Micronuclei frequencies in hospital workers occupationally exposed to low levels of ionizing radiation: influence of smoking status and other factors

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In the context of a medical surveillance program aimed at preventing cancer risk from exposure to ionizing radiation, we investigated chromosomal damage in peripheral lymphocytes from 37 hospital workers exposed to low levels of ionizing radiation and 37 controls. The micronucleus (MN) assay was used as a biomarker of genetic damage. The influence of confounding factors like smoking status, age and gender was investigated by multiple regression analysis. The results indicated that, overall, MN frequency was higher in exposed workers than in controls, although the difference was not statistically significant. Interestingly, smoking status significantly raised MN frequency among the exposed workers but not among controls. This suggests that smoking can influence chromosomal damage induced in humans by ionizing radiation. Among both exposed workers and controls, MN frequency was found to increase with age. Female gender influenced the increase in MN frequency in the exposed group. Our results suggest that the effect of cigarette smoking should be carefully factored into genetic monitoring studies assessing the risks associated with low level radiation exposure.

Introduction

The largest man-made contribution to human exposure to ionizing radiation comes from its diagnostic and therapeutic uses (UNSCEAR, 1982). Thus hospital workers constitute the group most consistently exposed to low doses of ionizing radiation (UNSCEAR, 1982). Occupational exposure to ionizing radiation now generally falls well within the currently accepted limits, and is much lower than it was some years ago. In particular, the limit of 20 mSv/year is seldom or never exceeded (Thierens et al., 1996). However, the low general levels of occupational exposure to ionizing radiation have shifted the focus of medical surveillance from the identification of dose-dependant effects to the prevention of possible stochastic effects, in particular of cancerogenic ones. In fact, some epidemiological studies have indicated that people occupationally exposed to ionizing radiation may show increased risk of leukaemia (Smith and Doll, 1981; Wang et al., 1988; Aoyama, 1989; Muirhead et al., 1999; IARC, 2000).

Today, surveillance is largely based on physical control of operators (dosimetry). While this method can effectively reveal any accidental overexposure, it provides no information on the real long-term risk of low level exposure. As agencies and organizations involved in important radioprotection programs have underlined (National Academy of Sciences–National Research Council, 1989; IRCP, 1991; UNSCEAR, 1993), there is an urgent need for new preventive initiatives for the surveillance of hospital workers chronically exposed to ionizing radiation.

In the last decade, biomarkers, such as cytogenetic alterations, have been proposed to reveal early carcinogenic events and to identify subjects at risk in time for preventive intervention (Perera and Whyatt, 1994; Hagmar et al., 1998). Chromosomal aberrations have been accepted as fairly reliable parameters for evaluating damage induced by ionizing radiation in humans. Earlier cytogenetic studies of hospital workers occupationally exposed to low doses of ionizing radiation revealed an increased baseline level of chromosomal aberrations, as compared with control populations (Evans et al., 1979; Lloyd et al., 1980; Bigatti et al., 1988; Jha and Sharma, 1991). Nowadays, although dose levels are reduced, exposure to ionizing radiation still presents the hazard of chromosome damage for hospital workers (Barquinero et al., 1993; Bonassi et al., 1997; Rozgaj et al., 1999; Cardoso et al., 2001). Biological dosimetry is largely based on cytogenetic analysis of dicentrics and, more recently, on the evaluation of chromosomal translocations. However, since the techniques involved are time consuming and require highly skilled personnel, the biomonitoring of large groups of workers is difficult. Thus, micronuclei (MN) analysis in human lymphocytes using the cytochalasin B technique (Fenech and Morley, 1985) has been proposed as a valid and less laborious alternative to chromosomal aberrations analysis for large-scale studies (Fenech et al., 1999). MN consist ofacentric chromosome fragments or whole chromosomes that are not found in the main nuclei during anaphase. Consequently, MN formation is a reliable biomarker of exposure to clastogenic and aneugenic hazards (Fenech, 1998). It has been demonstrated that MN are a reliable biomarker for biological dosimetry in human post-acute radiation exposure (da Cruz et al., 1994; Wuttke et al., 1996). A dose-dependent increase in MN frequency over a 50–500 mSv range of X-ray exposure has also been recorded in human lymphocytes in vitro (Fenech and Morley, 1986). Moreover, the MN assay has been employed to assess cytogenetic damage in populations living in areas with high levels of radioactivity (Chang et al., 1997, 1999; Tsai et al., 2001) and in groups occupationally exposed to ionizing radiation (Thierens et al., 1996; Cardoso et al., 2001). Recently, significantly elevated numbers of centromere-positive MN have been revealed in radiological workers by the fluorescence in situ hybridization technique with a pan-centromeric probe (Thierens et al., 2000).

