

EFFECT OF CEMENT AND ITS PARTICULATE MATTERS ON LIPID PEROXIDATION AND ANTIOXIDANT STATUS IN THE MASONS

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ABSTRACT

Air pollution is a significant factor in morbidity and mortality within industrial society. Lipid peroxidation levels studied in workers involved in construction. The antioxidant enzymes like glutathione peroxidase, superoxide dismutase, catalase and the antioxidant scavengers like Vitamin A, E, C and reduced glutathione activities revealed highly fascinating informations. This study has provided further support that component of particulate matter causes oxidative damage. The particulate matter sample is more active and possesses the potential prooxidant activity. The present investigation is aimed to study the effect of cement dust on the Masons.

KEYWORDS: *Catalase, glutathione peroxidase, lipid peroxidation, reduced glutathione, Superoxide dismutase, vitamin A, C and E.*

Introduction

Hazardous substances are distributed widely in ecosystems due to diverse human activities such as energy usage, industrial enterprises, agriculture, etc. Mankind is faced with air pollution; the common gaseous pollutants are CO, CO₂, SO₂, H₂S, and NO, NO₂ while the common particulates are carbon and dust particles.

Silica present in the cement results in an activation of alveolar macrophages, recruitments of polymorpho nuclear leukocytes into broncho alveolar spaces and production of free radicals. Nitric oxide is one of the free radicals generated by bronchoalveolar lavage cell population following either LPS or silica exposure¹. For many centuries polluted air has been considered to be hazards to health and

concern has been mounting during, the last few decades about the possible deleterious effects of the introduction of increasing number exobiotic substances into the environment and that many of these compounds can chemically alter DNA which in turn can lead to deleterious consequences². Occupational exposure to cement increased lipid peroxidation but increased antioxidants levels in masons. Increased lipid peroxidation, seems to be responsible for the reduction in RBC and Hb³. The combined effect of the many different types of particulate in the airborne mixture, form the biological basis of the observed epidemiological findings⁴. Building material applied in the form of a soft paste with sand and water contains a mixture of sulfates, chlorides, carbonates and oxides of

sodium, potassium, calcium, fly ash, lead, arsenic and gases like silicon dioxide, aluminum oxide.

Antioxidants are substances which react chemically with free radicals and render them harmless and at the same time break the vicious circle, which involves the decomposition of fatty acid and proteins, the creation of new free radicals and eventual cell death. Thus they act as weapons for combating free radicals. The various antioxidants exert their effect by scavenging superoxide or by activating a battery of detoxifying or defensive proteins⁵.

The antioxidants in the body can be derived from exogenous (dietary) or endogenous (biosynthesized) sources. They are both enzymic and non enzymic. Radical scavenging antioxidants have been exposed to have novel function by which they regulate gene expression of the cell peroxidation is to decrease membrane fluidity, destabilizing membrane receptors⁶.

The current aim of the present investigation is to analyze the change in blood, lipid peroxidation by antioxidant scavenging enzymes and non-enzyme antioxidants (vitamin A, E, C and GSH) levels in Masons who are exposed to cement dust.

Materials and Methods

Blood samples were collected from Masons in and around Chennai. Venous blood was collected into tubes containing EDTA as anticoagulant. The plasma was separated and used for further analyses. In all samples, general parameter are studied include lipid peroxidation and antioxidants in RBC's, the antioxidant enzymes superoxide dismutase and glutathione peroxidase were assayed in hemolysate while catalase was assayed in red cell membrane. Antioxidants vitamin A and E

were assayed in plasma and vitamin C in whole blood. Malondialdehyde was assayed in plasma. The workers were classified according to the age group, group I (20-35 yrs) and group II (35 and above). The particulars like name, age, height, food habits were noted. Non-parametric studies included blood pressure, sugar, hypertension and cholesterol. The subjects were compared with normal subjects. Erythrocyte membrane was isolated with a change in buffer. Packed cells remaining after the removal of plasma was washed three times with isotonic saline to remove the buffy coat. 4 ml of packed cells were then washed 3 times with isotonic tris-HCl buffer, pH 7.4. Hemolysis was performed by precipitating out the washed red blood cell suspension into polypropylene centrifuge tubes which contain hypotonic buffer, pH 7.2. Erythrocyte ghosts were sedimented in a high speed refrigerated centrifuge at 20,000g for 40 minutes. The supernatant or hemolysate was decanted carefully and ghost button was resuspended by swirling. Sufficient buffer of the same strength was added to reconstitute the sedimented membrane to the original volume. The ratio of the cells to washing solution is approximately 4:3 by volume. The ghosts or the membrane were washed three times subsequent to hemolysis. The supernatant after the last wash was either pale pink or colourless. The pellet of the erythrocyte membrane was resuspended in 10ml of tris-HCl buffer, pH 7.4 and aliquots of this reconstituted membrane preparation was taken for various analyses.

