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RESEARCH ARTICLE

CYTOGENETIC AND HAEMATOLOGICAL STUDIES ON SHIPYARD WORKERS OCCUPATIONALLY EXPOSED TO ASBESTOS IN CHENNAI SHIPYARDS

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Abstract

carcinogens in their complex occupational environment. The study made an attempt to investigate exposure to asbestos from the workers engaged in different shipyards at Chennai, Tamil Nadu and to thereby determine the genotoxic effects associated with it. The study included 30 shipyard workers and 30 control subjects with similar mean ages and smoking prevalence was analyzed for cytogenetic damage in blood leucocytes by Peripheral blood leucocyte culture (PBLC) and Micronucleus assay (MN) and the haematological parameters were assessed along with the mineralogical assessment for asbestos from the sputum samples. The mean total chromosomal aberrations, MN and haematological (RBC, WBC, Total haemoglobin concentration, MCHC, PCV, MCV) concentrations in shipyard workers were significantly higher than in controls. Linear regression analysis was performed and the parameters of the experimental and control subjects were compared and statistically significant difference was observed between the two groups. The results of our study indicated that the genetic damage was detectable in shipyard workers occupationally exposed to asbestos and also demonstrate the high sensitivity of PBLC and MN to evaluate early oxidative effects induced by exposure to asbestos even at low doses and confirm the suitability as a biomarker of asbestos exposure.

Shipyards workers are exposed to many known

Introduction

The ship construction sector is one among the fast developing industry in India with thousands of workers being employed as welders, fitters, painters, sandblasters, plumbers, machinists, electricians, sail makers, blacksmiths and carpenters. Shipyards are dangerous construction zones with many worker hazards. Shipbuilding and ship repair activities include painting, surface preparation, tank cleaning, and all aspects of marine construction (U.S National library of medicine 2010). The production and repair process involves numerous treatments such as painting and coating, metal plating and surface finishing, solvent cleaning and degreasing, machining and metal working, welding, vessel cleaning, and fiber glass operations. The consumption of various products results with production of different forms of pollutants (solid, liquid, and gaseous). The amount of outcome such as wastes and pollutants is a major risk from an environmental and ecological point of view. A basic questionnaire was designed to analyze the health defects of the workers working in the various shipyards of Chennai and based upon their information and past literatures, asbestos was identified as the conflict of interest. Chrysotile asbestos is a convicted mass killer (Lemen, 2004). Although India banned the import of asbestos waste in 1998, 74,398 kg were from Russia between April 2003 and February 2004 (Subramanian and

Madhavan, 2005). In the shipyards asbestos is used for insulation, roofing and sealing because of its heat resisting property. Asbestos mineral is composed of fibrils (about 0.03 µm diameter) that are packed together. When the mineral is broken apart mechanically, the material separates primarily between fibrils and the resulting fibres are usually bundles of fibrils. (Baron, 2001).When products containing asbestos are damaged or disturbed, tiny asbestos fibers are released into the air and can be inhaled into the lungs. Eventually, these asbestos fibers can cause serious illnesses, such as mesothelioma, asbestosis and lung cancer. (http://EzineArticles.com/?expert=Tara_Nagel). The shipyard workers mostly involve in scrapping and molding works during which the asbestos particles are disturbed. A sputum sample detected for asbestos bodies suggests a significant lung asbestos burden (Teschler et al., 1996). Lebovitis et al., 1983 reported about the risk information of smoking and asbestos and concluded that smoking cessation remains the only most effective preventive health measure to be adopted by an asbestos exposed individuals. Trosic et al., 1991 demonstrated the association between the haematological status of workers with occupational asbestosis and it is reflected in the peripheral blood parameters. Peripheral blood leucocyte culture is also good and a simple biomarker to indicate the chromosomal damage in the cells and it is a traditional parameter employed in the classical genetics. Micronuclei (MN) in peripheral blood lymphocytes appear to be one of the most suitable biomarker that can be used to measure the cytogenetic damages. MN originates from chromosome fragments or whole chromosomes that are not included in the main daughter nuclei during nuclear division. MN reflects chromosome damage and may provide a marker of early carcinogenesis (Fenech et al., 1999). Based on the above facts the present study was designed to analyze the following objectives to examine the [a]. Genetic alterations in the ship yard workers of Chennai and their exposure risks to asbestos [b]. To identify the chromosomal alterations in the ship yard workers and to determine whether there is sub-clinical evidence of cytological damage in the workers. [c].To use relevant multiple biomarkers to perform the cytogenetic and haematological analysis, for the conformation of toxic substances in the subjects exposed to the concerned area. [d].To identify the asbestos particles from the sputum of exposed workers through mineralogical analysis. [e] The study was to create awareness among the Chennai shipyard workers and the public to be vigilant about the polluted environment around them and though asbestos is prevented still it persists in the variable forms and needs more implementations to be eliminated at a full scale.

