RESEARCH COMMUNICATION

DNA Damage Induction and Repair Inhibition Among Building Construction Workers in South India

Sudha Sellappa1*, Shibily Prathyumnan1, Vellingiri Balachandar2

Abstract

Construction industry workers are exposed to many known carcinogens in their complex occupational environment. Since there are no past studies on genotoxicity among this group in the Indian subcontinent, workers engaged in different construction sites at Coimbatore, Tamil Nadu, India, were assessed here. We enrolled 96 workers and 68 control subjects with similar mean age, smoking, tobacco chewing prevalence and alcohol consumption, for analysis of DNA damage in blood leucocytes by micronucleus (MN) and comet assays. DNA repair inhibition was also analyzed by assessing the XPD gene. Construction workers showed a significant increase in MN and comet tail length compared to controls with adjustment for smoking habits, tobacco chewing, alcohol consumption and years of exposure (P<0.05). The results indicated that chronic occupational exposure to cement during construction work could lead to increased levels of DNA damage and repair inhibition.

Keywords: Cement exposure - genotoxicity - micronucleus assay - comet assay - XPD gene polymorphism

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Introduction

Construction Industry is one of the most booming industries in the whole world. Construction workers formed 4.97% of the total worker-force in India. Workers engaged in construction work are exposed to various hazardous substances. Portland cement is the most common type of cement in general usage, an important substance used in construction industry and it consists of many toxic constituents. Portland cement contain 0.75% insoluble residue. A fraction of these residues may be free crystalline silica. Respirable crystalline silica (quartz) can cause silicosis, a fibrosis of the lungs and possibly cancer (Brown et al., 1997; Hessel et al., 2000). There is evidence that exposure to respirable silica or the disease silicosis is associated with an increased incidence of Scleroderma, tuberculosis and kidney disorders. Carcinogenicity may be dependent on inherent characteristics of the crystalline silica or on external factors affecting its biological activity or distribution of its polymorphs (IARC, 1997).

Recent epidemiological surveys relate 2-8% of all cancer is due to exposure to carcinogens at the workplace and some studies have indicated that cement and concrete constituents might be carcinogenic. A case-control study in Denmark found that people working in concrete and cement manufacturing had an increased risk of laryngeal cancer (Olsen et al., 1984) and a Swedish cohort study reported an increased risk of colorectal cancer in cement exposed men (Jakobsson et al., 1993). An increased risk of lung cancer, gastrointestinal tumours and dermatitis were also reported in diverse studies (Vestbo and Rasmussen 1990; Jakobsson et al., 1993; Yang et al. 1996; Abu Dhaise et al., 1997; Noor et al., 2000; Algranti et al., 2001; Al-Naeimi et al., 2001; Laraqui et al., 2001; Stern et al., 2001).

Cement dust exposure occurs commonly in the cement production and construction industry. Most studies carried out in these industries, however, focus on silica exposure. There is no data published about genotoxicity among construction workers.

Millions of workers in India are potentially exposed each year to hazardous chemicals, dusts, or fibers in construction settings. Some of these agents are genotoxic and may cause genetic alterations in the somatic or germ cells of exposed workers. Such alterations may lead to the development of cancer. The risk of cancer is less easy to detect with traditional epidemiological methods in the construction industry than in other industrial sectors. It is not sufficient to rely upon broad epidemiological data to estimate the risk of cancer due chemicals in the construction industry (Järvholm, 2006).

The present study aimed to investigate the genotoxic effects associated with cement exposure on South Indian construction industry workers using the comet assay and the micronucleus (MN) test. The comet assay has been found to be a very sensitive, quick, reliable and fairly inexpensive way of measuring DNA damage (Schmid, 1975; Snow et al., 1989). It has a further advantage that the observations are made at the single cell level. Moreover, it is an invaluable tool for investigating DNA damage in human populations (Collins et al., 1997). To substantiate our results and to provide a cytogenetic parameter, the MN
Leukocyte cultures were set up by adding 0.5 ml whole blood to 4.5 ml RPMI 1640 medium supplemented with 15% heat-inactivated fetal calf serum, 1% antibiotics (penicillin and streptomycin) and L-glutamine (all obtained from Gibco). Leukocytes were stimulated by 1% phytohaemagglutinin (PHA; Gibco) and incubated for 72 h at 37°C. A final concentration of 6 µg/ml cytochalasin B (Sigma) was added to the cultures 44 h later to arrest cytokinesis. At 72 h of incubation, the cultures were harvested by centrifugation at 1000 rpm for 8 min and treated with a hypotonic solution (2–3 min in 0.075 M KCl at 4°C). Cells were centrifuged thereafter and a 3:1 (v/v) methanol: acetic acid solution was gently added. This fixation step was repeated twice and the resulting cells were resuspended in a small volume of fixative solution and dropped onto clean slides. Finally the slides were stained with Acridine orange (Merck) analyzed under a fluorescent microscope and scored.