2.1 Lipid Peroxidation

Lipid peroxidation was assayed as the thiobarbituric acid reactive substance, and malondialdehyde was used as a standard. Lipid peroxidation was measured in plasma and erythrocyte membrane. Lipid peroxide levels in plasma were determined by using thiobarbituric acid (TBA) reagent reaction. The

water soluble substances that react with TBA were eliminated by precipitating lipid peroxides along with plasma proteins in a phospho tungstic acid (PTA) system. The interference from sialic acid and bilirubin, both of which react with TBA, was avoided by performing TBA reaction in an acetic acid solution. Equal volumes of 0.67% thiobarbituric acid (aqueous solution) and glacial acetic acid were mixed. A stock standard solution of malondialdehyde was prepared in distilled water using 1, 1, 3, 3-tetraethoxypropane. This was stored at 4°C and diluted just before use such that the working standard contains 50 n moles/ml. To 0.5ml of plasma, 1ml of phosphotungstic acid was added, mixed well and centrifuged. The supernatant was discarded and the sediment was mixed with 2 ml of N/12 sulphuric acid and 0.3 ml PTA. The mixture was centrifuged and the sediment was dissolved in 4ml distilled water and 1ml of TBA reagent. The contents were heated in a boiling water bath for 60minutes. After cooling, 5ml of butanol was added and the contents shaken and centrifuged for 20minutes. The upper butanol layer was read at 515nm. A stock solution of malondialdehyde containing 2–10 n moles was taken mixed with TBA reagent and processed in the same manner as above. Lipid peroxidation values are expressed as n moles of MDA/dl plasma.

2.2 Antioxidant Enzymes

2.2.1 Superoxide Dismutase

Superoxidedismutase catalyses the dismutation of superoxide anion (O_2^-) to hydrogen peroxide and molecular oxygen. Superoxide dismutase in the hemolysate was assayed based on the oxidation of epinephrine–adrenochrome transition by the enzyme. 0.5ml haemolysate was diluted with 0.5ml of water. To this, 0.25ml of ethanol and 0.15ml of chloroform were added and

centrifuged. The enzyme activity in the supernatant was determined. To 0.5ml of the supernatant, 1.5ml of buffer was added. The reaction was initiated by the addition of 0.4ml of epinephrine and change in optical density per minute was determined at 480nm. The change in optical density per minute measured at 50% inhibition of epinephrine to adrenochrome by the enzyme is taken as one enzyme unit. The enzyme activity is expressed as units/mg hemoglobin.

2.2.2 Catalase

This enzyme catalyses the reaction and is assayed in the erythrocyte membrane suspension. 1ml of buffer, 0.4ml of water and 0.1ml of membrane suspension, were taken and brought to 37°C. The reaction was initiated by the addition of 0.5ml hydrogen peroxide and the reaction mixture was incubated at 37°C for 1 minute. Addition of 2ml of dichromate acetic acid reagent terminated the reaction. Standard hydrogen peroxide solution in the range of 4–20 μ moles were taken and treated in the same manner. The tubes were heated in a boiling water bath for 15 minutes, cooled and read at 510nm. Enzyme activity is expressed as μ moles of hydrogen peroxide utilised/mg protein/minute.

2.2.3 Glutathione Peroxidase

Glutathione peroxidase was assayed in the hemolysate. 0.2ml each of EDTA, sodium azide, glutathione and hydrogen peroxide incubation mixture containing 0.4ml of buffer and 0.1ml of lysate was incubated at 37°C for 10 minutes. The reaction was arrested by the addition of 0.5ml of TCA. Proteins were sedimented by centrifugation and 0.5ml of the supernatant was taken. 3ml of phosphate solution and 1ml of DTNB were added and the colour developed was read immediately at 412nm. Suitable aliquots of standard solution

were taken and treated in the same manner to obtain a standard curve for comparison. Glutathione peroxidase activity is expressed as $\mu\text{g GPx}$ utilised/mg haemoglobin/minute.