In Italy, recent legislation on radioprotection activity has also emphasized the need to introduce new biological indicators in cancer prevention programs for the health surveillance of workers exposed to ionizing radiation (AIRM, 1995; Italian
Parliament, 2000). In line with these recommendations, we have organized a research project on the evaluation of genetic markers in routine surveillance programs for the prevention of cancer risk in workers exposed to low levels of ionizing radiation.

Herein, we report a study of MN analysis on peripheral blood lymphocytes taken from hospital workers occupationally exposed to ionizing radiation and from control groups. To shed more light on the influence of some confounding factors on chromosomal damage, the effects of donor age, gender and smoking status on MN frequencies were also investigated.

Materials and methods

Subjects

The study population of professionals occupationally exposed to ionizing radiation comprised 37 individuals (21 physicians and 16 technicians) working in the Radiology, Radiotherapy and Cardiology Units at the Sant’Orsola-Malpighi Hospital, Bologna. None of these workers were professionally exposed to any carcinogenic agent other than ionizing radiation. The control group comprised 37 individuals working in the same hospital without any work-related exposure to hazardous agents. All the subjects of both groups lived in the same urban area. They were all provided with specific written information about cytogenetic tests and the aims of the study and gave their written consent. Prior to blood collection in the course of a routine occupational medical examination, each individual was extensively interviewed by a specialist physician who filled in a structured questionnaire specifying gender, date of birth, smoking status, dietary habits, alcohol consumption, work-related exposure to hazardous agents, previous exposure to diagnostic x-rays as a patient and use of therapeutic drugs. No member of either group had received diagnostic or therapeutic x-ray exposure or chemotherapeutic drugs in the 12 months prior to cytogenetic analysis. None of the subjects had a deficient diet or peculiar dietary habits. Seventy percent of the exposed workers and 78% of controls consumed wine and/or beer, and they had an alcohol intake range of 1–2 l/week. The remaining members of the two groups did not consume alcohol. The routine occupational medical examinations revealed no abnormal findings in any of the members of either group. All the blood samples were drawn by venipuncture by medical workers and were coded in the Occupational Medicine Unit. Processing and scoring of the samples of the two groups were then performed blind and concurrently in the laboratory of the Department of Pharmacology. At the end of the study the data from the questionnaire and the radiation burden records were linked to the code number for data analysis.

Exposure monitoring

The subjects in the occupational exposure group are all regularly exposed to x- and γ-rays. Their occupational exposure to ionizing radiation is routinely monitored by personal exposure measurement devices (film badges) that are read every 40 days.

Micronuclei assay

The MN analysis was performed using the cytochalasin B technique (Fenech and Moorley, 1985). Peripheral lymphocytes were isolated from heparinized blood by Histopaque gradient centrifugation (Sigma, St Louis, MO). Two replicate cultures were set up using 2×10⁶ lymphocytes for each sample in 5 ml of RPMI 1640 (Sigma) medium containing 15% fetal calf serum (Sigma), 1% phytomenadione (Sigma), 1 mM L-glutamine (Sigma), 100 U/ml penicillin and 100 μg/ml streptomycin (Sigma). They were incubated at 37°C in 5% CO₂ for 72 h. Cytochalasin B (Sigma) was added (final concentration 6 μg/ml) for the last 28 h. Cells were collected and treated with a mild hypotonic treatment (one part RPMI 1640 medium and one part distilled water) and fixed with a mixture of methanol/glacial acetic acid as described (Maffei et al., 2000). The slides were prepared by cytogenitification and air dried. The slides were stained by conventional May Grünwald Giemsa staining (Sigma). In accordance with standard criteria (Fenech, 1993), MN analysis was performed on coded slides by scoring 2000 binucleated (BN) lymphocytes for each subject. Cell cycle parameters were evaluated by classifying 1000 cells according to the number of nuclei. The nuclear division index (NDI) was calculated following the formula NDI = (M1 + 2M2 + 3M3 + 4M4)/n, where M1–M4 indicates the number of cells with 1–4 nuclei and n indicates the total number of cells scored (Eastmond and Tucker, 1989).