**Comet assay:** An aliquot of 40µl of whole blood was used to quantitate basal DNA damage. Cell viability was determined by the trypan blue exclusion technique (Maxild et al., 1978) ranged from 90 to 95% (data not shown). Slides were prepared in duplicate per person according to Singh et al., (1988).

The cell suspension was centrifuged, the pellet obtained was mixed with 0.7% low melting agarose (LMA) and placed on fully frosted roughened slides previously coated with 1% normal melting point agarose. To the solidified agarose, a third layer of 0.1% LMA was applied and were immersed in freshly prepared ice cold lysis solution for 1 hour. The slides were then electrophoresed, neutralized, dried and stained with ethidium bromide.

A total of 100 randomly captured comets from each slide were examined at 400X magnification using an epifluorescence microscope (Zeiss) connected through a black and white camera to an image analysis system (Comet Assay II; Perceptive Instruments Ltd, UK). A total of 100 randomly captured comets from each slide were examined at 400X magnification using an epifluorescence microscope (Zeiss) connected through a black and white camera to an image analysis system (Comet Assay II; Perceptive Instruments Ltd, UK).
computerized image analysis system acquires images, computes the integrated intensity profiles for each cell, estimates the comet cell components and then evaluates the range of derived parameters. To quantify the DNA damage, tail length (TL) and tail moment (TM) were evaluated. Tail length (length of DNA migration) is related directly to the DNA fragment size and presented in micrometers. It was calculated from the centre of the cell. Tail moment was calculated as the product of the tail length and the fraction of DNA in the comet tail.

An undamaged cell resembles an intact nucleus without a tail and a damaged cell has the appearance of a comet. The length of the DNA migrated in the comet tail, which is an estimate of DNA damage, was measured.

**Restriction Fragment Length Polymorphism of XPD Gene:** Polymorphism of XPD gene was analyzed by a PCR-RFLP procedure with the following oligonucleotide primer: FP: 5'-GCGTGCCTCTGATATTACG-3' RP: 5'-CTATCATCTCGTGGCCCG-3' which gave a 436 bp product. The PCR conditions were standardized as initial denaturation at 94°C for 3 min, followed by 38 cycles of denaturation at 94°C for 45 sec, annealing at 57°C for 45 sec, extension at 72°C for 60 sec and a final extension of 72°C for 7 min. A negative control without template DNA was used in each run. The amplified products were then resolved in 2% agarose gel stained with ethidium bromide.

The products were then digested with restriction endonuclease, PstI (Invitrogen Life Technologies), as recommended by the manufacturer. The fragments obtained were analyzed in a 1.2% agarose gel stained with ethidium bromide and photographed under UV light. The bands obtained were confirmed in triplicate along with undigested sample and DNA marker.

**Statistical analysis**

The samples were coded at the time of preparation and scoring. They were decoded before statistical analysis for comparison. Mean and standard deviation (SD) were calculated for each biomarker. The significance of the differences between control and exposed end-point means were analyzed using Student’s t-test. Mean values and standard deviations were computed for the scores and the statistical significance (P<0.05) of effects were determined.

**Results**

The effect of occupational exposure to cement on the levels of genetic damage in building construction workers and control subjects was assessed by the comet assay and MN assay. Table 1. represents the distribution of subjects with respect to age, years of exposure, smoking habits, tobacco chewing and alcohol consumption. The age, alcohol consumption and smoking status distributions were similar among exposed workers and controls. Among the smokers and alcoholics, the years of smoking/ alcohol consumption and daily cigarette/alcohol consumption were similar in the two groups. The mean age of the exposed group was 36.4±11.5, ranging from 21 to 60 years, and that of controls were 34.9±10.82.

Table 2 represents mean MN and DNA damage in

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### Table 1. Demographic Characteristics of Control and Experimental Subjects

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Exposed</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>36.4±11.5</td>
<td>34.9±10.82</td>
</tr>
<tr>
<td>Work exposure (years)</td>
<td>10-30</td>
<td>-</td>
</tr>
<tr>
<td>Smoking</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Tobacco chewing</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Alcohol Consumption</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Duration of work (years)</td>
<td>13.3±9.6</td>
<td>-</td>
</tr>
<tr>
<td>Duration of work (years)</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Range (years)</td>
<td>21-60</td>
<td>21-60</td>
</tr>
<tr>
<td>Range (years)</td>
<td>10-30</td>
<td>-</td>
</tr>
<tr>
<td>Smoking</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Tobacco chewing</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Alcohol Consumption</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

