



Increased micronucleated cell frequency related to exposure to radiation emitted by computer cathode ray tube video display monitors

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Abstract

It is well recognized that electromagnetic fields can affect the biological functions of living organisms at both cellular and molecular level. The potential damaging effects of electromagnetic fields and very low frequency and extremely low frequency radiation emitted by computer cathode ray tube video display monitors (VDMs) has become a concern within the scientific community. We studied the effects of occupational exposure to VDMs in 10 males and 10 females occupationally exposed to VDMs and 20 unexposed control subjects matched for age and sex. Genetic damage was assessed by examining the frequency of micronuclei in exfoliated buccal cells and the frequency of other nuclear abnormalities such as binucleated and broken egg cells. Although there were no differences regarding binucleated cells between exposed and control individuals our analysis revealed a significantly higher frequency of micronuclei ($p < 0.001$) and broken egg cells ($p < 0.05$) in individuals exposed to VDMs as compared to unexposed. We also found that the differences between individuals exposed to VDMs were significantly related to the sex of the individuals and that there was an increase in skin, central nervous system and ocular disease in the exposed individuals. These preliminary results indicate that microcomputer workers exposed to VDMs are at risk of significant cytogenetic damage and should periodically undergo biological monitoring.

Key words: micronucleus test, mutagenicity, electromagnetic fields.

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Introduction

Constant advances in the electronics industry and the increasing use of electrical appliances have resulted in consumers being increasingly exposed to the effects of extremely low frequency electromagnetic fields (ELF-EMF). Concerns have been raised (Ivancsits *et al.*, 2002) about the possible health hazards of chronic exposure to extremely low frequency (ELF) and very low frequency (VLF) electromagnetic radiation from various appliances including computer cathode ray tube (CRT) video display monitors (VDMs). Over the past few decades several studies (Wertheimer, Leeper, 1979; Li and Theriault, 1997) have demonstrated that exposure to ELF-EMF may be related to an increased risk of certain types of cancer, including leukemia, central nervous system cancer, and lymphoma, although other studies (Verkasalo *et al.*, 1993; Schreiber *et al.*, 1993) have failed to find such association.

Chromosomal mutation is a key event in carcinogenesis (Fenech, 2000) and the study of DNA damage at the chromosome level is an essential part of genetic toxicology. Micronuclei (MN) are acentric fragments or complete chromosomes which fail to attach to the mitotic spindle during cytokinesis and are normally excluded from the nuclei (Maluf and Erdtmann, 2000). The quantification of micronuclei by the micronucleus test (MT) is an important non-invasive cytogenetic method which is a good indicator of chromosome mutation (Majer *et al.*, 2001) and has been extensively used for monitoring populations exposed to known mutagens and carcinogens. The major advantages of the micronucleus test over other techniques are that it can be applied to interphase cells and does not require cell culture or the preparation metaphase cells, a further advantage being that because of the low cost of this test it is suitable for the large-scale screening of populations (Titenko-Holland *et al.*, 1994).

The major aim of our study was to use the presence of investigate a possible link between genotoxic effects and

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microcomputer users occupationally exposed to CRT VDMs through the quantification of cells with micronucleus (MNC), binucleated (BNC) and broken egg (BEC) cells.

Material and Methods

Subjects

The study was carried out on 10 male and 10 female microcomputer users who were occupationally exposed to CRT VDMs and 20 control subjects matched for age and sex. At least five years of working was the criteria used for sample selection of the exposed individuals. To obtain necessary data on lifestyles and personal factors (age, working period, diets, etc.), all subjects were interviewed, according to the protocol published by the International Commission for Protection against Environmental Mutagens and Carcinogens (Carrano, 1988). The subjects were either Caucasian or mixed race Brazilians of African and Caucasian descent living in the city of Pelotas in the southern Brazilian state of Rio Grande do Sul. The exposed group worked principally as copy typists, word processor operators or computer programmers while the unexposed subjects were selected from the general population of the same city.

The effects of occupational exposure to VDMs were investigated by the micronucleus test applied in exfoliated buccal cells from the exposed and unexposed individuals.

Cytological preparations

The buccal mucosa of each individual tested was washed with distilled water and then swabbed with a wooden tongue depressor. Two coded slides were prepared for each individual by smearing the exfoliated buccal cells onto pre-cleaned slides. The smears were then air-dried, fixed with methanol, stained by the Feulgen reaction and counterstained with fast-green as described by Stich and Rosin (1982) and examined using bright-field microscopy. For each individual 2,000 cells were assessed for genetic damage by observing the frequency of micronucleated, binucleated and broken egg cells in exfoliated buccal cells and the frequency of. The number of cells assessed was selected to ensure an untruncated spontaneous dispersion of micronucleated, binucleated and broken egg cells.

Statistical analysis

Independent variables such as age, working period, ocular, skin, and central nervous system (CNS) diseases, theoretically expected to be associated with the occurrence of MN (dependent variables), were classified as qualitative (no parametrical). These non-parametric factors were analyzed between controls and exposed individuals by Mann-Whitney test, while the Student t-test was used for age and time comparisons. The frequencies of MNC, BNC and BEC between exposed and control groups were compared through Mann-Whitney U test. The association of inde-

pendent variables and the occurrence of MNC, BNC and BEC was analyzed by Mann-Whitney U test, and further evaluation, if necessary, performed by non-parametric Spearman rank correlation coefficient. The hypothesis was considered to be 5%.

