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EFFECT OF OCCUPATION ON LIPID PEROXIDATION AND ANTIOXIDANTS' STATUS IN MASONS

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Abstract : Effect of occupation on haematological factors, lipid peroxidation and antioxidants' status was studied in masons and compared with normal subjects. Red blood corpuscles (RBC), haemoglobin (Hb), Vitamin C, Vitamin E, β-carotene levels and glutathione peroxidase (GSHPx), superoxide dismutase (SOD) and catalase (CAT) activities decreased. Thiobarbituric acid reacting substances (TBARS) level increased. Occupational exposure to cement increased lipid peroxidation but decreased antioxidants' levels in masons. Increased lipid peroxidation seems to be responsible for the reduction in RBC and Hb.

Key words : masons lipid peroxidation antioxidants haematological factors

INTRODUCTION

In an industrial setting, inhalation and skin contact are the most important route of entry of chemicals into the body to produce lethal effects (1). Occupationally masons are continuously being exposed to cement dust which contains silicates and calcium oxide as its major constituents. Occupational exposure to silicates has been reported to cause lung diseases like pneumoconiosis, irritant bronchitis (2) and also augment free radicals production (2, 3). Exposure to calcium oxide causes irritation of eyes, skin, mucous membrane, brittleness of nail and skin burns (4, 5). Though reports are available on various aspects of health and disease status in cement workers (2–5), no study has been made on lipid peroxidation and antioxidants' status in masons. Hence we have investigated TBARS, as a measure of lipid peroxidation and enzymatic and nonezymatic antioxidants' status as defence mechanism against lipid peroxidation. In addition RBC and Hb also investigated to know the effect of lipid peroxidation.

METHODS

The study comprised 35 (male) masons, from in and around of Chidambaram, a Taluk Headquarters of Cuddalore District, Tamil Nadu, India. They were compared with age matched normal subjects (n = 35). All the participants were examined by a physician and were free from chronic illness and were normotensives. They ranged in the age group between 20 and 30 years and had more than 5 years experience in their occupation. Oral consent was obtained from all the participants.

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Venous blood was collected after an overnight fast into heparinised tubes. Total RBC was enumerated by haemocytometer (6). Plasma was separated by centrifugation at 3000 rpm for 15 minutes and stored at 4°C until analysis. The packed cells were washed thrice with physiological saline and stored at 4°C until analysis. We estimated haemoglobin (7), thiobarbituric acid reactive substances (8), reduced glutathione (9), glutathione peroxidase (GSHPx, 10), superoxide dismutase (SOD, 11), catalase (12), vitamin C (13), vitamin E (14) and β-carotene (15). Statistical analysis was done by Student's t test (16) and values were expressed as means ± SD.

RESULTS

Haematological factors and biochemical parameters of masons and normal subjects are given in Table I. Total RBC count, haemoglobin, vitamin C, vitamin E, β-carotene levels and SOD, glutathione peroxidase and catalase activities decreased significantly. TBARS level increased significantly in the experimental group when compared to normal. Reduced glutathione did not show any variation.

TABLE I: Haematological factors and plasma biochemical parameters in normal subjects and masonry workers in the age group 20–30 years.

S. No.	Parameters	Normal n = 35	Experimental n=35
1.	RBC (million/cu.mm)	5.11±0.15	3.8±0.33*
2.	Hemoglobin (%)	84.22±2.85	76.75±4.80*
3.	TBARS (nmol/ml)	1.68±0.62	6.89±2.11*
4.	Glutathione peroxidase (μg GSH/min/mg Hb)	12.43±2.33	6.98±2.76*
5.	Reduced glutathione (mg/dl)	24.82±4.06	23.24±5.26
6.	Vitamin C (mg/dl)	1.28±0.17	0.85±0.21*
7.	Vitamin E (mg/dl)	2.09±0.3	0.91±0.3*
8.	β-Carotene (mg/l)	0.55±0.07	0.15±0.08*
9.	Superoxide dismutase (E, units/mg Hb)	3.55±0.6	1.68±0.42*
10.	Catalase (EM, μmol H_2O_2 consumed/s/mg protein)	7.45±0.87	4.85±1.0*

Values represent mean ± SD *P<0.001
E: Erythrocyte Em: Erythrocyte membrane

DISCUSSION

Our study showed a decrease in RBC count and Hb level which may be due to chronic exposure to cement. Cement contains calcium hydroxide as its important constituent (2) and chronic exposure to calcium hydroxide has been reported to decrease RBC count and haemoglobin level (17). Micronutrients such as iron, vitamin C, etc. were reported to be essential for the synthesis of RBC and haemoglobin. The decrease in the level of RBC and haemoglobin may also be due to decrease in the level of vitamin C which is observed in our study.

