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Radon-induced Chromosome Damage in Blood Lymphocytes of Smokers

C. Meenakshi and Mary N. Mohankumar

Indira Gandhi Centre for Atomic Research, Radiological Safety Division, Kalpakkam-603102, Tamil Nadu, India

Corresponding Author: Mary N. Mohankumar, Indira Gandhi Centre for Atomic Research, Radiological Safety Division, Kalpakkam-603102, Tamil Nadu, India Tel: +91 44 27480500x23439 Fax: +91 44 27480235

ABSTRACT

In order to investigate the synergistic effect of smoking and radon in the absence of confounding factors, an *in vitro* study was carried out to compare chromosome aberrations in blood lymphocytes of smokers and non-smokers. Blood samples were exposed to different concentrations of radon ranging from 0 to 35643.223 kBq m⁻³ corresponding to mean doses of 2.9±0.27 mGy (non smokers) and 2.7±0.25 mGy (smokers) using a simple, portable irradiation assembly. Chromosome aberrations in giemsa stained first division metaphase preparations were scored. The results show that compared to nonsmokers there is a significant increase in radon-induced dicentrics, acentric fragments and chromatid breaks in smoker cells. The study implies that chromosomes in smoker cells are more unstable to radon exposure and thus the effect acts synergistically to the risk of cancer.

Key words: DNA damage, radon, smokers, synergistic effect, chromosome aberrations

INTRODUCTION

Radon is a naturally occurring alpha emitting radioactive gas present in the atmosphere, homes and workplaces causing a great deal of health concern. It is emitted from uranium, a naturally occurring mineral in rocks and soil. The natural decay of uranium in building stones, bricks, soils and bedrocks produces the radioactive gas, therefore radon seeps into and accumulates inside buildings (Salameh *et al.*, 2011). It has been pointed that the concrete slab of a house is more exposed to weathering and therefore more susceptible to cracking, favorising the radon suction inside the building (Gasó *et al.*, 2005).

Radon has been identified to be the second largest cause of lung cancer next to smoking. Increasing radon concentration in respiratory gases and also in respiratory tract will cause the related tissues to be exposed to the alpha radiation produced by radon decay and its products and finally will increase the risk of lung cancer (Talaepour *et al.*, 2006). Environmental tobacco smoke emanating from cigarette consumption is one of the most common indoor air pollutants (Ebisike *et al.*, 2004). The incidences of lung cancer are significantly higher in smokers and female smokers are more prone compared with the male smokers (Zafar *et al.*, 2003). Most lung cancer and emphysema, as well as high percentage of heart attacks are caused by cigarette smoking (Colagar *et al.*, 2007). Strong epidemiologic evidence links smoking and cancers, suggesting that smoking is the leading cause of lung cancer related mortality (Naddaf, 2007; Eichholzer, 2000; Tomida *et al.*, 2005).

Smokers living in environments with high radon concentrations are estimated to be at an elevated risk of lung cancer. However, this estimate is solely based on epidemiological studies and the association between radon and smoking alone has not been well established. Thus the combined effect of smoking and radon still remains controversial in risk assessment. Besides, as most studies have been conducted on miners it is not certain if the synergistic effect often contended to be multiplicative (Lee *et al.*, 1999) in nature is solely due to the interaction of radon gas with smoker cells or to other factors such as mineral dusts present in the mining environment.

Cytogenetic damage has proved to be a trustworthy biomarker of cancer risk. Moreover, there are sufficient reports to prove that radon causes cytogenetic damage in peripheral blood cells suggesting that radon inhalation can cause DNA damage in cells other than those in the lungs (Hagmar *et al.*, 1998; Zaire *et al.*, 1996; Lehnert and Goodwin, 1997; Alavanja, 2002). The present study was conducted to investigate the synergistic effect of smoking and radon using cytogenetic biomarkers.