Methodology:

Subject recruitment and sample collection

Totally 60 samples including experimental subjects (n=30) and controls (n=30) were recruited in the present investigation. Equal numbers of control subjects were selected, who were normal, healthy and they did not expose themselves to any kind of chemicals or radiation. Controls were matched to the respective subjects in terms of age. Both the experimental subjects and controls were sorted out based on the age wise manner (±2 years relaxed) and were divided into group I (35 and below) and group II (36 and above). Smoking status was also analyzed based on the pack years as, above and below 10 years of smoking. All subjects were informed of the objective of the study and their consent was obtained. Blood (7.0 ml) was collected from the subjects aseptically using heparinised disposable syringes. The sample blood was brought to the culture laboratory by placing in an icebox to perform the hematological and cytogenetic analysis. The work was performed in accordance with the ethical standards of the 1964 Declaration of Helsinki.

Sputum collection:

The sputum sample was collected after inhalation of an isotonic saline aerosol generated by an ultrasonic nebulizer for 10 min and a short expiratory physiotherapy, in a carefully cleaned flask containing 10 mL of 10% formalin, previously filtered through a membrane filter (Cellulose acetate membrane filter of 0.45 mm pore size). A morning sputum sample was collected at home by each subject over the next 2 days in two different flasks cleaned and prepared with 10% formalin in a similar way, for mineralogical analysis.

Sputum analysis for asbestos:

Sputum analysis was conducted according to the method described by (Paris et al., 2002). Sputum sample was allowed to react at room temperature for 1–2 h with 20 mL of freshly filtered sodium hypochlorite (commercial laundry bleach) with gentle shaking until complete digestion of the organic material. The suspension was filtered through a membrane filter and the membrane was washed (five times with distilled water) and dehydrated using isopropyl alcohol. The membrane was then cleared using toluene (5 mL) and mounted on carefully cleaned microscope slides. Slides were examined by light microscopy (x100 magnification).

Chromosome Aberration Assay

Chemical reagents were purchased from Sigma Chemical (St. Louis, MO) and colcemid, was obtained from Gibco Laboratory (Grand Island, NY). According to standard procedures of our laboratory (Moorhead et al. 1960) the blood samples were established for cell cultures. In brief, 0.5 ml whole blood was added to 4.5 ml RPMI 1640 medium supplemented with 10 % fetal bovine serum, 2 mM L-glutamine, 1 % streptomycin–penicillin antibiotics, and 0.2 ml reagent-grade phytohemagglutinin and then incubated at 37°C. At 71 h, cultures were treated with 0.1 µg/ml colcemid to block mitosis. Lymphocytes were harvested at 72 h by centrifuging cells to remove culture medium (800–1,000 rpm/7 min) and adding hypotonic solution (KCl 0.075 M) at 37°C for 20 min to swell the cells after treatment twice with fixative (methanol and acetic acid [3:1 vol/vol]). Cytological preparations were made by placing two to three drops of the concentrated cell suspension onto slides wetted with ice-cold acetic acid (60 %). Slides were carefully dried on a hot plate (56°CC for 2 min). For CA analysis, 100 complete metaphase cells of the first cell cycle were evaluated under a microscope (9100) to identify numerical and structural CAs according to the International System for Human Cytogenetic Nomenclature. The data was registered and later transferred to a computer file.

MN Assay:

MN assay was performed using the cytochalasin B technique (Fenech and Morley 1985). Lymphocytes were cultured in the same manner as described previously. Cytochalasin B (6 µg/ml) was added at 44 h of incubation. After a total incubation time of 72 h at 37°C, cells were harvested by centrifugation, rinsed, and submitted to mild hypotonic treatment followed by fixation with methanol and acetic acid. Slides were prepared according to standard cytogenetic procedures and stained with 4 % Giemsa. Slides were coded and scored by light microscopy at 9400 magnification. For each experiment, 1,000 binucleated lymphocytes with well-preserved cytoplasm were scored. MN was identified according to the criteria of Fenech, et al. (2003).

