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Nucleoplasmic bridges and acrocentric chromosome associations as early markers of exposure to low levels of ionising radiation in occupationally exposed hospital workers

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Abstract

Ionising radiation, with the contribution of telomere shortening, induces DNA double-strand breaks that result in chromosome end fusion, nucleoplasmic bridges (NPBs) and chromosome aberrations (ChAbs) as well as dicentric chromosomes. In order to investigate the chromosomal damage induced by occupational ionising radiation at low exposure levels, and to find early markers of health hazard, peripheral lymphocytes of occupationally exposed hospital workers were cytogenetically analysed. Results showed a significant difference in the frequency of ChAbs in exposed subjects relative to controls. A significant number of NPBs between nuclei of binucleated cultured lymphocytes from exposed subjects were also observed, as well as a consistent amount of acrocentric chromosomes with associations of their short arms. Excluding confounding factors, the frequencies of all these three biological endpoints differed significantly in exposed subjects from those in controls. Because the absence of telomeres and/or their short length could be a common root for both the findings, we utilised fluorescence *in situ* hybridisation technique with telomeric repeat as probe to demonstrate that, in exposed subjects, chromatin of short arms of involved acrocentric chromosomes did not exhibit a telomeric shortening but appeared strongly decondensed. This finding suggests that NPBs and telomeric acrocentric association should be regarded as early markers of exposure to low levels of ionising radiation and their increase should be seen as an early warning for the health of the involved workers.

Introduction

It is well known that ionising radiation induces DNA double-strand breaks that result in ChAbs when the cell progress through the cell cycle to form the next metaphase, but it is also reported that the generation of chromosome rearrangements may be related to telomere shortening (1). In fact, chromosome ends are capped by telomeres, tandem repeated (TTAGGG)n sequences added by telomerase and the telomeres protect chromosomes from end-to-end fusion and degradation (2). Alterations in the telomere–telomerase mechanism contribute to loss of telomeric functions and induce telomeric associations that, in turn, can produce chromosome instability (3); in fact, telomeric associations of metaphase chromosomes have been found in human tumours and in senescent cells (4). It is widely described that dicentric chromosomes originate either from misrepair of chromosome breaks or telomere-to-telomere end fusions (5,6). More recently, it was reported that the yields of ionising radiation-induced nucleoplasmic bridges (NPBs) in human cells are dose dependent and it has been proposed recently that NPBs are an important biomarker of early chromosome damage events induced by ionising radiation (7).

In order to investigate the chromosomal damage induced by occupational exposure to low-level ionising radiation, and to find early markers of health hazard, peripheral lymphocytes of occupationally exposed hospital workers were analysed after short-term culture.

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Materials and Methods

Cell culture

Peripheral blood lymphocytes were collected from 56 hospital workers (18 clinicians, 20 technicians and 18 attendants, mean age 42.03 years) and from 54 healthy unexposed subjects, considered as controls (mean age 30.18 years). All individuals lived in the same country. After providing informed consent, each subject filled a

Table I. Frequency of ChAbs

Subjects	ChAbs % (±SD)
Unexposed	1.15 (0.05)
Exposed	2.87 (0.17)*

*$P < 0.05$ according to Student's t-test.