Lipid peroxidation is a chain reaction initiated by the attack on the membrane lipids by free radicals that has sufficient reactivity to abstract a hydrogen atom from the methylene group. This leaves behind an unpaired electron on the carbon atom. The carbon radical is stabilised by molecular rearrangement to produce conjugated diene, which then reacts with an oxygen molecule to form a peroxy radical. Peroxy radical can form cyclic peroxide and cyclic endoperoxide. Fragmentation of these peroxides leads to formation of aldehyde like 4-hydroxy-2, 3-transonenal and malondialdehyde (MDA) (18). These aldehydes react with thiobarbituric acid forming TBARS. 4 hydroxy-2, 3-transnonenal attacks essential sulfhydryl group of many proteins (19) and MDA attacks

amino groups of the protein molecule to form intra and intermolecular cross links (20). Increase in the level of TBARS may be due to exposure of masons to cement which may lead to increased production of free radicals (2, 3). Further masons are constantly exposed to sunlight and hence the electromagnetic radiation from the sunlight can also cause increased free radical formation in the system (21) thereby increasing TBARS.

The first line of cellular defence against radicals consists of enzymes such as superoxide dismutase, catalase and peroxidases. These enzymes react directly with the oxidizing radicals to yield non-radical product. Selenium dependent glutathione peroxidase removes both H_2O_2 and lipid peroxides by catalysing the conversion of lipid hydroperoxides to hydroxy acids in the presence of reduced glutathione. Decrease in the activity of glutathione peroxidase in our investigation may be due to exhaustion or inactivation of the enzyme by reactive oxygen species since oxidative damage to haemoglobin and cell membrane has been reported to reduce the activity of glutathione peroxidase (19, 20).

Superoxide dismutase is a copper containing enzyme, occurring widely in cells and tissues such as erythrocytes, liver and brain. It is a free radical metabolising enzyme, catalysing dismutation of superoxide anion to hydrogen peroxide. This protects the cell membrane from damage by highly reactive oxygen species. The low activity of SOD in the experimental group could be due to inactivation of the enzyme by cross linking or damage to DNA (19, 20) by lipid peroxidation and hence causing decrease in expression of the enzyme.

Catalase catalyses the dismutation of hydrogen peroxide. Our study shows a significant decrease in the activity of membrane bound catalase. This decease in the activity of CAT could be due to increase in MDA which can cross link with amino group of protein to form intra and intermolecular cross links thereby inactivating several membrane bound enzymes (23).

Glutathione plays a major role in cellular protection against oxidative damage. Condition that perturb intracellular levels of glutathione have been shown to result in significant alteration in cellular metabolism. In our study, reduced glutathione level in the plasma is not altered though there is an elevation of lipid peroxidation. It has been reported that the levels of total glutathione (GSH + GSSG) in the plasma do not increase at the onset of exercise for the trained animals and the possible reason might be that endurance training which has resulted in an increased ability for rapid glutathione cellular turnover and uptake by those tissues where it plays a vital role during periods of increased physical activity. During the initial stages of physical exercise, endurance-trained animals show none of the evidence (as judged from the glutathione status of tissues) of increased oxidative stress as shown for untrained animals undergoing comparable workloads (24). In our study also, as masons are continually involved in physical activities the system would have adopted for increased synthesis and repletion so that the plasma levels are kept unaltered as in the case of endurance-trained animals.

A principal biological function of vitamin C is to scavenge oxygen free radical and in that it is converted dehydroascorbate. In animal tissue the dehydroascorbate is reduced back to ascorbate by glutathione resulting in recycling of ascorbate. Vitamin C has been reported to contribute up to 24% of the total peroxy radical trapping antioxidant activity in human plasma (25). Decrease in the level of vitamin C compared with normal may be due to increased utilisation to trap the oxy radicals.

Vitamin E is a well accepted as natures' most effective lipid soluble, chain breaking antioxidant in the biological system protecting cell membrane from peroxidative damage. Decrease in its level may be due to excessive destruction of vitamin E by free radicals or due to decrease in the level of vitamin C, since vitamin C and vitamin E are synergistic antioxidants (26).

Beta-carotene acts as a radical trap whereby the chain carrying peroxy radicals add covalantly to the conjugated system of β-carotene, thus breaking the chain reaction of lipid peroxidation (27). Decrease in the level of β-carotene in our study may be due to increased utilization to trap the reactive oxygen species.

Thus our study shows that masons have increased lipid peroxidation and decreased antioxidants revealing, that occupation of masons has a definite effect on these parameters and they need supplementation of micronutrients.