2.3 Antioxidant Scavengers

2.3.1 Vitamin A

Vitamin A was assayed in plasma. 3ml of light petroleum ether, 1.5ml of ethanol and 1.5ml of plasma was added and shaken well for about 10 minutes and allowed to stand to obtain a clear supernatant. 2.5ml of the supernatant as well as standard in the range 150-350 μg was used for the assay. 2ml of TCA-chloroform reagent was added and the optical density was read at 620nm within 15 seconds. Values are expressed as $\mu\text{ moles/L}$ plasma.

2.3.2 Vitamin E

Vitamin E was assayed in plasma after extraction. After saponification and solvent extraction of lipids to remove the interfering substances, α -tocopherol was estimated spectrophotometrically using bathopenanthroline reagent. 1ml of plasma, 2ml of 2% solution of pyrogallol in purified ethanol was mixed thoroughly. The mixture was heated at 70°C for 2 minutes. Add 0.3ml of saturated potassium hydroxide and mixed again and incubated at 70°C for 30 minutes. The tubes were shaken and centrifuged for 10 minutes to separate the two phases. The hexane extract (upper phase) was estimated for vitamin E. 3ml of hexane extract was evaporated to dryness under vacuum. 3ml of absolute ethanol was added to the residue. 0.2ml of 2% bathophenanthroline reagent was added and mixed thoroughly. Care was taken not to expose the tubes to direct light. 0.2ml of orthophosphoric acid reagent and ferric chloride reagent was added and mixed well. Standard vitamin E in the range of 50–100

μg was also treated in a similar way. The absorbance of the pink colour was read at 536nm against a reagent blank with ethanol. The values are expressed as $\mu\text{ moles/L}$ of plasma.

2.3.3 Vitamin C

Vitamin C (ascorbic acid) was estimated in an aliquot of whole blood after protein precipitation. 1ml of blood was mixed with 2ml 10% TCA and allowed to stand for 5 minutes. 1ml of the supernatant was taken after centrifugation and made upto 3ml with 5% TCA then treated with 0.1ml of the DTC reagent. The tubes were shaken well and incubated for 3 hours at 37°C. 0.75ml of 65% sulphuric acid reagent was added and the colour developed was read after 30 minutes at 520nm. Blank contained 3ml of 5% TCA. Standard ascorbic acid solution in the range of 10–50 μg was treated in the same way. Vitamin C values are expressed as $\mu\text{ moles}$ of ascorbic acid/L of blood.

2.3.4. Reduced Glutathione

Reduced glutathione in blood was measured. 0.2ml of blood was collected with EDTA as anticoagulant and then hemolysed with 1.8ml of EDTA solution and then 3ml of precipitating reagent was added and mixed thoroughly. It was allowed to stand for 5 minutes before filtering. Then 2ml of filtrate was added to 4ml of 0.3M disodium hydrogen phosphate solution and 1ml of DTNB reagent. Blank was prepared in a similar manner using distilled water instead of blood filtrate. A set of standards containing 20–70 μg of reduced glutathione were taken and made upto 2ml with water. To each tube 4ml of DSHP and 1ml of DTNB reagent was added and the optical density was read at 412nm. Values were expressed as $\mu\text{ moles/L}$ of blood.

2.4 Statistical Analysis

All quantitative measurements are expressed as mean $\pm$ SD for the different groups. Statistically significant differences between the different groups studied using student 't' test.

Results and Discussions

This investigation aims to study the effect of cement and its particulate matters on lipid peroxidation and antioxidant status in Masons. There is a significant association between particulate air pollution and biomarkers of oxidative stress. These associations suggest that personal exposure to fine particles in ambient air can lead to changes and damage to several components of blood.

Table 1:

Lipid Peroxidation Product (TBARS) Expressed as Malondialdehyde (MDA) in Plasma (Mean $\pm$ SD)

Subjects	Plasma (n moles / dl)
Healthy Control (n = 25)	0.013 $\pm$ 0.005
Group – I (n = 26)	0.088 $\pm$ 0.005*
Group – II (n = 27)	0.16 $\pm$ 0.105

Statistically significant differences are expressed as *P<0.001, when compared with normal subjects.