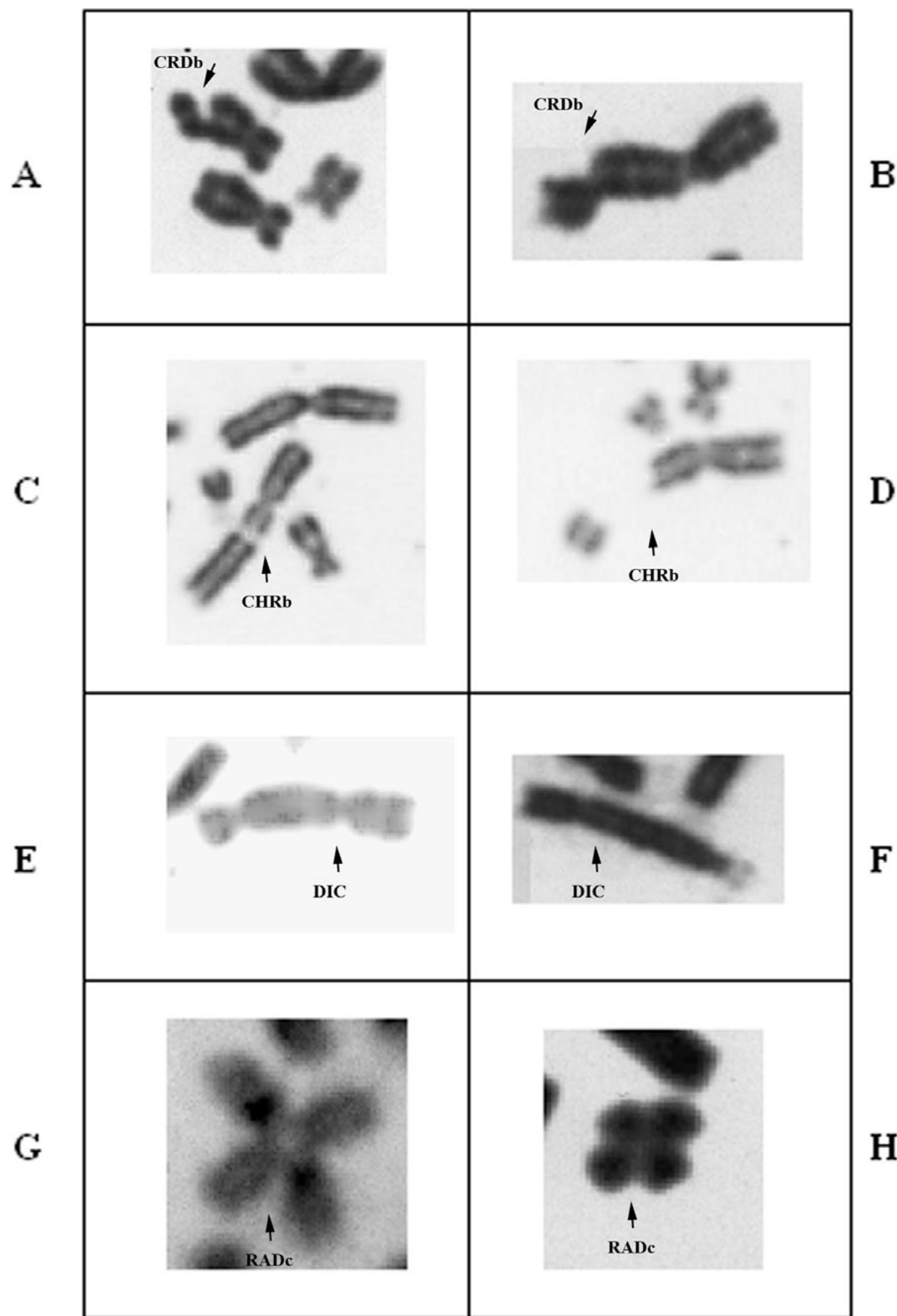

Figure 1. Chromosome rearrangements observed in metaphases of exposed subjects. Chromatid breaks (CRDb in **A** and **B**); chromosomal breaks (CHRb in **C** and **D**); dicentrics (DIC in **E** and **F**); radial configurations (RADc in **G** and **H**).

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detailed questionnaire containing some information about lifestyle and eventual diseases or therapy.

Conventional lymphocyte cultures were performed in complete medium additioned with 10% bovine foetal serum for 48 h at 37°C; incubation with 300 µg/ml of cytochalasin B for 28 h was performed in parallel lymphocyte cultures in order to obtain binucleated cells. Colcemid (final concentration 0.1 µg/ml; Sigma) was added for the last 2 h. At least 200 well-spread metaphases whose chromosome number was 46 ± 2 and 1000 binucleated cells were scored for each subject by two independent observers.