Statistical analysis

The Wilcoxon rank sum test was used to compare the frequencies of MN in exposed workers and controls. This non-parametric test allows problems related to equal variances, normality assumptions or those regarding the use of frequencies to be obviated (Wilcoxon, 1945; D’Agostino et al., 1990). Linear regression analysis was applied to assess the correlation between the dose equivalent of ionizing radiation to the whole body (Hwb) and MN frequency in exposed workers. Multiple regression analysis was used to evaluate the influence of age, gender and smoking status on MN frequencies of both groups. Single and multiple regression analyses were performed after logarithmic transformation of non-normally distributed variables. All statistical analysis was performed using the STATA-6 program (Stata Corp., 1999).

Results

The demographic characteristics of the study subjects are presented in Table I. The age, gender and smoking status distributions were similar among exposed workers and controls. Among the smokers, the years of smoking exposure and daily cigarette consumption were similar in the two groups. Hwb of ionizing radiation among the exposed hospital workers ranged from 0.45 to 141.77 mSv (35.06 ± 40.76); none of them had recorded doses above the annual limit of 20 mSv or the 5 yearly limit of 100 mSv. The mean Hwb and qualities of radiation did not differ between the never smokers (39.77 ± 46.22 mSv) and current smokers (30.09 ± 34.57 mSv) in the group. The results of the MN assay, reported as total number of MN per 1000 BN cells, are shown in Table II. MN frequencies were greater in exposed workers than in controls (6.78 ± 4.92 versus 5.54 ± 2.99). Although the increase in MN frequency in the exposed group was 22% with respect to the control group, it did not reach statistical significance. In exposed smokers, Hwb of ionizing radiation did not influence the MN frequency observed in exposed workers (β = −0.022, P = 0.774, 95% CI = −0.179–0.134). Among the current smokers, a higher frequency of MN was found in the exposed worker group with respect to controls (8.83 ± 5.94 versus 6.00 ± 1.94), but this difference was not significant. The mean MN frequency observed in non-smokers was similar in exposed workers and controls (4.84 ± 2.61 versus 5.15 ± 3.67). MN frequencies were significantly higher in exposed smokers than in exposed never smokers (8.83 ± 5.94 versus 4.84 ± 2.61, P = 0.011). As regards NDI, no significant overall difference was found between exposed workers and controls (1.63 ± 0.17 versus 1.60 ± 0.16). However, in both groups the mean NDI of current smokers was significantly lower than that of never smokers (exposed workers, 1.57 ± 0.14 versus 1.70 ± 0.18, P = 0.041; controls, 1.68 ± 0.12 versus 1.52 ± 0.15, P = 0.007).

Table III shows the results of multiple regression analysis including age, gender and smoking status. A borderline association between age and MN frequency was observed in the exposed group, while a significant one was found in the controls. Female gender influenced the increase in MN frequencies among exposed workers (β = −0.255). This effect did not reach statistical significance, probably because of the low statistical power due to the relatively low number of female subjects. Smoking status affected MN frequency in both groups studied, but a statistical association was detected only in the exposed group.

Discussion

In this study we investigated the feasibility of adopting the MN assay for routine medical surveillance of chromosomal alterations in the peripheral lymphocytes of hospital workers occupationally exposed to low levels of ionizing radiation. Moreover, the influence of confounding factors like smoking
Low level ionizing radiation and chromosome damage