Haematological parameters

Total Erythrocyte (RBC) Count (Samuel, 1986):

This method involves an accurate dilution of 5 to 6 drops of blood with diluting fluid which is isotonic with the blood and prevents coagulation. The diluted blood is placed over the specified grids of the haemocytometer which is marked with Neubauer counting chamber. The number of cells counted in a circumscribed volume is enumerated under a microscope with immersion oil.

Calculation:

No. of cells counted = ------ x;
Area of small squares = 1/400 sq.mm;
Depth of the squares = 1/10 sq.mm;
Volume of the square= 1/400 x 1/10
Dilution of blood = 1/200

Total number of RBC's
= x/80 x 4000/1 x 200/1
= ---------- cells/cu.mm of blood.

Total Leucocyte (WBC) Count (Samuel, 1986):

For leucocytes cell identifictaion a dilution of 1 in 20 is used and the diluents (WBC diluting fluid (Turk's fluid) is usually the one which destroys the red blood corpuscles. The diluted blood is placed over the grids of the haemocytometer and the number of cells is counted under a microscope.

Calculation:

Area of four large squares = 4 sq.mm;
Depth of the chamber = 0.1 mm
Volume of fluid in four squares = 0.1x4 = 0.4cu.mm
Dilution of fluid = 1/20
No. of WBC/cu.mm blood

$$= \frac{\text{No. of cells counted x dilution x chamber}}{\text{Area of squares}}$$

Total Haemoglobin Content (Oser, 1965):

0.5 ml of EDTA is added to blood samples; the standard was prepared from haemoglobin (Hb) powder (0.1mg/ml) and distilled water is used as blank. 2.5ml of haemoglobin reagent was added and mixed well. The test tubes were kept at room temperature for 20 minutes and the optical density (O.D) was measured at 540 nm.

Calculation:

Haemoglobin content (g/ 100 ml)

$$= \frac{\text{O.D of sample}}{\text{O.D of standard}} \text{ X mg Hb of standard X } 0.15$$

Packed cell volume (Samuel, 1986):

Oxalated blood was taken and mixed thoroughly by repeated inversion. Wintrobe's tube was filled with oxalated blood up to the 100 mark, the original column of blood in the tube being 100mm. The volume of packed cells can be used directly as percentage.

Mean Corpuscular Volume (Samuel, 1986)

This was performed to determine the average volume of a single red cell in cubic microns.

Calculation: $\frac{\text{Packed cell volume X 10}}{\text{Red blood cells in millions/cu.mm}} = \text{MCV}$

Statistical analysis:
Linear regression analysis was performed by SPSS version 20 for the variables of the experimental subjects by taking the dependent variable as years of exposure in the shipyard and the independent variables were age, smoking, chyrsotile fiber numbers from the sputum and total CA and MN. Mean and standard deviation calculations were carried out for the haematological parameters.

Results:
Demographic details: Among the 60 experimental and control samples, group I comprised of 11 members (36.66%) and group II comprised of 19 members (63.34%). The health defects of the subjects are also depicted and it was documented from the questionnaire reported from the workers. Of them the respiratory defects were more prominent comprising of 46.6%. Dermal defects were observed in 10 members comprising of 33.3 % and reproductive defects were seen in 6 members comprising of 20 %. Smoking status of the subjects was based on the pack years, as above and below 10 years of smoking. Among group I members, 4 were below 10 years, 3 were above 10 years and 4 were non-smokers. Among group II members, 4 were below 10 years, 7 were above 10 years and 8 members were non-smokers. Asbestos particle detected from the sputum samples was mainly chrysotile and the total numbers of persons detected for asbestos are depicted in the Table 1.

Sputum Analysis:
Among the 60 sputum slides analysis, along with the asbestos bodies, some macrophage cells and other debris was observed. 20 slides were detected positive for asbestos bodies. The examined asbestos bodies were of the chrysotile forms. Among the 20 slides, the experimental subjects were reported for 17 (5 from group I and 12 from group II) and the rest 3 was from the control subjects. Though the control groups were none exposed to the shipyards, it could have been through the other sources of environmental pollution.
More number of particles was detected from the group II experimental subjects and the older age groups who had more years of exposure in the shipyards had more asbestos particles. The presence of asbestos bodies in the sputum appears to be significantly related to either years of exposure or age and smoking status. The relationships between the presence of asbestos bodies in the sputum and individual parameters (age and exposure parameters) were analyzed on the population of subjects by linear regression analysis and the mean and SD values are depicted. In the experimental subjects, the duration of exposure was considered as the dependent variable and in the control group age was considered as the dependent variable since the control subjects were not exposed to shipyard environments (Table 2).

