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OXIDATIVE STATUS IN WORKERS ENGAGED IN RECYCLING OF PLASTIC: OCCUPATIONAL HAZARD

PRAKASH CHANDRA SATI*, RAVI KAUSHIK, VINOD KUMAR, FARAH KHALIQ AND NEELAM VANEY

Department of Physiology,
University College of Medical Sciences (University of Delhi) and GTB, Delhi, India

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Abstract : Recycling plastic industry is on rise. Plastic waste in environment is a pollutant so recycling of it can save environment and is economical too. However its recycling is associated with harmful effects on workers engaged in it. The present study was designed to elucidate the role of free radicals and cytochrome c in pathogenesis of polypropylene associated diseases. Thirty workers from plastic recycling factory occupationally exposed to polypropylene between the age of 18-40 years and working for atleast 8 hours a day for more than a year but less than 10 years were selected for the study. A trend in increase of FRAP and decrease of MDA was observed but they could not reach the level of significance. The level of serum cytochrome c, which is an indirect marker of oxidative stress, was also detectable in only two subjects. Since the number of subjects in the study was less, the result needs to be confirmed on larger number. More over cause of pulmonary dysfunction and carcinomas in these workers needs to be investigated.

Key words : oxidative stress, recycling plastic industry, cytochrome c

INTRODUCTION

The global usage of plastic is increasing, as it is inert and cheap material. Plastic product consists of various types of component depending upon the use like teflon, polyvinylchloride, polypropylene, polyethylene, polystyrene. This is a cross sectional study done in workers who were engaged in recycling process of polypropylene. The raw material (plastic scrap of polypropylene) is made to pass through a machine (Pilot plant) in which after various processing, finally melted polypropylene is given the shape of granules that can be used for plastic product synthesis. Various gases like CO, NO_2, SO_2, polypropylene etc. are produced in the environment, which can be inhaled by workers, engaged in the recycling process. Polypropylene is also a product of combustion of organic matter (biomass burning, motor vehicle exhausts and

*Corresponding Author : Dr. Prakash Chandra Sati, Department of Physiology, University College of Medical Sciences (University of Delhi) and GTB, Delhi, India; E-mail: pc_mamc@yahoo.co.in, Phone No.: 09873799525

tobacco smoke) and is released during production and use. The most probable route of exposure to humans is by inhalation (1). Once absorbed, a major route of metabolism for propylene is through the cytochrome P-450 system to propylene oxide, a known carcinogen in experimental animals (IARC group 2B) (2). Cytochrome P- 450 enzymes in both the liver and nasal epithelium can convert propylene to its toxic metabolite.

The current literature supports the possibility that xenobiotic exposure results in increased reactive oxygen species (ROS) or free radical generation (3-6). Excessive free radical or reactive oxygen species generation has been implicated as an initiator of apoptosis (7) and number of events associated with immune cell regulation, such as respiratory burst, intracellular calcium regulation and cytochrome c release from mitochondria. Also, apoptosis has been found to be inhibited by the addition of antioxidants (8). Oxygen free radicals (OFRs) are involved in the toxicity of numerous chemicals and also pathogenesis of many diseases. These free radicals are scavenged by the reducing agents present in the body. Lipid peroxidation products i.e. Malondialdehyde (MDA), Ferric Reducing Ability of Plasma (FRAP) are important markers to assess Oxygen free radicals (OFRs) generation. Cytochrome c, marker of apoptosis, has been found to be associated with decreased levels of GSH (9).

In the era where plastic use is increasing day by day, there is need to study occupational hazard associated with its recycling. So, this study has been designed to elucidate the role of free radicals and cytochrome c in pathogenesis of polypropylene associated diseases.

MATERIALS AND METHODS

Thirty workers from plastic recycling factory occupationally exposed to polypropylene between age group 18-40 years and working for atleast 8 hours a day for more than a year but less than 10 years were short listed. The made of machine used for polypropylene recycling was such that from starting point till melting zone, it was a closed structure and gases produced in this zone get sucked into pollution control gadget while after this zone the melted polypropylene come out from where vapors got escape into the surrounding environment in which workers were working. Thirty age and sex matched subjects not exposed to polypropylene served as controls. Subjects suffering from chronic illness like diabetes, hypertension, and heart disease were excluded from the study. Subjects who were taking antioxidants prescribed by doctor were not included in the study. Informed written consent was taken from subjects before starting the study. The study was approved by institution Research and Ethical Committee. Detailed history along with general physical examination and systemic examination for each system was carried out.