Table I. Demographic characteristics of the study population

<table>
<thead>
<tr>
<th></th>
<th>Sample size</th>
<th>Age (mean ± SD)</th>
<th>Gender</th>
<th>Smoking years (mean ± SD)</th>
<th>Cigarettes/day (mean ± SD)</th>
<th>Hwb (mSv) (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Female</td>
<td>Male</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exposed workers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All subjects</td>
<td>37</td>
<td>43.67 ± 8.90</td>
<td>13</td>
<td>24</td>
<td></td>
<td>35.06 ± 40.76</td>
</tr>
<tr>
<td>Never smokers</td>
<td>19</td>
<td>42.58 ± 8.04</td>
<td>5</td>
<td>14</td>
<td>39.77 ± 46.22</td>
<td></td>
</tr>
<tr>
<td>Current smokers</td>
<td>18</td>
<td>44.00 ± 9.44</td>
<td>8</td>
<td>10</td>
<td>30.09 ± 34.57</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All subjects</td>
<td>37</td>
<td>41.62 ± 8.25</td>
<td>14</td>
<td>23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smokers</td>
<td>20</td>
<td>41.70 ± 8.81</td>
<td>8</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smokers</td>
<td>17</td>
<td>41.53 ± 7.81</td>
<td>6</td>
<td>11</td>
<td>21.47 ± 7.45</td>
<td></td>
</tr>
</tbody>
</table>

Table II. Frequencies of MN in peripheral lymphocytes of exposed workers and controls

<table>
<thead>
<tr>
<th>Group</th>
<th>MN/1000 BN cells Mean ± SD</th>
<th>NDI Mean ± SD</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposed workers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All subjects</td>
<td>6.78 ± 4.92</td>
<td>1.63 ± 0.17</td>
<td>1.58-1.69</td>
</tr>
<tr>
<td>Never smokers</td>
<td>4.84 ± 2.61</td>
<td>1.70 ± 0.18</td>
<td>1.61-1.78</td>
</tr>
<tr>
<td>Current smokers</td>
<td>8.83 ± 5.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.57 ± 0.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.50-1.64</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All subjects</td>
<td>5.54 ± 2.99</td>
<td>1.60 ± 0.16</td>
<td>1.55-1.65</td>
</tr>
<tr>
<td>Never smokers</td>
<td>5.15 ± 3.67</td>
<td>1.68 ± 0.12</td>
<td>1.62-1.74</td>
</tr>
<tr>
<td>Current smokers</td>
<td>6.00 ± 1.94</td>
<td>1.52 ± 0.15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.44-1.59</td>
</tr>
</tbody>
</table>

BN, binucleated cells; NDI, nuclear division index.
<sup>a</sup>Significantly different from the exposed never smokers (Wilcoxon test, \( P = 0.011 \)).
<sup>b</sup>Significantly different from the exposed never smokers (Wilcoxon test, \( P = 0.041 \)).
<sup>c</sup>Significantly different from the control never smokers (Wilcoxon test, \( P = 0.007 \)).

Table III. Multiple regression analysis of confounding factors on MN frequencies in peripheral lymphocytes of the study groups

<table>
<thead>
<tr>
<th>Confounding factors&lt;sup&gt;b&lt;/sup&gt;</th>
<th>( \beta ) coefficient</th>
<th>( P )</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposed workers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.777</td>
<td>0.068</td>
<td>-0.061-1.615</td>
</tr>
<tr>
<td>Gender (1,2)</td>
<td>-0.255</td>
<td>0.163</td>
<td>-0.619-0.109</td>
</tr>
<tr>
<td>Smoking status (0,1)</td>
<td>0.459</td>
<td>0.011</td>
<td>0.114-0.804</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>1.458</td>
<td>0.002</td>
<td>0.582-2.333</td>
</tr>
<tr>
<td>Gender (1,2)</td>
<td>0.040</td>
<td>0.824</td>
<td>-0.325-0.406</td>
</tr>
<tr>
<td>Smoking status (0,1)</td>
<td>0.317</td>
<td>0.061</td>
<td>-0.016-0.650</td>
</tr>
</tbody>
</table>

<sup>b</sup>Multiple regression analyses were performed after logarithmic transformation of MN frequencies and age.
<sup>1</sup>Gender: 1, females; 2, males; smoking status: 0, never; 1, current.

Our results indicated that, overall, MN frequency was higher in exposed workers than in controls, although the difference is not statistically significant. This finding is in agreement with other studies reporting a slight, but not significant, increase in MN frequency in workers occupationally exposed to low level ionizing radiation (Thierens et al., 1996; Cardoso et al., 2001). In our study no association between MN frequency and \( Hwb \) was found. Other reports have also indicated that it is difficult to establish a dose–effect relationship between chromosome damage and exposure to low levels of ionizing radiation (Barquiner et al., 1993; Thierens et al., 1996; Cardoso et al., 2001). The lack of a dose–effect relationship could be attributed to various factors, which may, in combination, influence the yield of cytogenetic damage. It has been hypothesized that the absence of an appreciable dose–effect relationship at low doses could be due to induction of repair enzymes until saturation is reached (Pohl-Rülig, 1990; Bauchinger, 1995). Another contributory mechanism could regard the physiological elimination of lymphocytes bearing chromosome damage (Bigatti et al., 1988).