Table 1 shows that lipid peroxidation levels are significantly elevated for group I and II as compared to healthy controls. Peroxidation and formation of thiobarbituric acid reactive substance in tissue is induced by free radicals formed due to external stressors

lead to chain reaction, generation of free radicals acting on polyunsaturated fatty acid (PUFA) and lipid hydro peroxides. The higher susceptibility of erythrocytes to lipid peroxidation arises out of its high levels of molecular oxygen and iron content.³ has reported that occupational exposure to cement increased lipid peroxidation, but decreased antioxidant levels in Masons. Increased lipid per oxidation seems to be responsible for the reduction in red blood cells and hemoglobin.

Table 2:

Antioxidant Enzyme Activities in the Erythrocyte (Mean $\pm$ SD)

Subjects	GPx (lysate) (μ g GSH utilized/min /mg Hb)	SOD (lysate) (IU/mg Hb)	Catalase (membrane μ Mole H ₂ O ₂ consumed/min/mg protein)
Healthy Control (n = 25)	0.14 $\pm$ 0.025	0.11 $\pm$ 0.070	0.45 $\pm$ 0.115
Group – I (25 to 35 years) (n = 26)	0.11 $\pm$ 0.039*	0.10 $\pm$ 0.101*	0.55 $\pm$ 0.43*
Group – II (35 and above) (n = 27)	0.09 $\pm$ 0.01*	0.09 $\pm$ 0.347*	0.56 $\pm$ 0.40*

Statistically significant differences are expressed as *P<0.001, when compared with normal subject.

In table 2, antioxidant enzymes like glutathione peroxidase and superoxide dismutase showed a significant decrease, while catalase showed a marked increase on exposure to dust. Glutathione peroxidase is the main detoxification agent destroying peroxides in the erythrocytes⁷. The level of SOD was found to be increased 4 times. SOD depleted

cells have been found to be more sensitive to lipid peroxidation and hemolysis than normal cells confirming the protective role of SOD from oxidative damage. Catalase is inactivated by hydrogen peroxide and superoxide radicals. Reduction in residual catalase levels confer increased susceptibility for the cells to undergo lipid peroxidation as compared to controls.

Table 3:

Antioxidant Scavengers Levels in Blood (Mean $\pm$ SD)

Subject	Vitamin ($\mu\text{mol/L}$ plasma)	Vitamin ($\mu\text{mole/L}$ plasma)	Vitamin ($\mu\text{mole/L}$ whole blood)	GSH ($\mu\text{Mole/L}$ whole blood)
Healthy Control (n = 25)	0.05 $\pm$ 0.142	0.40 $\pm$ 0.286	0.03 $\pm$ 0.014	1.17 $\pm$ 0.487
Group –I (n = 26)	0.06 $\pm$ 0.040*	0.26 $\pm$ 0.043*	0.02 $\pm$ 0.012*	1.27 $\pm$ 0.344*
Group –II (n = 27)	0.08 $\pm$ 0.04*	0.38 $\pm$ 0.115	0.02 $\pm$ 0.01*	1.52 $\pm$ 0.322*

*Statistically significant differences are expressed as *P<0.001, when compared with normal subjects.*

Antioxidant scavengers are shown in table 3, vitamin E and C are decreased compared to the normal subjects. But vitamin A and reduced glutathione levels are elevated to the healthy control. Vitamin A, E, C is important antioxidant vitamins that can directly scavenge free radicals. The β carotene functions as a radical trapping antioxidant⁸. The efficient biological radical trapping antioxidant activity of β -carotene was demonstrated through its inhibition of lipid peroxidation induced by xanthine oxidase system⁹. Ascorbic acid functions as an important component of cellular defense against oxygen toxicity and

lipid peroxidation caused by free radical mechanism¹⁰. GSH is the principal cellular non-protein sulfhydryl compound it has variety of functions in bio-reduction and detoxification process¹¹.

Lipid peroxidation levels studied in workers involved in construction were found to be increased. The antioxidant enzymes like glutathione peroxidase, superoxide dismutase were decreased, while catalase levels are elevated. The antioxidant scavengers vitamin E and vitamin C are decreased while vitamin A and reduced glutathione levels are elevated. This study has provided further support that component of particulate matter causes oxidative damage. The particulate matter sample from the Masons, which proved approximately three times as active and possess the potential pro oxidant activity.

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