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RESEARCH ARTICLE

An attempt to search the health status of garage workers---A neglected part in India

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Abstract

In many developing countries awareness of occupational hazards is still in the budding stage. The objective of this study is to find out the occupation related health hazards in automobile repair shops specially for those workers who randomly handled toxic waste water during battery wash, car wash, and oil grease wash. This water is polluted by lead and cadmium and in the course of their work they are exposed to these two exogenous elements which are agents of DNA damage. From the study area lead (Pb) , Cadmium (Cd) concentration of garage waste water was estimated. whose, whole blood Pb and Cd as well as Selenium(Se) concentration is estimated by Graphite Furnace Atomic Absorption and by ED-XRF technique both in control and occupationally exposed workers, and DNA damage was measured by single cell gel electrophoresis (alkaline comet assay). Results shows there is a significant ($p<0.001$) difference between the blood lead and cadmium concentration in control and workers. Workers were also found suffering from Se deficiency and this may be the cumulative reason for their higher level of DNA damage (significantly high, $p<0.001$) and put them in more susceptible condition

INTRODUCTION

What makes a car stand out in the crowd of millions of others? Is it the style? Is it the paint? Or is it the price? All of these can make a car appeal to people, but do they take the time to think of the work and efforts involving making a car look the way it does? The professionals in auto industry know what it takes to make a car look and stay brand new. This is a profession that most people do not attach any importance to. They do not know the health risks and hazards involved in completing very simple to complex jobs. Many workers in this field are exposed to health and environmental risks everyday on the job. Due to the amount of potential health hazards involved in the auto repairing and maintenance profession, workers and technicians need to be educated in the regulated use, storage, and disposal of hazardous chemicals, and in proper safety procedures to promote a safe work environment.

Automobile workers are in the increased risk of handling carcinogenic substances (Ghhebreyohannes.2005). In the recent years, especially for the last two decades number of garages or different automobile shops have increased(Shende.2004) . In the many low income group countries illegal or unauthorized garages or number of temporary

garages are high, and most of the workers are illiterate and work without any safety measure. Though for the last decade use of unleaded gasoline is mandatory worldwide but there are different types of mobile oil and grease which is rich in Pb (2006) and battery liquid is one of the main sources of both Pb and Cd(Palus,etal.2003). Workers are randomly handling these liquid and grease (containing Lead Nathanael) and oils without any heavy gloves, but mostly with bare hands and previous researchers observed that this grease and battery waste can penetrate skin(Dongre, etal.2011) Different routes of exposure of Pb are through inhalation, ingestion and minor amount through skin absorption. During servicing of battery, workers are directly exposed to Lead which is carcinogenic and also responsible for chromosomes damage(Leston,etal.2010) . In most studies, lead caused micronucleus formation, chromosomal aberrations, and DNA damage, though studies on sister chromatid exchange gave conflicting results. Previous researchers have already established that automobile workers have high blood lead level and delta-aminolevulinic acid were higher while the activity of delta-aminolevulinic acid dehydrates were also decreased(Papanikoulou,etal.2005). Parallel to this situation Cd is also a xenotoxic as well as cytotoxic element and automobile workers are in the increased risk of Cd exposure(Schwertle,etal.2010) Previous researchers have already proved that Cd induced tail moment in comet assay (DNA damage study) and it is also responsible for oxidative stress. Burning of automobile tires is also responsible for increased Cd concentration in automobile workers (Shakya,etal.2006) . Apart from this car body spray is water soluable and contains Cd and Pb (Vitayavirasuk,2005) . This paint is toxic to human skin and also penetrates skin (Hino,etal.2008) . The major difficulty in small scale automobile repair shops this, they are not generally grouped and perform those duty which they find compatible (Annavajhula,2010) . Specifically workers who handled only paint waste water or who handled only battery waste water is literally impossible to seek out. So, in this present study we wish to investigate the overall health hazards of automobile repair shop waste water on workers. Opposed to the role of Pb and Cd, Se is one of the essential trace element and important as an antioxidant (Mehdi,etal.2013) . Se is conjugated with proteins to make selenoproteins, which are important antioxidant enzyme. There is evidence that Se deficiency may contribute to a form of heart disease, hypothyroidism and weakened immune system. Its deficiency makes body more susceptible to illness.

In this present study the main goal is to find out the health effect of garage waste water, relatively rich in Pb and Cd and manually washed out during car repairing and servicing. This water is some times also contaminated with metallic color spray which is rich in Cadmium and workers are exposed to this and ingest unknowingly through having food in unwashed hands during their occupation hours. Kolkata is a megacity in eastern India, with a large population and rising pollution. The present study the whole blood Pd, Cd and Se concentration is measured and correlated with DNA tail moment to evaluate the possible adverse health effects of the workers.