Centromeric–telomeric fluorescence *in situ* hybridisation

Using the fluorescence *in situ* hybridisation (FISH) technique, telomeric repeats were detected by using (TTAGGG)n probe, whereas centromeric repeats were highlighted with 'all centromere α-satellite DNA probe' (ONCOR, Gaithersburg, MD, USA). Both the probes were labelled with digoxigenin-11-dUTP (Roche Applied Sciences), according to the protocol of Antonacci *et al.* (8) with modifications. (9) Briefly, labelled probes were hybridised with metaphase spreads (or interphase nuclei) in 2× standard saline citrate solution, 50% formamide, 10% dextran sulphate, 0.5 mM Tris–HCl, pH 7.6, 0.1 mM EDTA and 0.1 mg/ml of sonicated salmon sperm DNA at 42°C overnight. Detection of digoxigenin was performed by using monoclonal anti-digoxigenin antibody fluorescein isothiocyanate-conjugated (Roche Applied Sciences, Europe). Chromosomes or interphase nuclei were counterstained with 2 µg/ml of propidium iodide and the preparations, mounted in antifade solution (200 mM 1,4-diazabicyclo octane [DABCO], 20 mM Tris–HCl, pH 8, 90% glycerol) were observed under a Nikon fluorescence microscope equipped with a 100-W mercury lamp and image catching system. At least 100 well-spread metaphases whose chromosome number was 46 ± 2 and 500 binucleated cells were scored for each subject by two independent observers.

Results and Discussion

Results from cytogenetic analysis showed a significant difference in the frequency of ChAbs found in metaphases from exposed and control subjects (Table I). Chromosome rearrangements included chromosomal and chromatid breaks, dicentric chromosomes and quadriradial configurations (Figure 1).

Even though only three dicentrics and three quadriradials were observed in six different exposed subjects (0.06%, respectively), a consistent and significant number of NPBs between nuclei of binucleated cultured lymphocytes from exposed subjects was found (Table II, Figure 2A and B). Thus, although the frequency of the chromosomal interchange figures found in our investigation is low, comparable with that reported in the literature for control subjects (10,11), taking into account that the NPBs are indicators of

Table II. Frequency of NPBs

Subjects	NPBs % (±SD) (*n* = scored binucleated cells)	
	Giemsa stain (*n* = 1000)	Centromeric FISH detection (*n* = 500)
Unexposed	1.42 ± 0.15	1.27 ± 0.16
Exposed	5.18 ± 0.23**	5.05 ± 0.24**

***P < 0.006 according to Student's *t*-test.

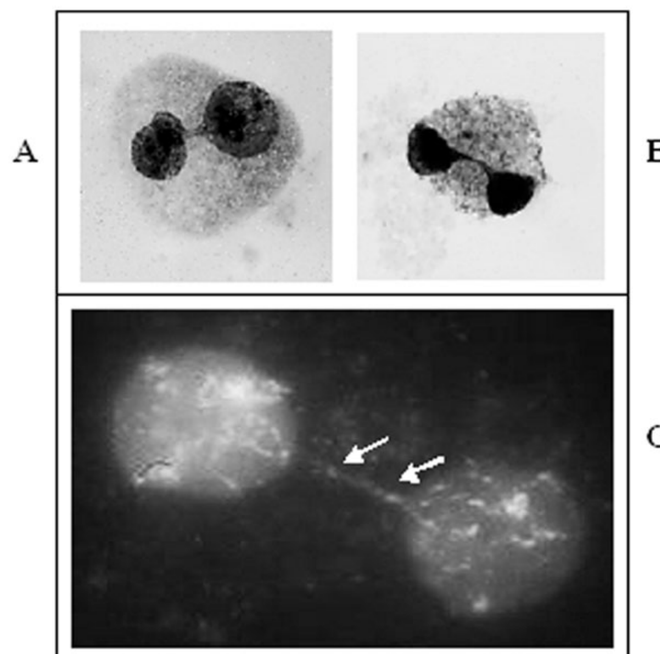

Figure 2. NPBs between nuclei of binucleated cultured lymphocytes from exposed subjects. (**A** and **B**) Conventional Giemsa staining; (**C**) Centromeric FISH with α-satellite DNA probe. The arrows indicate the signals of the two centromeres of the dicentric stretched between the two nuclei.

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Table III. Frequency of association between two or more acrocentric chromosomes

Subjects	ACSAA % (±SD) (n = involved chromosomes)						
	$n = 2$	$n = 3$	$n = 4$	$n = 5$	$n = 6$	$n = 7$	$n = 8$
Unexposed	10.40 (0.59)	2.26 (0.45)	0.40 (0.09)	0.13 (0.06)	0.04 (0.02)	0 (0.00)	0 (0.00)
Exposed	15.20 (1.39)	4.40 (1.48)	1.01 (0.16)	0.40 (0.14)	0.12 (0.04)	0.02 (0.01)	0.04 (0.02)