Blood sample collection

Blood (5ml) was collected in EDTA (Ethylenediaminetetraacetic acid) containing and plain vial for the estimation of FRAP, MDA and cytochrome c. Serum was then separated from the clotted blood in plain vial by centrifuging the sample at 3000 rpm for 10 min. The entire tests were performed within 12 hours of sample collection.

Measurement of Ferric reducing ability of plasma

Ferric-reducing ability of plasma (FRAP) in plasma was determined by measuring the ability of plasma to reduce Fe^{3+} to Fe^{2+} by the method of Benzie et al (10). The complex between Fe^{2+} and 2,4,6- tri(2-pyridyl)-1,3,5-triazine (TPTZ) gives a blue colour with absorbance at 593 nm. Concentration of FRAP was expressed in µmol/l.

Measurement of lipid peroxidation

The lipid peroxide levels in serum were measured using a thiobarbituric acid reactive substances (TBARS) assay, which monitors MDA production based on the method of Satoh et al (11). The MDA - TBA adduct formation was measured spectrophotometrically at 532 nm. The concentration of MDA was expressed as nmol/ml.

Estimation of Cytochrome C

Cytochrome C was estimated by using Bender Medsystems kit, Austria (Human Cytochrome C BMS 263) as per manufacture's protocol. Briefly, in the appropriate wells of microtiter plates, serum samples, standard and controls along with biotin-conjugate antihuman cytochrome c monoclonal antibody were added (except blank) and incubated for 2 hour at room temperature. The solutions were then aspirated, and wells were washed four times. Streptavidin-HRP was added to each well except blanks and incubated for 1 hour at room temperature. 100µL of stabilized substrate solution was added into the wells and incubated approximately for 10 min. at room temperature. After adding stop solution to each well, the absorbance at 450 nm was recorded using a micro plate reader (Biorad-680, USA). The readings were plotted on standard curve thus obtained and expressed in ng/ml.

Statistical analysis

Analysis was done by Microsoft Office Excel 2007. Unpaired 't' test was performed to compare oxidative stress between polypropylene exposed subjects and controls.

RESULTS

The anthropometric measurements in two groups (polypropylene exposed subjects vs. controls) are shown in Table I. No significant difference was observed between the two groups for age, height and weight (Table I).

On comparing the two groups (polypropylene exposed subjects and controls), MDA was observed to be high while FRAP was low but none of the value could reach the level of significance ($P<0.05$) (Table II).

TABLE I: Anthropometric measurements of controls and subjects working in recycling plastic factory.

Parameter	*Controls (n=30)*	*Exposed Subjects (n=30)*
Age (year)	29.85±6.04	31.23±5.67
Height (cm)	168.82±5.18	169.21±5.23
Weight (kg)	66.18±8.62	63.72±9.26
BMI (Kg/m^2)	23.45±4.24	22.40±3.96

Data presented are mean±SEM.0

TABLE II: Oxidative stress of controls and subjects working in recycling plastic factory.

Oxidative stress variables	*Controls (n=30)*	*Exposed Subjects (n=30)*	*P value (unpaired 't' test)*
MDA (nmole/ml)	2.46±0.68	2.48±0.79	0.91
FRAP (µmole/L)	399.43±36.16	340.81±68.78	0.15

Data presented are mean±SEM. MDA: Malondialdehyde, FRAP: Ferric-reducing ability of plasma.

The level of serum cytochrome c was detectable in only two subjects exposed to polypropylene while in control its levels were under detection limit (0.05 ng/ml).

DISCUSSION

An earlier study done by Khaliq et al (12) has shown deranged pulmonary function in workers recycling polypropylene. Chronic exposure of propylene is associated with increased inflammation of nasal mucosa characterized by an influx of lymphocytes, macrophages and granulocytes into the submucosa and granulocytes into the lumen (13). A significant infiltration of neutrophils, macrophages, mast cells during pathogenesis of interstitial pulmonary disorder and the increased release of IL-8 and TNF-á has been observed in Flock workers (14). The increased release of IL8 and TNF-α are indicative of ongoing pro-inflammatory process in these workers. Incidence of colorectal carcinoma has been found to be increased in polypropylene workers (15). In the present study workers from plastic factory recycling polypropylene were selected, who were exposed to polypropylene fumes working in the factory for at least one year (6 days a week and 8 hours a day). Earlier pulmonary functions were found to be deranged in these workers (12), it was hypothesised that oxidative stress might be one of the reason for this, as recent studies have demonstrated that important changes may occur in oxidative status and immune system in host after xenobiotics ingestion (3-6). Free radicals have been implicated in pathways of metabolism of drugs and environmental chemicals. Interestingly inhalational exposure to styrene, ethylene glycol and other solvents has been found to be associated with increased free radicals imposing oxidative stress on cell (16, 17). In the present study a trend in increase of FRAP and decrease of MDA is observed but they could not reach the level of significance. The level of serum cytochrome c was also detectable in two subjects. Since the number of subjects in the study was less, the result needs to be confirmed on larger number. Moreover the most sensitive employees may have changed their occupation, so not evaluated in the present study. The study can be extended to over a large group of workers with ambient level of various constituents in air. It suggests that fumes in recycling plastic factory (polypropylene) do not raise the free radicals. The flock might be able to provoke inflammatory response by sticking to alveoli but vapors are not found to cause oxidative stress in cells. Specific inhalation challenges with polypropylene heated to 250°C resulted in a late asthmatic reaction. FEV1%, which was found to be 43% in worker, engaged in polypropylene industry return to 89% after 2-month leave from job (18). Heated polypropylene might induce the respiratory damage but the author gave no explanation. This might be due to damage to mucosa by high temperature.