Materials and Method

Study area

The selected study area was Central part of Kolkata (India), and only those particular areas were selected where persons were involved in different temporary workers/ heavy laborious works including automobile works.

Analysis of waste water

To analyze the waste water (garage waste water), the followings steps are observed

Sample collection

Water sample was collected from garages (two different spots in a single area), and collected in sealed container. They were stored in 4ºC for further analysis.

Estimation of Heavy Metals

Water samples were digested by Nitric Acid Perchloric Acid Mixture(3:1) and heavy metals, Lead(Pb) and Cadmium(Cd) was estimated in Graphite Furnace Atomic Absorption spectrophotometer(AAS)(perkin- Elmer) for both garage waste water which is manually washed out and normal tap water.

Questionnaires preparation and Blood Collection

The subjects to be eligible for the study must be Indian adult men and have been living for a continuous time (at least 5 years) in central Kolkata region and working strictly on garage work, and handeled garage waste water, and before their occupational carrier never exposed to any such polluted or toxic water knowing fully. Candidates(garage workers of Kolkata),and control population(living in the same area but never being exposed to such polluted and toxic water) asked to answer questions in order to verify any possible additional exposure through ingestion of food, smoking, use of medicine, and other. The information was particularly useful in the evaluations of final data. 41 exposed male workers are selected for our experiment who are engaged in garage workers and during their working periods handled garage waste water, as water after battery washing, water after car painting and mixture of the overall waste water in bare hand but must have no continuous addiction to either alcohol or tobacco, use

hygienic toilet (corporation toilet), drink corporation purified water, and also have a sound monthly income. After Ethical committee clearance 5 ml of blood was drawn from each subject under proper medical supervision and care. The blood obtained were kept in a citrate (3.8%) container and stored in 4 º C refrigerators for further experiment. Similarly venous blood (5ml) was taken from 25 healthy male and this served as control group.

DNA damage Study in Human Peripheral Blood

The alkaline single cell gel electrophoresis (comet assay) was performed by a small modification of Tice *et al*1988 . 10 µL of fresh peripheral blood was diluted with 1ml of Phosphate Buffer Saline. After dilution, 100 µL were mixed with 100 µL of 20% low melting agarose (gene I), 75 µLof this was layered on 1% low melting agarose (LMP agarose). After preparation, the slides were immersed in lysis solution and refrigerated at 4ºC for 4 hours. From this lysis solution (pH>12), the slides (Blue star) were placed in alkaline solution (pH ≈11), for electrophoresis sides were kept for 15 minutes.This is essential for salt equilibration. After this, electrophoresis was continued for 30 minutes at 30V and 330 Ma at 4ºC.experiment was carried out at dark condition. For neutralization, Slides are then coated with 0.4M Tris buffer (pH 7.5) for 10 minutes, this step was repeated. The cells were stained with 80 micro liters Ethidium Bromide (20 microgram/ml water) for 5 minutes and dipped in distilled water for few seconds. The slides were dried and covered with a covers lip. Comet images were analyzed using a fluorescence microscope (Leica) equipped with CCD camera. One hundred cells were examined randomly for each sample for . The tail movement of DNA was measured by using Comet Score Software (TriTEk Corporation).

Estimation of whole blood Selenium (Se) concentration

The blood samples (2ml) were lyophilized at -80◦ C. The blood samples were dried and powdered using mortar and pestle. 150 mg of powdered sample was taken and compressed to form a pellet of 13mm diameter, with a table top pelletizer using 100 kg/cm2 pressure. The pallets were subjected to energy dispersive X-ray fluorescence (EDXRF) technique. The concentration of Se in the blood samples was measured by EDXRF (Jordon Valley, Netherlands) which consists of an oil-cooled Rh anode X-ray tube (maximum voltage 50 kV, current 1 mA). The measurements were carried out in vacuum. Different filters were used between the source and sample for optimum detection of elements. The concentration of trace in the blood samples was measured by EDXRF technique –a highly sensitive, non destructive technique .The main principle of the EDXRF is based on the principle of bombardment of X-rays onto the samples followed by analysis of the characteristic X-rays using a liquid-nitrogen-cooled Si (Li) detector having a resolution 150 eV at 5.9 KeV. The quantitative estimation of the trace elements were carried out by analyzing the spectra using the nEXT software integrated with the EDXRF system. The spectrometer was standardized for quantification of the elements using different Standard reference materials (SRM) such as SRM1515-Apple leaf; SRM 1577b- Bovine liver from National Institute of Standards and Technology and IAEA-A-13- Animal Blood reference material from Analytical Quality Control Services, International Atomic Energy Agency (IAEA).