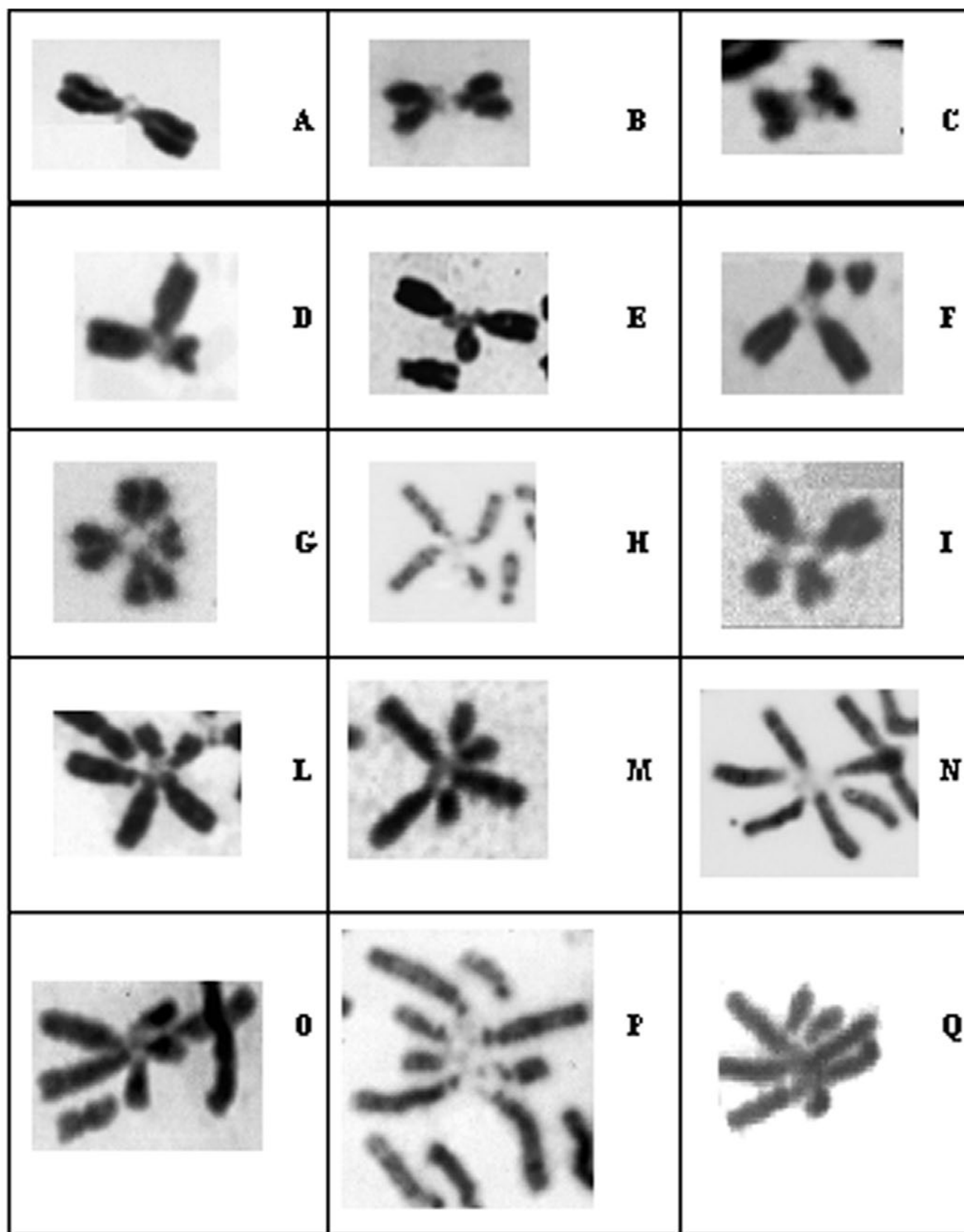

Figure 3. Associations between the short arms of acrocentric chromosomes (ACSAA) observed in exposed subjects metaphases: between two (**A**–**C**), (**D**–**F**) three, (**G**–**I**) four, (**L**, **M**) five, (**N**, **O**) six, (**P**) seven, (**Q**) eight chromosomes.