REFERENCES

1. Shukla NP. Industrial emissions and particulate control. Environment protection information. *Ind J Envi Prot* 1995; 15 : 2.
2. Ghio AJ, Kennedy JP, Schapija RM, Crumbliss AL, Hoidal JR. Hypothesis: Is lung disease after silicate inhalation caused by oxidant generation? *Lancet* 1990; 20 : 927–929.
3. Yang CY, Huang CC, Chang IC, Lee CH, Tsai JT, Ko YC. Pulmonary functions and respiratory symptoms of portland cement workers in Taiwan. *Kao Hsuch Ko Huch Tsa Chih* 1993; 9 : 186–192.
4. Grant WM. Toxicology of the Eye. 3rd edition. Springfield, Illinois, Charles C Thomas 9186; 173–180.
5. Fisher AA. Contact Dematitis. Lea & Febizer, Mosby Philadelphia 1973; 17–20.
6. Gopalen MK. Notes on clinical Lab. technology. Laboratory procedure and their interpretation. 3rd edition, edited by K.M. Samuel Publishers, Madras. 1982; 104–110.
7. Drabkin DL, Austin JM. Spectrophotometric studies; spectrophotometric constants for common haemoglobin derivatives in human, dog and rabbit blood. *J Biol Chem* 1932; 98 : 719–733.
8. Nichans WG Jr, Samuelson B. Formation of malondiadehyde from phospholipid arachidonate during microsomal lipid peroxidation. *Eur J Biochem* 1968; 6 : 126–130.
9. Ellman GL. Tissue sulphydryl groups. *Arch Biochem Biophys* 1959; 82 : 70–77.
10. Rotruck JT, Pope AL, Ganther HE, Swanson AB. Selenium : Biochemical roles as a component of glutathione peroxidase. *Science* 1973; 197 : 588–590.
11. Kakkar P, Das B, Visvananthan PN. A modified spectrophotometric assay of SOD. *Ind J Biochem Biophys* 1984; 21 : 130–132.
12. Sinha KA. Colorimetric assay of catalase. *Anal Biochem* 1972; 47 : 289–394.
13. Roe JH, Kuether CA. Detection of ascorbic acid in whole blood and urine through the 2, 4 DNPH derivative of dehydroascorbic acid. *J Biol Chem* 1943; 147 : 399–407.
14. Baker H, Frank O, De Angelis B, Feingold S. Plasma tocopherol in man at various time after ingesting free or acetylated tocopherol. *Nutr Res Int* 1980; 21 : 531–536.
15. Bradley DW, Hombeck CL, Cecil L. Clinical evaluation of an improved trifluoroacetic acid

(TFA) micromethod for plasma and serum. *Vitamin A Biochem Med* 1973; 7 : 78–86.

16. Snedecor GW. Statistical methods. Ames : Iowa State College Press 1948; 182–184.
17. Wands RC. Alkaline materials. In : Industrial Hygiene and Toxicology Clayton GC, Clayton FE, (eds.) 3rd edition, New York, Wiley-Interscience 1981; 2B : 3052–3058.
18. Wills ED. Evaluation of lipid peroxidation in lipids and biological membranes. In : Biochemical Toxicology Snill K and Mullock B (eds), IRL Press, 1987; 127–157.
19. Pfafferoot C, Meiselman HJ, Hochstein P. The effect of MDA on erythrocyte deformability. *Blood* 1982; 59 : 12.
20. Svadlenka I, Davidkova E, Rosmus J. Interaction of MDA with collagen. *Z Lebensm Unters Forsch* 1975; 157 : 263.
21. Halliwell B. Free radicals, antioxidants and human disease : Curiosity, cause or consequence. *Lancet* 1994; 344 : 721–724.
22. Flohel J. Glutathione peroxidase fact and fiction. In : Oxygen free radicals and tissue damage. *Excerpta Medica* 1979; 95–122.
23. Kikugawa K, Kosugi H, Asakura T. Effects of MDA, a product of lipid peroxidation on the function and stability of heamoglobin. *Arch Biochem Biophys* 1984; 229 : 7.
24. Alexandre T Quaintanilha. Effects of exercise-induced oxidative stress on liver glutathione. CRC Handbook of Free Radicals and Antioxidants in Biomedicine 1988; 11 : 133–143.
25. Chatterjee IB, Anuradha Nandi. Ascorbic acid : A scavenger of oxyradicals. *Indian J Biochem Biophys* 1991, 28 : 233–236.
26. Infers H, Sies H. The production by ascorbate and glutathione against microsomal lipid peroxidation is dependent on vitamin E. *Eur J Biochem* 1988; 174 : 353–357.
27. Burton W, Ingold KU. Beta Carotene : An unusual type of lipid antioxidant. *Science* 1984; 224 : 569–573.