Concerning the effect of smoking on MN frequency, the data reported in biomonitoring studies are contradictory (Sorsa et al., 1988; Au et al., 1991; Migliore et al., 1991; Tomanin et al., 1991; Norppa et al., 1993; Holmen et al., 1995; Bolognesi et al., 1997; Barale et al., 1998). In our study multiple regression analysis showed that smoking status affected genetic damage in both groups studied, but a significant association emerged only among exposed workers. Furthermore, exposed smokers showed greater frequencies of MN than control smokers, although this difference did not reach statistical significance. Moreover, as compared with exposed non-smokers, the exposed smokers exhibited significantly higher frequencies of MN in lymphocytes. These findings suggest that smoking can influence the genetic damage induced in humans by ionizing radiation. Interestingly, when Wang et al. (2000) measured the frequency of chromosomal aberrations after in vitro exposure to \( \gamma \)-rays in peripheral lymphocytes taken from a large population of healthy subjects they found that cigarette smoking affected the radiosensitivity of the cells. Using a challenge assay, Au et al. (1991) showed that chromosome aberration frequency was consistently higher after X-ray irradiation in lymphocytes from smokers than in those from non-smokers. Based on these data, the authors postulated that the cells from smokers have infidelity of DNA repair. However, the effects of smoking on genetic damage in humans exposed to ionizing radiation remain unclear. Among the studies that detected a significantly increased frequency of chromosomal damage in subjects exposed to ionizing radiation, only a few studies were able to identify the association between genetic damage and smoking status (da Cruz et al., 1994; Slozina et al., 1997).

We recorded a significant reduction in NDI among both exposed and non-exposed current smokers. This could suggest that cigarette smoking also influences lymphocyte proliferation. There is evidence that exposure of human T cells to cigarette tar or its major phenolic components, hydroquinone and catechol, causes a cessation of DNA synthesis without cytotoxicity (Li et al., 1996; Karla et al., 2000). Moreover, in vitro studies have demonstrated that hydroquinone and
catechol inhibit lymphocyte proliferation by acting on some essential and rate limiting enzyme in DNA synthesis, such as DNA polymerase or ribonucleotide reductase (Li et al., 1996; McCue et al., 2000).

In our study MN frequencies tended to rise with age in both groups of subjects, although a significant association emerged only in the control group. Our current knowledge of the effect of age on MN frequencies has come from biomonitoring studies (Barale et al., 1998; Fenech et al., 1998; Bolognesi et al., 1999); the age effect has recently been confirmed by data on nearly 7000 subjects of the Human MicroNucleus Project (Bonassi et al., 2001). It has been postulated that the age effect could reflect a progressive increase in spontaneous chromosome instability, associated with an accumulation of DNA damage due to an age-related decline in DNA repair capacity (Wei et al., 1993; Barnett and King, 1995). The effect could also be explained by an increase in chromosome loss during aging (Richard et al., 1994; Guttenbach et al., 1995; Stone and Sandberg, 1995; Catalan et al., 1998).

Another possible confounding factor is gender: higher frequencies of MN have previously been reported in females than in males (Barale et al., 1998; Fenech, 1998). In our study the gender exerted an influence on the yield of MN in the exposed group. The absence of any statistically observable effect may have been due to the relatively low number of females in both study groups.

In summary, our data suggest the presence of increased chromosomal damage (in terms of MN) in smokers, but not in non-smokers, occupationally exposed to low levels of ionizing radiation. These findings suggest that the effect of cigarette smoking should be considered in genetic monitoring for assessing risk associated with low level radiation exposure. Studies are needed to investigate the existence of a possible influence of smoking on genetic damage induced by ionizing radiation. The present study also indicates the usefulness of MN analysis as a biological exposure index for individual risk assessment in medical surveillance programs. Biological indicators of radiation damage are also essential for dose estimation after radiation accidents or in the presence of operational hitches in physical dosimetry.

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Low level ionizing radiation and chromosome damage


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