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Table IV. Influence of confounding factors on frequency of ChAbs, NPBs and ACSAA

Subjects	No.[a,b]	ChAbs[a,b] % (DS)	NPBs[a,b] % (DS)	ACSAA[a,b] % (SD)
Unexposed				
Selected	18	0.50 (0.22)	1.12 (0.19)	11.65 (0.15)
All	54	1.15 (0.05)	1.34 (0.15)	13.21 (0.20)
Exposed				
Selected	20	2.56 (0.34)*	4.72 (0.55)*	19.30 (0.42)*
All	56	2.87 (0.17)*	5.12 (0.23)**	21.19 (2.77)

[a]Selected subjects.
[b]All subjects.
*$P < 0.05$ according to the Student's *t*-test.
**$P < 0.006$ according to the Student's *t*-test.

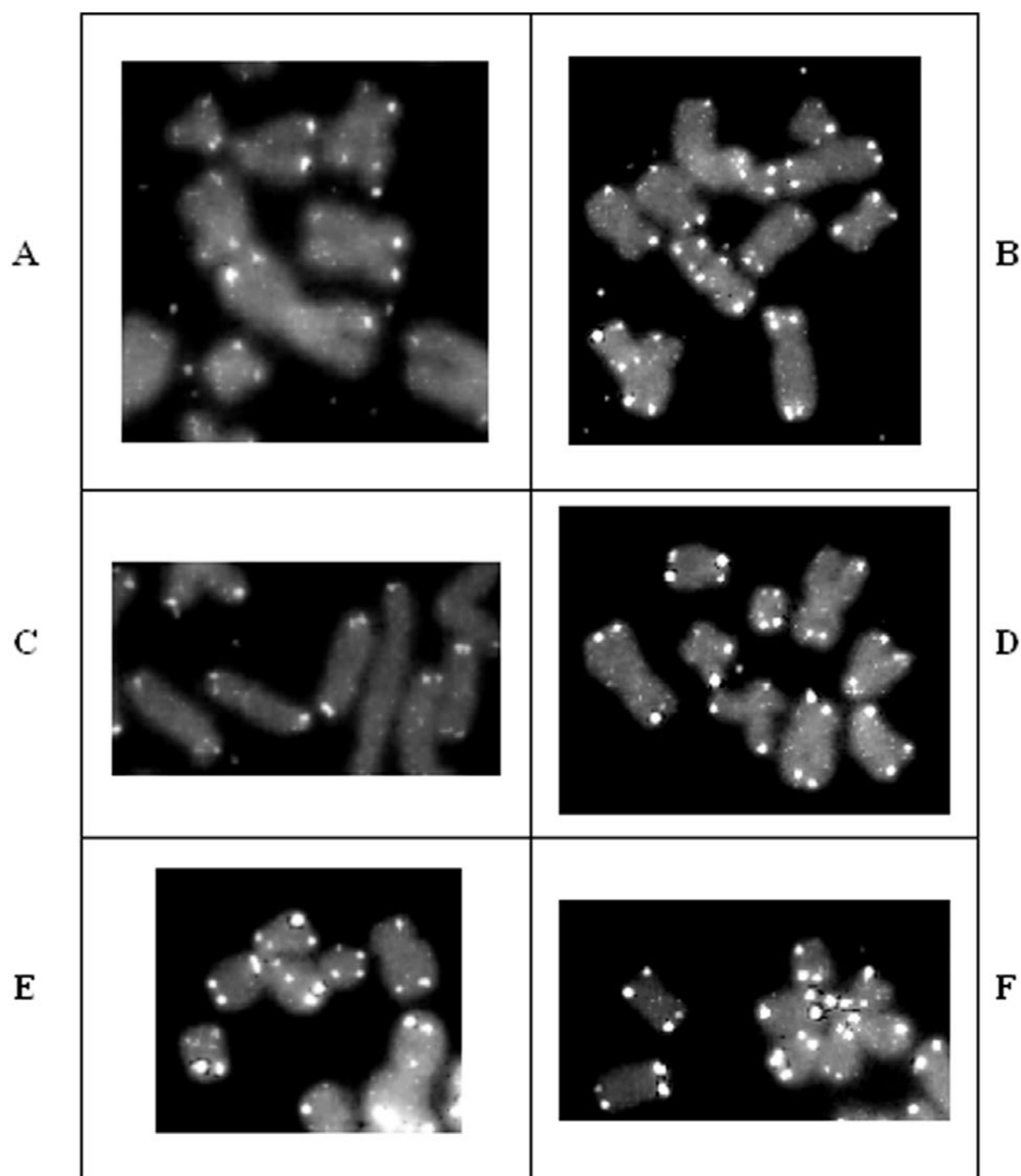

Figure 4. FISH localisation of telomeric repeats in some chromosome associations of exposed subjects metaphases. (**A** and **B**) telomeric associations; (**C** and **D**) ACSAA between two acrocentric chromosomes; (**E**) ACSAA between three acrocentric chromosomes; (**F**) ACSAA between six acrocentric chromosomes.