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### Table 2. Micronucleus Frequency and Mean Comet Tail Length (µm) with Respect to Age, Smoking, Tobacco Chewing Habit, Alcohol Consumption and Years of Exposure in Controls and Building Construction Workers

<table>
<thead>
<tr>
<th>Study group</th>
<th>n=164</th>
<th>MN (Mean ± SD)</th>
<th>Comet tail length (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls n=68</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>≤40</td>
<td>4.56±1.49</td>
<td>9.90±0.92</td>
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<tr>
<td></td>
<td>≥41</td>
<td>3.85±0.99</td>
<td>8.09±1.18</td>
</tr>
<tr>
<td>Smoking</td>
<td>Yes</td>
<td>5.96±1.37</td>
<td>10.40±2.42</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>3.24±1.01</td>
<td>9.21±1.32</td>
</tr>
<tr>
<td>Tobacco chewing</td>
<td>Yes</td>
<td>4.84±1.20</td>
<td>10.12±2.71</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>2.98±0.96</td>
<td>8.85±2.33</td>
</tr>
<tr>
<td>Alcohol Consumption</td>
<td>Yes</td>
<td>3.95±0.82</td>
<td>9.96±2.44</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>3.12±1.13</td>
<td>9.23±2.30</td>
</tr>
<tr>
<td>Workers n=96</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>≤40</td>
<td>8.84±1.45</td>
<td>16.85±2.08a</td>
</tr>
<tr>
<td></td>
<td>≥41</td>
<td>8.92±1.71</td>
<td>14.12±2.33a</td>
</tr>
<tr>
<td>Smoking</td>
<td>Yes</td>
<td>9.64±1.67b</td>
<td>15.97±2.61a</td>
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<tr>
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<td>No</td>
<td>6.70±2.13b</td>
<td>13.71±2.89b</td>
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<tr>
<td>Tobacco chewing</td>
<td>Yes</td>
<td>7.74±1.83c</td>
<td>15.71±2.34c</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>6.78±1.36c</td>
<td>12.94±1.77c</td>
</tr>
<tr>
<td>Alcohol Consumption</td>
<td>Yes</td>
<td>8.28±1.23d</td>
<td>14.05±2.59c</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>6.82±1.22d</td>
<td>12.90±2.98d</td>
</tr>
<tr>
<td>Work exposure (years)</td>
<td>≤10</td>
<td>8.56±1.88e</td>
<td>14.36±1.10e</td>
</tr>
<tr>
<td></td>
<td>≥10</td>
<td>10.97±2.83f</td>
<td>16.73±2.45f</td>
</tr>
</tbody>
</table>

*Significantly different from controls (P<0.05); †Significantly different from the exposed never smokers (P<0.05); ‡Significantly different from the exposed never smokers (P<0.05)
peripheral blood leukocytes with age, smoking, tobacco chewing, alcohol and exposure period of experimental and control subjects. The results of MN frequency of construction workers increased significantly, as compared with controls (P<0.05). The difference in MN frequency between ≤40 years and ≥40 year subgroups of exposed was insignificant (8.84 versus 8.92). Similar result was seen in control group also (4.56 versus 3.85). Duration of exposure to cement had a significant effect on MN frequency in lymphocytes of workers who had been working in building construction for ≥10 years over those who worked for ≤10 years (10.97 versus 8.56; P<0.05).

The comet tail length significantly increased in exposed group (P<0.05) than control groups. Subjects who were ≤40 years of age group showed a statistically significant increase in mean DNA damage than those who were ≥40 years in exposed group (16.85 versus 14.12, P<0.05). Similar result was seen in control group also (9.90 versus 8.09, P<0.05). The comet tail length significantly increased in exposed group with ≥10 years exposure than ≤10 years of exposure (16.73 versus 14.36, P<0.05).

Alcohols of experimental subjects showed significant (P<0.05) amount of MN and DNA damage than alcohol users of control subjects and non alcohol users in experimental subjects. An increased level of DNA damage was observed in the workers with smoking habits when compared to controls with smoking habit. A clear and statistically significant (P<0.05) increase in DNA damage was observed in experimental group when compared to control groups. As analyzed smoking, tobacco chewing and alcohol differences shows that exposed subjects carry more number of MN and DNA damage than control subjects.

XPD gene (exon 23) was amplified by polymerase chain reaction using specific primer. The XPD gene exon 23 has an amplification product at 436bp. The XPD gene polymorphism was analyzed by PCR-PFLP. Bands of 227, 146 and 63bp products were observed for the presence of normal XPD gene. Samples with an altered gene failed to give bands at these sites. Our results also indicate an association between the frequencies of MN, mean comet tail length and polymorphisms in DNA repair gene (XPD, exon 23), involved in NER.