MATERIALS AND METHODS

Study group: Blood samples were collected using heparinised Vacuette tubes (Greiner Labortechnik, Austria) by vein puncture, after prior consent, from apparently healthy smoking and non-smoking individuals working at the Indira Gandhi Centre for Atomic Research (IGCAR), Kalpakkam. A detailed questionnaire containing information about occupational exposure to radiation, personal habits, alcohol consumption, frequency and period of smoking etc., was prepared. There were 25 nonsmokers aged between 24 and 56 years and 25 smokers aged between 21 and 54 years who smoked about 6-16 cigarettes per day for periods ranging between 7 and 30 years. All participants belonged to the same ethnic group and were neither engaged in radiation work for over 3 years nor did they differ significantly in their dietary habits. Among the non-smokers, none had ever been a smoker.

Irradiation procedure: A novel irradiation technique developed at Radiological Safety Division of IGCAR was used for irradiation of blood sample. Details of the experimental set-up and irradiation are given elsewhere (Hamza *et al.*, 2008; Hamza and Mohankumar, 2009). About 5 mL blood sample collected from 25 smoking and 25 non-smoking volunteers was divided into two aliquots of equal volume. One was treated as control and the other exposed to radon doses ranging between 0.9 and 5.2 mGy with a mean dose of 2.9 mGy (non-smokers) and 2.7 mGy (smokers). Doses were estimated using a Lucas cell and an alpha counter.

Chromosome aberrations assay: Cultures for the chromosome aberration assay were initiated by adding 1 mL of whole blood to 9 mL RPMI 1640 containing 100 U mL⁻¹ penicillin, 100 µg mL⁻¹ streptomycin and 1 mL of fetal bovine serum. Bromodeoxyuridine to a final concentration of 10 µM was added to differentiate first division cells. PHA was added to a final concentration of 5 µg mL⁻¹ phytohemagglutinin and incubated for 48 h in 5% CO₂ atmosphere. At 45th hour, colchicine (Sigma) to a final concentration of 0.04 µg mL⁻¹, was added to arrest cells at metaphase. Cultures were harvested at 48 h and subjected to a hypotonic treatment of 0.56% KCl. Cells were washed and suspended in Carnoy's fixative, cast on microscope slides, air-dried, stained with giemsa and scored for aberrations in first division metaphases.

Metaphases were captured using an automated metaphase finder system (Metasystems, Germany). Individual metaphases obtained from non-exposed as well as *in vitro* radon exposed smokers and control samples were carefully analyzed and the aberrations were noted onto scoring sheets. Aberrations present in radon exposed cells were subtracted from their respective controls.

Statistical evaluation: The percentage of aberrations for each individual was calculated. The mean and standard error for individuals falling within a selected group was computed and the data was tested for significance using the InStat program.

RESULT

Table 1 shows the frequencies of chromosomal aberrations induced in smokers and nonsmokers. Acentric fragments and chromatid breaks were observed in the controls of smokers and non-smokers whereas dicentrics were observed only in smoker controls. The mean percentages of dicentrics, acentric fragments and chromatid breaks among the exposed smoker group were 1.60 ± 0.202 , 1.83 ± 0.186 and 1.486 ± 0.211 , respectively and these values for exposed non-smokers group were 0.923 ± 0.156 , 1.128 ± 0.159 and 0.842 ± 0.139 , respectively.

The mean percentages of dicentrics, acentric fragments and chromatid breaks among the control smokers group were 0.0047 ± 0.0047 , 0.055 ± 0.021 and 0.081 ± 0.031 , respectively. The mean percentage of acentric fragments and chromatid breaks among the control non-smokers group were 0.053 ± 0.027 and 0.049 ± 0.031 , respectively.

Frequencies of dicentrics, acentric fragments and chromatid breaks were significantly elevated in exposed smoker group than that of control smoker group ($p < 0.0001$). The frequency of acentric fragments and chromatid breaks of exposed non-smoker group were also extremely significant compared to control non-smoker group ($p < 0.0001$). Figure 1a shows metaphase spread from a normal cell and Fig. 1b shows metaphase spread with aberrations. Figure 2 shows regression plot of total aberrations in smoker controls and non-smoker controls as a function of age.