Chromosomal and MN analysis:
Peripheral blood leucocytes cells were examined for major and minor chromosomal aberrations and MN cells independently. The major and minor chromosomal aberrations were summed up as total chromosomal aberrations and the mean and SD values are indicated separately for each age group for the experimental and control subjects in the Table 2. Total CA was more commonly observed in the group II experimental groups than in the other groups and this is probably due to the exposure to the asbestos particles and hence there was a subclinical evidence for the relationship between asbestos exposure and cytogenetic damage. Similarly more number of micronuclei was detected from the exposed individuals than the control ones which indicated the occupational exposure to asbestos particle and the damage to the genetic material. The comparison between the exposed and the control groups for MN values are depicted in the Table 2 as mean and SD values. Linear regression analysis showed a statistically a significant difference between the experimental and control individuals. The predictive variables were strongly correlated with each other (age with duration of exposure, smoking, asbestos bodies in sputum, total CA and MN) and the analysis confirmed the results of linear regression, since the R values (0.959; 0.982; 0.821; 0.885) were nearing to 1 (Table 2).

Haematological parameters:
The various haematological parameters were analyzed as any changes in the body would be well revealed through the blood haematological parameters. As said above, significant differences was observed between the experimental and control subjects and the RBC, total haemoglobin, MCHC and PCV values were significantly lower in the experimental subjects whereas the WBC and MCV values were found in an increased range than the normal which indicated the diseased state in the exposed individuals. The values are depicted in the form of Mean and SD in Table 3. These changes indicate the occupational exposure to asbestos particles in their bodies

Table 1: Demographic features of Experimental subjects

Particulars		Total no of samples	Percentage (%)
Age Groups			
Group I (< 35 years)		11	36.66
Group II (> 36 years)		19	63.34
Health defects			
Respiratory defects		14	46.66
Dermal defects		10	33.34
Reproductive defects		6	20.0
Smoking status			
Group I	Below 10 years	4	36.36
	Above 10 years	3	27.28
	Non smokers	4	36.36
Group II	Below 10 years	4	21.05
	Above 10 years	7	36.84
	Non smokers	8	42.11
Asbestos particle(Chrysotile) detected			
Group I	Control subjects	1	9.09
	Experimental subjects	5	26.31
Group II	Control subjects	2	18.18
	Experimental subjects	12	63.15

Table 2: Linear regression results for the experimental and control subjects

Variables	Experimental subjects*		Control subjects#	
	Group 1 (Mean ± SD)[95% CI]	Group 2 (Mean ± SD)[95% CI]	Group 1 (Mean ± SD)[95% CI]	Group 2 (Mean ± SD)[95% CI]
Age	(29.54±5.1)[-0.15 - 0.76]	(46.89±8.3)[0.46 – 1.10]	(29.54±5.0)	(46.63±8.2)
Yrs of Exp	(5.81±3.86)	(23.31±8.31)	NA	NA
Smoking	(2.09±0.83)[-2.16 – 1.60]	(2.05±0.91)[0.16 – 2.4]	(2.09±0.83)[-3.22 – 6.40]	(2.05±0.91)[-2.5 – 2.40]
Chrysotile fibers	(0.63±0.80)[-0.89 - 3.31]	(1.89±1.99)[-1.025 – 0.41]	(0.09±0.30)[-1.90 – 15.47]	(0.15±0.50)[0.03 – 9.11]
Total CA	(1.63±0.67)[-1.57 - 5.45]	(4.10±1.62)[-1.57 – 1.42]	(0.54±0.82)[3.95 – 15.34]	(1.68±0.94)[4.87 – 10.94]
MN	(3.36±1.36)[-1.30 - 2.36]	(7.63±3.23)[0.02 – 1.49]	(0.81±1.25)[-8.16 - 1.90]	(1.21±0.97)[-3.89 – 2.04]
R	0.959	0.982	0.821	0.885
R^2	0.920	0.965	0.674	0.783
Adjusted R^2	0.841	0.951	0.566	0.722
F	11.564	71.504	6.208	12.665
P	0.009	0.000	0.006	0.000

** Years of exposure (Yrs of Exp) to asbestos fibers (Chyrsotile) were considered as the dependent variable for the regression analysis*