Estimation of heavy metals from blood samples

1mL blood sample was digested by nitric acid perchloric acid mixture (1:3) following little alteration of the method of Ibito Cynthia Nkolika, 2009 and analyzed the concentration of Pb and Cd by Aomic Absoprtion spectrophotomerer (Make: Perkin-Elmer; Model Analyst 200).

Result

Normal tap water which is used by the control population shows no detectable Pb and Cd level. But garage waste water was found to be highly contaminated by Pb and Cd with average concentration is 4.22 mg L^{-1} and 1.32 mg L^{-1} .Both are considerably higher than the recommended Pb and Cd level in discharge waste water (1 mg L^{-1}) and this toxic water is always handled with the garage workers in bare hands which reflects in their blood Pb and Cd concentration.

In control population it is observed, average Pb concentration is 0.201±0.13 mg $L^{-1}$and average Cd , Se concentration is 0.003±0.004 mg $L^{-1}$and 3.25±1.6 mg L^{-1}respectively. Average tail moment is 1.44±0.33.In control population (n=26) 50% are below detection limit for Cd concentration measurement but all are above detection limit during Pb measurement. In the study group i.e the garage workers highly exposed to Pb and Cd contaminated water during their working periods , elevated level of blood Pb and Cd concentration have been observed (0.4±.184 mg L^{-1} , 0.02±0.022 mg L^{-1}) respectively and have a depleted blood Se concentration 0.98±0.77 mg L^{-1} and the tail moment is 5.97±1.2. In garage workers blood Pb level is ~50% higher than the control population and it is highly significant ($p<0.001$) .The same event is followed by the Cd concentration , garage workers have 85%

more Cd compared to their corresponding control population and it is statistically significant too ($p< 0.001$). Garage workers show a considerable depletion in their Se concentration ~70% which is statistically significant($p<0.001$) followed by the intensity of DNA damage , which is also ~76% high in garage workers and this is also statistically significant ($p< 0.001$). Peripheral blood DNA adducts have been considered an acceptable surrogate for target tissues and possibly predictive of cancer risk (table 1)

The regression study among tail moment and Pb and Cd is shown accordingly

Tail moment=5.378 (<0.01) + 1.097(0.405) Pb + 8.910(0.413) Cd

Here, R^2: 0.03, p-value=0.492, H_o:$\beta_{1=}\beta_2=0$

So, the p value corresponding to H_{o1}:$\beta_{Pb}=0$ is 0.405 which means we accept the hypothesis. For Cd the p-value is 0.413 which means that we accept the hypothesis that the slope corresponding to Cd is 0. Finally the p value for the test H_{o2} :β_{Cd}- $\beta_{Pb}=0$ is 0.492 which mean that both Pb and Cd is not significant in explanation of DNA damage in garage workers.

Table 1 Levels of Blood Lead, Cadmium and Selenium in Blood, (TM= Tail Moment)

Population	Pb (M±SD) in mgL^{-1}	Cd (M±SD) in mgL^{-1}	Se(M±SD) in mgL^{-1}	TM(M±SD)
Control	0.201±0.13*	0.003±0.004*	3.25±1.6*	1.44±0.33*
Garage	0.4±.184*	0.02±0.022*	0.98±0.77*	5.97±1.2*

*indicates values are statistically significant ($p< 0.001$)

Discussion

On Garage waste water is highly contaminated by Cd and Pb because automobile shops are important sources of anthropogenic Pb and Cd contamination. The present study attempts to the interpret that genotoxicity results, especially DNA damage by the presence of chemical contaminants like lead and cadmium in automobile wash water in garages in comparison to normal tap water. Generally formations of comets indicate primary DNA lesion leading to genetic damage in any cell. According to the isoelectric point of skin, it is negatively charged (Baroli,etal.2007). Thus, heavy metals can get attached to skin and a very small amount can penetrate skin. As most of the workers are poorly educated and sense of health awareness is very poor, they ingest these heavy many during the course of their work as they don't follow the safety guideline . It is noteworthy to mention here that though the tap water samples handled by control population have heavy metal ,Pb, below detection limit (0.001ppm) .The control population selected were non addicted, have no history of routine drug intake and occupational hazards, yet the lead concentration in their blood samples were far above detectable limit, which could be due to the fact that control population sample taken were urban population residing in proper Kolkata for a long period, where automobile pollution and others sources of pollution were found to be higher (Asheville,2005) . Previous Studies have already pointed out that in South east Asian Countries non occupational exposure to Pb is high due to dietary habit and due to the induction of air pollution (Ikeda,etal.2000) . Automobile workers without any history of addiction were still higher blood Pb than the control population could be due to the fact that the water and different oil sample contains higher lead concentration and during work lead particles get attach with their skin. It can penetrate the skin and may enter the body through oral routes when they have food without proper hand washing. In case of control population though it is significantly less than the exposed workers, Cd concentration was found higher than the detection limit. This may be due to some food habit. Besides, as workers work for a long time in a close environment where presence of chemicals like spray car color may increase the Pb and Cd concentration in ambient air. Workers inhalation of the same is may be one of the reason for elevated Pb and Cd concentration in their blood (Brooman) .