REFERENCES

1. Central control Pollution Board. Plastic waste management issues & options. Delhi: *Minist Environment Forests Bull* 2007; 4–32.
2. Maples KR, Dahl AR. Blood levels of propylene oxide during propylene inhalation and effect on hepatic and nasal cytochrome P-450 concentrations. *Drug Metab Dispos* 1991; 19: 835–837.
3. Videla LA, Tapia G, Varela P et al. Effects of

acute gamma-hexachlorocyclohexane intoxication in relation to the redox regulation of nuclear factor-kappaB, cytokine gene expression, and liver injury in the rat. *Antioxid Redox Signal* 2004; 6: 471–480.

4. Banerjee BD, Seth V, Ahmed RS. Pesticide-induced oxidative stress: perspectives and trends. *Rev Environ Health* 2001; 16: 1–40.

5. Koner BC, Banerjee BD, Ray A. Organochlorine pesticide-induced oxidative stress and immune suppression in rats. *Ind J Exp Biol* 1998; 36: 395–398.

6. Yarsan E, Tanyuksel M, Celik S, Aydin A. Effects of aldicarb and malathion on lipid peroxidation. *Bull Environ Contam Toxicol* 1999; 63: 575–581.

7. Corcoran GB, Fix L, Jones DP et al. Apoptosis: molecular control point in toxicity. Toxicol Appl Pharmacol 1994; 128: 169–181.

8. Atroshi F, Biese I, Saloniemi H et al. Significance of apoptosis and its relationship to antioxidants after ochratoxin A administration in mice. *J Pharm Pharm Sci* 2000; 3: 281–291.

9. Ghibelli L, Coppola S, Fanelli C et al. Glutathione depletion causes cytochrome c release even in the absence of cell commitment to apoptosis. *FASEB J* 1999; 13: 2031–2036.

10. Benzie IF, Strain JJ. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement for total antioxidant power and ascorbic acid concentration. *Meth Enzymol* 1999; 299: 15–27.

11. Satoh K. Serum lipid peroxide in cerebrovascular disorders determine by a new colorometric method. *Clin Chim Acta* 1978; 90: 37–43.

12. Khaliq F, Singh P, Chandra P, Gupta K, Vaney N. Pulmonary Functions in Plastic Factory Workers: A Preliminary Study. *Ind J Physiol Pharmacol* 2011; 55: 60–66.

13. Quest JA, Tomaszewski JE, Haseman JK, Boorman GA, Douglas JF, Clarke WJ. Two-year inhalation toxicity study of propylene in F344/N rats and B6C3F1 mice. *Toxicol Appl Pharmacol* 1984; 76: 288–295.

14. Atis S, Tutluoglu B, Levent E et al. The respiratory effects of occupational polypropylene flock exposure. *Eur Respir J* 2005; 25: 110–117.

15. Acquavella JF, Douglass TS, Phillips SC. Evaluation of excess colorectal cancer incidence among workers involved in the manufacture of polypropylene. *J Occup Med* 1988; 30: 438–442.

16. Sati PC, Khaliq F, Vaney N et al. Pulmonary function and oxidative stress in workers exposed to styrene in plastic factory. *Hum Exp Toxicol* 2011; 30: 1743–1750.

17. Dlugosz A, Sawicka E, Marchewka Z. Styrene and ethylene glycol have a synergetic effect on lipid peroxidation that is better protected than repaired by CoQ10. *Toxicol In Vitro* 2005; 19: 581–588.

18. Malo JL, Cartier A, Pineault L, Dugas M, Desjardins A. Occupational asthma due to heated polypropylene. *Eur Respir J* 1994; 7: 415–417.