acid reactive substances accompanied by altered antioxidant defense systems were confirmed by Adanaylo and Oteiza.10 Lead is shown to bind strongly to phosphatidylcholine membranes in vitro.11 An alteration of the

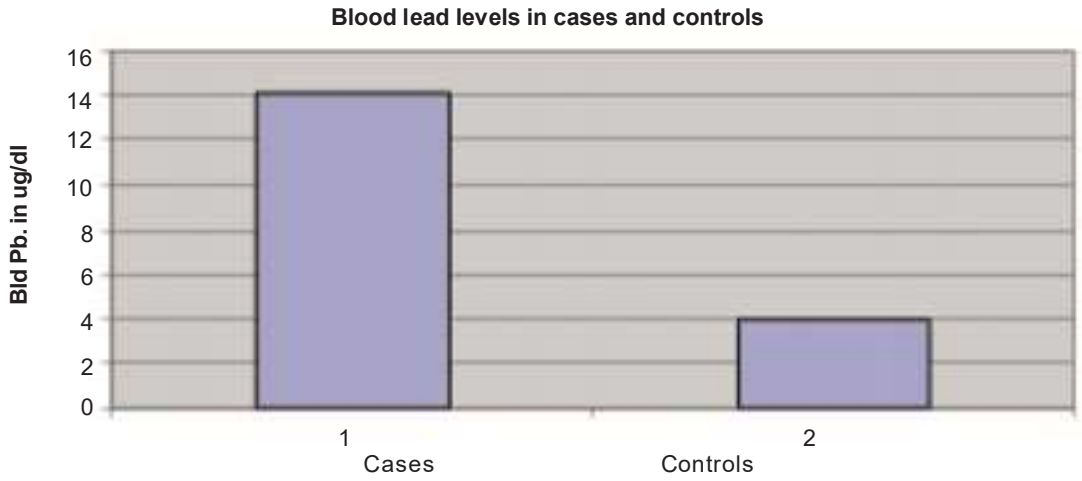

Fig. 1

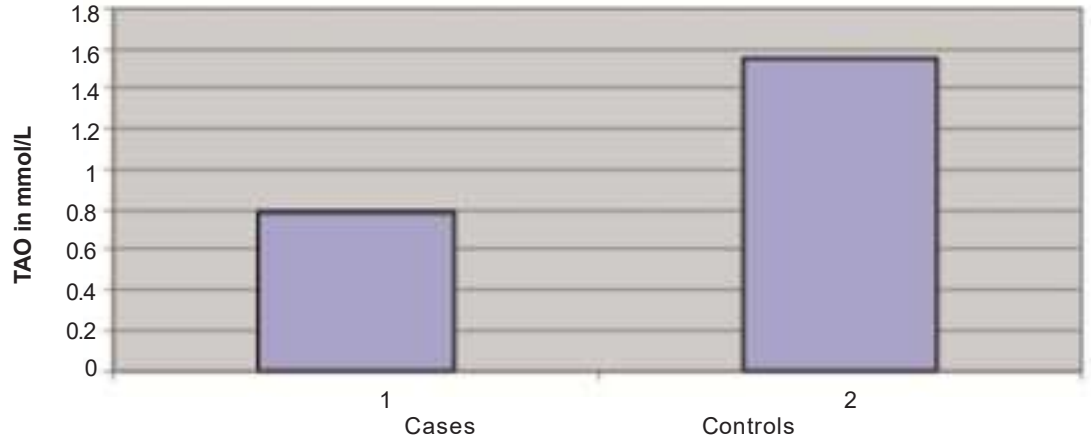

Fig. 2

composition of membrane phospholipids, indicating a decrease in the levels of phosphatidylcholine has been reported.12 At low levels of lead in blood, the enzyme d aminolevulinic acid dehydratase (ALAD) is inhibited, which causes an increase in the levels of ALA. ALA undergoes enolisation and autoxidation, which generates superoxide anions.[13,14]

Lead is also known to inhibit enzymes having functional –SH groups.15 Glucose-6-phosphate dehydrogenase (G6PDH) is one such enzyme, and this enzyme supplies most of the extramitochondrial NADPH. NADPH keeps GSH at a constant level. GSH is an important antioxidant. Thus lead, by its action on (G6PDH) reduces the level of GSH and in this way causes oxidative damage. Another antioxidant, lipoic acid, has been suggested to be able to abate the toxic effects of lead.[16]

Conclusion

At high levels, lead is known to cause oxidative stress and damage. This study was aimed at looking at the levels of the total antioxidant status, to see if they were affected by low levels of lead in blood. From the results we see that, even at blood lead levels as low as 14.15µg/dl ± 4.8µg/dl, which is seen in the general population, not exposed occupationally to lead, the total antioxidant status has decreased. Studies by Villeda et al support this finding.17 Studies have shown that antioxidants enhance the efficacy of lead antidotal treatments.[18]

This study supports the introduction of antioxidants along with chelation, as a regular treatment for lead poisoning cases, as well as a preventive measure against lead poisoning in persons specially exposed to different sources of lead.

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