Table 1: Frequencies of chromosome aberrations induced in smokers and non-smokers (controls and *in-vitro* radon exposed)

Group of workers	No. of donors	No. of cells	No. of dicentrics (% \pm SEM)	No. of acentric fragments (% \pm SEM)	No. of chromatid breaks (% \pm SEM)
Controls (NS)	25	12719	0	0.053 ± 0.027	0.049 ± 0.031
Controls (S)	25	18249	0.0047 ± 0.0047	0.055 ± 0.021	0.081 ± 0.031
Non smoker (exposed)	25	14973	0.923 ± 0.156	$1.128 \pm 0.159^{*b}$	$0.842 \pm 0.139^{*b}$
Smoker (exposed)	25	13362	$1.60 \pm 0.202^{*b, *c}$	$1.83 \pm 0.186^{*a, *b}$	$1.486 \pm 0.211^{*b, *c}$

S: Smoker, NS: Non smoker, ^{*,*}: Very significant, ^{*,*}: Extremely significant, ^{*,*}: Significant, non-smokers control and *in vitro* radon exposed shows extremely significant ($p < 0.0001$), exposed non-smokers and smokers shows very significant ($p < 0.005$) and significant ($p < 0.01$) results

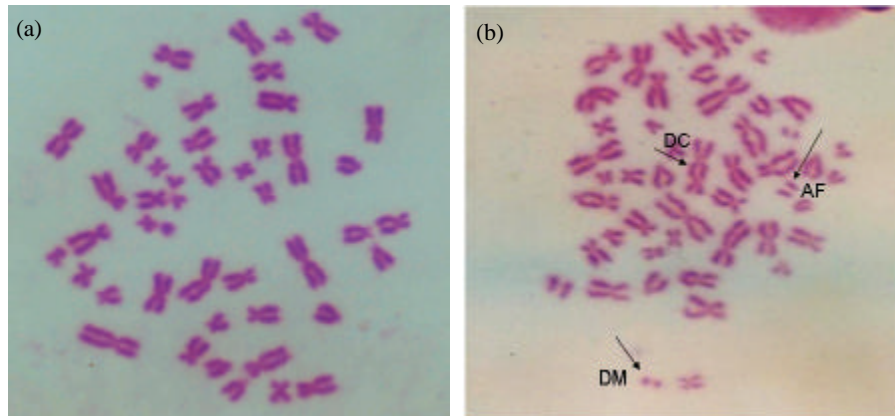


Fig. 1(a-b): (a) Metaphase spread from normal cell and (b) Metaphase spread with dicentric (DC), acentric fragment (AF) and double minute (DM) aberrations

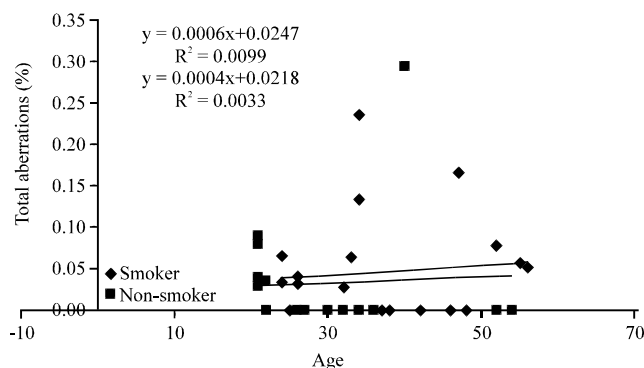


Fig. 2: Frequencies of total aberrations in control smokers and non-smokers as a function of age