Discussion

The main objective of the study was to evaluate if the exposure to complex mixture of chemicals in construction, induced increase in the level of genetic damage. The study was carried out in parallel with exposed and control group both from the same area and with similar individual characteristics. To evaluate the genetic damage two of the most common biomonitoring methods (MN assay and Comet assay) were chosen.

Biomarkers also permit enhanced analysis of health risk in humans exposed to carcinogens and because determinations are performed directly in human organism, uncertainties inherent in epidemiologic studies are avoided. There is no study available on the biomonitoring of construction workers. The current investigation reports genotoxicity in building construction workers from South India.

Peripheral lymphocytes have been typically used for detecting genotoxic effects in a great number of studies since they are considered to be adequate for detecting general exposure (Murray and Edwards, 1999).

The MN assay and Comet assay test has been increasingly accepted as a reliable biomarker of genotoxicity in occupationally exposed groups (IARC, 1990).

The present investigation recommended that construction workers under their particular conditions of exposure (tobacco and alcohol) reveal clear evidence of genotoxicity in lymphocytes when evaluated by MN test. Our study revealed a significant induction of MN and Comet tail length in construction workers when compared to controls with respect to their age, years of exposure, smoking, tobacco chewing and alcohol consumption. Similarly, an increased incidence of chromosomal aberrations was observed in the cement factory workers with smoking habit (Shehla et al., 2001).

These results are in agreement with our previous study which reported an increased chromosomal aberration among cement factory workers (Jude et al., 2002) and an increased MN induction in cement exposed tobacco chewers (Sudha et al., 2009). An enhanced sister chromatid exchange and chromosomal aberration in peripheral blood lymphocytes of asbestos factory workers were reported by Fathima et al (2001) and also cement particulate extracts in invitro showed an increased chromosomal aberration (Hadnagy et al., 1989).

The building construction workers with smoking habits and tobacco chewing habit also shows more DNA damage, which shows tobacco has synergistic effect on inducing DNA damage. Some previous findings reported similar results on bidi, and smokeless tobacco users. In addition, the present study correlates with smoking and cement exposure. Smoking-related DNA adducts have been detected by a variety of analytical methods in the respiratory tract, urinary bladder, cervix and other tissues. On terms of biological activity, cigarette smokers and its conductors have been shown to form adducts with DNA protein and to induce chromosome aberrations (Sasikala et al., 2003; Manikantan et al., 2010).

The genetic susceptibility of cancer may result from inherited polymorphisms in the genes involved in carcinogen metabolism and DNA damage repair (Park et al., 2002). The DNA repair system plays an important role in protecting against mutagenesis and carcinogenesis. The defect of DNA repair often results from gene mutations and such defects in DNA repair causes several hereditary cancers.

DNA repair systems play a critical role in protecting the genome from insults caused by carcinogenic agents (Hoeijmakers, 1993). So far, over a hundred proteins implicated in DNA repair have been found in human cells. These proteins are concerned with four major DNA repair pathways, including nucleotide excision repair (NER), base excision repair (BER), double-strand break repair (DSBR) and mismatch repair (MMR) (Yu et al., 1999; Wood et al., 2001).
Polymorphisms in NER genes have also been associated with individual susceptibility to develop cancer. The XPD gene is abundant with polymorphisms (Claerkson et al., 2005). The XPD gene encodes an ATP-dependent DNA helicase involved in NER and in basal transcription as part of the transcription factor TFIIH (Laine et al., 2007). The presence of the variant alleles 312Asn and 751Gln of XPD have been associated with relatively high risk of cancer (Dybdahl et al., 1999; Sturigs et al., 2000; Hemminki et al., 2001). A recent meta-analysis concludes that the variant genotypes 312Asn/Asn and 751Gln/Gln are associated with lung cancer risk (Hu et al., 2004). Although XPD polymorphisms cannot be considered as a crucial factor for cancer susceptibility, our results suggested that XPD is a highly suspected candidate gene without considering the role of environmental factors cautiously.

In conclusion, biomonitoring studies of workers exposed to construction industry are rather vague because each population has a different life style factors but same occupation in different areas under different climatic and environmental conditions and are exposed to indistinguishable mutagen. Therefore, there is a need to educate those who work in construction sites about the potential hazard of occupational exposure and the importance of using protective measures. Since DNA damage is an important step in events leading from carcinogen exposure to cancer disease, our study represents an important contribution to the correct evaluation of the potential health risk associated with exposure.

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References