Parallel to these facts, it is also observed that garage workers have very poor in whole blood Se concentration. This is may be due to continuous exposure to direct smokes (as they randomly inhale different types of smokes) in their work place(Kummer,etal.2009) . Previous researchers already suggested that increased Cd concentration replaces the Se level which may result in dysfunction in immunosystem by stimulation of oxidative stress and premature aging(Badiello,1996) . But in control population though blood Cd concentration is above detection limit , the concentration is quite low and opposed to garage workers their blood Se concentration is significantly high which neutralizes the toxic effect of Cd .Se is a micronutrients which is known to provide ability to defense against the cellular and tissue dysfunction and damage. Se has similar chemical characteristics to sulfur and therefore, it has a high affinity toward many heavy metals such as Pb and Cd. Pb and Se has antagonistic behavior (Zhang,1998) . In

these workers Pb accumulation in blood is high and due to less Se concentration Pb accumulation in blood may have been accelerated. Similarly Se deficiency in garage workers is responsible for Cd accumulation as the solubility of Cd in biological system has been retained to be increased in Se depleted condition (Giorgio,2005). As they are very poor in Se, so strong Cd-Se interaction is not possible and the workers is to a vulnerable condition as it is known that Se could ameliorate Cd-induced toxicity since it reduced malondialdehyde (MDA) levels and increased the activities of antioxidant enzymes in tissue. This high Pb and Cd level and low Se concentration may be responsible for the significantly high tail moment in the garage workers (Bruce,2001) .

Though these garage workers have high tail moment though the effect of Pb and Cd in blood is not elevated enough to explaining the DNA damage. According to previous literature the probable reason for this result is , as the workers are not only exposed to a toxic level of Pb and Cd, but there are lots of other xenotoxics which are directly handled by then regularly in addition to Pb and Cd .It is possible that there is a synergistic action of other elements along with Pb and Cd cumulatively responsible for this DNA damage in workers and thus the regression is accepting the hypothesis.

Exposure to Polycyclic Aromatic Hydrocarbons (PAHs) has been reported in a number of occupational settings. Several carcinogenic agents have been identified in contaminated garage air; PAHs are among the most prevalent air pollutants. Automobile processes produce many hazardous chemicals such as metal dusts, toxic fumes, silica, polycyclic aromatic hydrocarbons and metals that are released into the workplace and may have damaging effects on the health of the workers. PAHs are produced from the incomplete combustion of organic materials and have been considered as a possible cause of lung cancer among garage workers (Ramalangam,2013) .

Previous studies have demonstrated that increased concentration of PAHs in the workplace environment could induce the formation of DNA damage of exposed workers at road paving, bitumen and fire-proof plants . An increased risk for oxidative damage has been found in PAH exposed coke-oven workers. It has already been reported that PAH are associated with single and double standed DNA break. PAHs induced significant DNA damage in blood cells

of automobile emission inspectors has also been reported(Eunha,etal.2006) . Apart from PAH, Benzene is randomly found in automobile workshops and is also a sound agent to enhance the DNA damage and DNA–protein crosslink

It was not possible in the present report to identify what were the putative chemicals which caused the increased tail moment in automobile workers.workers exposed to a single chemical would be ideal for the examination of the effects of DNA damage of the specific chemical. As workers are often exposed to more than one chemical, biomarkers of exposures to each specific chemical may also be measured in comet assay studies. Then,

whether there is any correlation between the biomarker level of each chemical and the extent of DNA damage can be examined to clarify the role of each chemical

The main limitation of the present study was that not measure individual exposures using personal air sampling devices. More valid estimates, such as risk estimate, of the relationship between DNA damage and occupational exposures to specific agents can be obtained from studies using personal air sampling and other biomarkers to quantify the exposure.

Thus, in short garage workers are in a high risk situation, as they are continuously exposed to Pb, Cd contaminated water and chemicals and other toxic chemicals and their blood Se concentration is depressed them in putting a very susceptible condition and future researchers should try to find out their complex heath as well as xenotoxic effects.

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