 # Age was considered as the dependent variable for the regression analysis.

CA – Chromosomal aberrations; MN – Micronucleus cells

Table 3: Haematological parameters of controls and experimental subjects

Particulars and groups	RBC(million cells/cu.mm)	WBC (Thousand cells/cu.mm)	Total Haemoglobin (g/100ml)	MCHC %	PCV %	MCV (fl)
Controls I	4.9 ± 0.26	8572.72 ± 142.06	13.93 ± 0.27	35.0 ± 1.18	41.81 ± 1.25	81.18 ± 0.75
Experimentals I	4.13 ± 0.11	8945.45 ± 160.75	11.47 ± 0.59	27.0 ± 1.73	39.09 ± 1.70	82.90 ± 1.37
Control II	4.88 ± 0.17	8510.52 ± 128.64	14.05 ± 0.46	34.68 ± 1.15	41.89 ± 1.14	81.26 ± 0.99
Experimentals II	4.03 ± 0.16	9157.89 ± 167.71	11.24 ± 0.47	26.0 ± 1.63	38.94 ± 1.22	83.21 ± 0.91

RBC – *Red blood corpuscles (Normal value: 4.5 – 5 million cells/cu.mm);*

WBC – *White blood corpuscles (Normal value: 8000 – 10,000 cells/cu.mm);*

Total Haemoglobin – *(Normal value = 12-14 g/ 100 ml)*

MCHC – *Mean cell haemoglobin concentration (Normal value: 32 – 36 g/dl)**;***

PCV – *Packed cell volume (Normal value: 40 to 45 %)*

MCV – *Mean corpuscular volume (Normal value = 80 to 100 femtolitre)* ***; fl -*** *femtolitre*

Discussion:

Exposure to asbestos is an important criterion for the diagnosis of asbestos related diseases to obtain qualitative information about the working atmosphere and its state of pollution along with the health condition status of the workers employed in those areas. Shipyards are one among those polluted area for the occupational exposure to asbestos. Though asbestos is banned, its existence cannot be eradicated completely since it gets entry through various forms and is bonded closely to the working environments. The sputum samples analyzed for the mineral asbestos fibers were of chrysotile forms which were predominant in the older age exposed groups who had more years of exposure. A sputum sample positive for asbestos bodies suggests a significant lung asbestos burden (Teschler et al., 1996). The frequency of detection of asbestos bodies in sputum differed from one occupational cohort to another in highly exposed subjects (Sebastien et al., 1998; Sulotto et al., 1997). The asbestos particles gets slowly accumulated in the regions of lungs leading to asbestos related diseases and sometimes leads to cancer such as mesothelioma in the pleural layers of the lungs. The workers in the shipyards were exposed to the asbestos particles through the various substances they handle such as the cables, insulations on hull and pipes, hanger liners, ship blasting releases huge amount of asbestos particles and through many other sources. Asbestos and smoking have been possible confounders in most of the studies (Langard, 1986). The persons who had smoking habits were reported for more asbestos fibers from their sputum and hence smoking increases the chances for more asbestos exposure. Dermal and respiratory allergies along with reproductive defects were also related to the asbestos fibers. Exploration of correlations between biomarkers will contribute to the development of human biomonitoring to genotoxic exposures and will help to select optimal biomarkers for more efficient monitoring of various human exposures (Erika et al., 2008). Long term accumulation of asbestos leads to cytogenetic damages and it is confirmed from the present study which indicated chromosomal and increased Micronuclei damage in the peripheral blood cells of the workers of the shipyards.

Percentage of increased neutrophils is associated with inflammations caused due to asbestos fibers with active alveolitis (Renke et al., 1982). The local action of asbestos is reflected in peripheral blood parameters (Trosic et al., 1991) which demonstrates the haematological status of workers with occupational asbestosis, chronically exposed to asbestos dust over 20 years defined by systemic host relations and the effect of inhaled fibers could be reflected in a significantly decreased percentage of peripheral blood segmented leucocytes and increased percentage of monocytes (Ivancica Trosic and Zoran Pisl, 1995). Similarly in the study, increased state of WBC and low levels of RBC are seemed to be associated with the accumulation of asbestos fibers in the body and the present study has shown that the haematological and cytogenetic findings are much useful for screening the risky population of the workers exposed to asbestos in the shipyards.

In conclusion, the study indicates that shipyard workers exposed to asbestos contribute to an increase in micronuclei and increased cytological and haematological damage. These workers may not be aware of the genotoxic agents they are exposed nor the type and amount of agent to which they have been exposed. Hence, there is a need to instruct them about the potential hazard of occupational exposure and the importance of using protective measures. In general, the introduction of comprehensive exposure control measures in shipyard is a challenging task but can be attained to an extent by the use of protective equipment such as disposable respirators face masks, gloves and safety shoes throughout the work shift.

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