DISCUSSION

Chromosome aberrations are considered to be precursors and early biomarkers of cancer, aiding in early diagnosis and disease prevention. Tobacco consumption is positively correlated with accumulation of DNA damage. The DNA damaging agents found in tobacco include benzo(a)pyrene (B(a)P) and tobacco-specific N'-nitrosamines that exhibit carcinogenicity (Huberman *et al.*, 1976; Hoffmann *et al.*, 1982; Preston-Martin and Correa, 1989). Thus tobacco constituents are proven carcinogens and are known to induce significant chromosome aberrations in blood cells of smokers (Bilban and Jakopin, 2005; Obe and Herha, 1978; Vijayalaxmi and Evans, 1982; Littlefield and Joiner, 1986; Sinues *et al.*, 1990). Similarly, radon being a radioactive gas is a proven inducer of DNA damage. Reports indicate statistically significant increase of chromosome aberrations in uranium miners occupationally exposed to ^{222}Rn compared to controls (Leggett and Eckerman, 2001). Bauchinger *et al.* (1994) and others have shown an increase in dicentric and ring chromosomes in blood lymphocytes of persons living in houses with very high radon concentrations (Mai, 2007).

Although, smokers exposed to radon are estimated to be at an elevated risk of cancer the extent of synergism may be dependent on the frequency of smoking and other environmental and lifestyle factors to which individuals may be exposed. Besides, data available on the synergistic effect of radon and smoking comes exclusively from miners. Cytogenetic studies on occupational workers including miners and those employed in nuclear fuel manufacturing, indicate, exposure to low levels of ionizing radiation and smoking cause significant increase in all types of chromosomal aberrations such as gaps, breaks, acentric fragments, exchanges, dicentrics and polyploids (Kandar and Bahari, 1995; Martin *et al.*, 1991; Prabhavathi *et al.*, 2000; Al-Sabti *et al.*, 1992; Dias *et al.*, 2007). Prabhavathi *et al.* (1995) observed significant increase in SCEs among smokers and non-smokers exposed to uranyl compounds compared to their respective controls. However, Chung *et al.* (1996) and Tawn and Whitehouse (2003) did not observe such an increase in smokers occupationally exposed to radiation.

The present study is the first of its kind to investigate the interaction of radon and smoking sans confounding factors, as smoker cells were exposed *in vitro* to radon. The elevated frequencies of chromosome aberrations noted in this study are sufficient proof of the synergistic mode of action. Similar effects have been reported in smokers occupationally exposed to pesticides (Rupa *et al.*, 1988, 1989) and chemicals (Sorsa *et al.*, 1983).

In the present study there was one smoker (control) who had dicentric chromosome aberration and this could be attributed to radioactive polonium and lead in cigarette smoke (Tahir and Alaamer, 2008; Skwarzec *et al.*, 2001; Khater, 2004).

Many studies reveal an age related increase in aberration frequencies. In the present study, a regression analysis with respect to age did not reveal such an increase in chromosome aberrations with and without smoking. This may be due to the fact that the ages of the study group ranged between 21 and 54 while most studies showing age related increase in aberration frequencies included subjects aged 60 and above. Neither did smokers show elevated frequencies compared to nonsmokers with respect to age (p-value = 0.924).

Our study fails to show elevated frequencies of aberrations in control smokers compared to non-smoker controls. It may be noted that only light to moderate smokers were included in this study (<18 cigarettes/day). A higher frequency of micronuclei was observed in subjects that smoked a higher number of cigarettes per day when compared with healthy non-smokers or low level smokers (Fenech, 1993; Tsai *et al.*, 2001). Tawn and Binks (1989) identified a significant effect of heavy smoking (>20 cigarettes/day) but found it difficult to establish a significant effect of moderate or light smoking.

Further, enhanced levels of chromatid aberrations due to radon is attributed to genomic instability (Smerhovsky *et al.*, 2002) and genomic instability correlate well with decreased DNA repair capacity (Bailey and Bedford, 2006). Moreover, reports show that smokers have a reduced capacity to repair DNA damage (Myllyperkio *et al.*, 2000; Duell *et al.*, 2002; Fracasso *et al.*, 2006).

CONCLUSIONS

The present study reveals significantly higher frequency of chromosome aberrations in smoker cells exposed *in vitro* to radon compared to non-smokers. Thus the synergistic effect of smoking and radon is well established.

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