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the presence of dicentric chromosomes stretched between the two nuclei (Figure 2C), it is not consequently rigorous, in my opinion, to conclude that the occupational exposure of the subjects could be irrelevant in terms of genomic instability. Probably, the NPBs are an earlier instability marker than dicentric chromosomes because the latter, to affirm themselves in a metaphase, need a viable cell for more than one mitotic cycle, in respect to a binucleated cell that, in principle, could not live until it get to the next mitosis.

An interesting finding was the presence of a number of Associations between the Short Arms of Acrocentric Chromosomes (ACSAA). This kind of rearrangement has been widely described in the past as satellite associations (12–14), but no reports have been published more recently on this matter.

It is known that telomere length in somatic cells depends strictly on ageing (15,16). Subjects reported here belonged to a large extent to the same age group, even if controls were on average younger; therefore, the prevalence of ACSAA in exposed subjects seemed uncorrelated with the age parameter. As expected, the differences between ACSAA frequencies in unexposed and exposed were insignificant, but it is worth noting that all the exposed values were ~2.5-fold greater than the unexposed ones and, in addition, some ACSAA involving seven or eight acrocentric chromosomes were observed only in chromosome preparations of exposed subjects, while at most six associated acrocentric chromosomes were scored in metaphases of controls also (Table III, Figure 3).

In order to assess the real effect of the occupational exposure, ChAbs, NPBs and ACSAA frequencies were calculated also after the elimination of confounding variable factors related to lifestyle of exposed and control subjects, namely smoking or drinking coffee, personal exposure to medical X-rays or therapeutic treatment for any disease. For this purpose, we selected 20 exposed subjects and 18 controls in which the confounding factors were not present. In their metaphases, we found ChAbs frequencies of 2.56% and 0.50%, NPBs frequencies of 4.72% and 1.12% and ACSAA frequencies of 19.30% and 11.65%, respectively (Table IV). Excluding confounding factors, it should be noted that the frequencies of all the three biological endpoints differed significantly in exposed subjects from controls, suggesting that the presence of these three cytogenetic findings is attributable exclusively to exposure to ionising radiation.

To evaluate the influence of occupational exposure on the telomere presence and length, we utilised FISH technique with telomeric (TTAGGG)n repeat as probe. Results demonstrated that fluorescent spots were present at the end of all chromosomes, including at the terminus of short arms of acrocentric chromosomes involved in ACSAA, and that the size and the fluorescence intensity of acrocentric chromosome telomeres were similar to those of other chromosomes (Figure 4).

It has been suggested that the fusigenic potential of chromosomes in mammalian cells may be determined by both telomere length and chromatin structure at telomeric level, in the sense that telomeric chromatin undercondensation can result in terminal fusion of chromosomes also if they have relatively long telomeres (17). In telomeric associations observed in exposed subjects, chromatin of short arms of involved acrocentric chromosomes did not exhibit a true fusion but appeared strongly decondensed (Figure 4).

This finding suggests that telomeric acrocentric association should be regarded as an early marker of exposure to low levels of ionising radiation similarly to the NPBs. It is worth remembering that this kind of rearrangement can be maintained clonally, whereas the classical dicentric marker is an unstable aberration that comes after many exposures to low doses. Moreover, the dicentric chromosomes, as unstable markers, may be underestimated in a cytogenetic assay; instead, telomeric associations represent chromosomal damage that are still early, reversible and, by their nature, cannot be underestimated.

In conclusion, on the basis of these results, we propose a new two cytogenetic endpoint on which to base the advisability of increasing precautions in low-level ionising radiation workplaces, namely the personnel turnover. In particular, the presence of significant amount of NPBs and telomeric associations between acrocentric chromosomes deserves attention: as the maintenance of telomere function is essential for genomic stability, their increase should be seen as an early warning of health hazard for the involved workers.

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