Results

The main characteristics of the exposed and control subjects (average of age, working time, sex and ocular, skin and CNS diseases) are summarized in Table 1. None of the groups studied showed significant difference in age, period of work, ocular and CNS diseases, except for skin diseases found in a higher incidence (65%) in the exposed individuals ($p < 0.001$).

Table 2 shows the average numbers of MNC, BNC and BEC of both exposed and control individuals included in this investigation. Assessment of MN frequencies in exfoliated buccal cells revealed a significantly higher frequency of micronuclei ($p < 0.001$) in individuals exposed to VDMs (6.20 ± 4.44) compared to unexposed (2.05 ± 2.16). In the same way, we observed a significant difference ($p < 0.05$) in BEC frequency, between exposed workers (2.80 ± 2.50) and controls (1.40 ± 2.30). However, no statistically significant differences regarding BNC frequency between exposed (3.40 ± 3.89) and control individuals were observed (2.15 ± 2.54).

The number of cells (per 2,000 cells) with micronucleus, binucleated, and broken egg cells of each individual is showed in Figure 1. Within the control group, 35% showed no cells with micronucleus, and 40% of them showed only one to three MNC, resulting in mode zero. Six MNC was the maximum number found in non-exposed individuals. In contrast, in the exposed group, we found a variable distribution of cells with micronucleus, ranging from zero to 18, resulting in mode five. Similarly, the distribution of BEC was different between control and exposed

Table 1 - General characteristics of 10 male and 10 female workers occupationally exposed to cathode ray tube computer video display monitors (VDMs) as compared to unexposed control individuals matched for age and sex.

Characteristics of the subjects	Subjects	
	Exposed (n = 20)	Unexposed (n = 20)
Average age	37.75 \pm 8.62	38.0 \pm 8.84
Age range	24-49	24-50
Average time working with VDMs	14.45 \pm 7.44	11.7 \pm 8.29
Variation	5-25	1-28
Ocular disease present	10 (50%)	4 (20%)
Skin disease present	13 (65%)*	2 (10%)*
Central nervous system disease present	10 (50%)	5 (25%)

*High significant at $p < 0.001$, by the Mann-Whitney U test.

Table 2 - Cytological observations made on 10 males and 10 females occupationally exposed to cathode ray tube computer video display monitors (VDMs) as compared to unexposed control individuals matched for age and sex. We assessed 2,000 cells for each individual.

Cytological observation	Subjects	
	Exposed (n = 20)	Unexposed (n = 20)
Micronucleated cells		
Mean	6.20**	2.05**
Standard deviation	± 4.44	± 2.16
Minimum number	0	0
Maximum number	18	6
Binucleated cells		
Mean	3.40	2.15
Standard deviation	± 3.89	± 2.54
Minimum number	0	0
Maximum number	13	9
Broken egg cells		
Mean	2.80*	1.40*
Standard deviation	± 2.50	± 2.30
Minimum number	0	0
Maximum number	9	9

*Significant at $p < 0.05$ by the Mann-Whitney U test.
 **High significant at $p < 0.001$ by the Mann-Whitney test.

groups. While 90% of the controls showed zero to three BEC, resulting in multiple modes (zero and three), only 70% of the exposed individuals showed the same range of BEC. In contrast, the distribution of BNC found was similar between both groups, resulting in mode zero. Zero to three was the maximum number of BNC found in more than 50% of exposed and unexposed individuals.

Comparisons between independent variables age (within the following intervals: ≤ 35 and > 35 years), working time (≤ 10 and > 0 years) and the frequencies of MNC, BEC and BNC was investigated among exposed and controls. Our results suggest that there is no association between the age, working time, of subjects, and the number of MN and nuclear abnormalities (Table 3).

Among the exposed females the MNC, BEC and BNC frequency in the above cited intervals, did not differ ($n = 5$; 8.00 ± 4.64 , and $n = 5$; 8.80 ± 5.36). Among the exposed males, alike the females, in the same intervals, presented a small increase, however, not significant ($n = 3$; 2.00 ± 2.65 , and $n = 7$; 4.86 ± 2.73).

Exposed individuals presented an increased frequency of ocular (50%), skin (65%), and CNS (50%) diseases, however association between these diseases and the frequency of MNC, BNC and BEC was only obtained for binucleated cells among exposed individuals with ocular and CNS diseases ($p < 0.05$) (Table3).

The frequency of MNC, BNC and BEC between males and females was also compared, and, in both exposed

Table 3 - Cytological observations made on 10 males and 10 females occupationally exposed to cathode ray tube computer video display monitors (VDMs). We assessed 2,000 cells for each individual.

Exposed individuals	Cytological observations		
	Micronucleated cells (Mean ± SD)	Binucleated cells (Mean ± SD)	Broken egg cells (Mean ± SD)
Age (years)			
≤ 35 (n = 8)	5.75 ± 4.89	3.63 ± 3.62	2.13 ± 1.46
> 35 (n = 12)	6.50 ± 4.32	3.25 ± 1.46	3.25 ± 2.99
Number of years working with VDMs			
≤ 10 (n = 7)	4.57 ± 3.84	3.57 ± 3.91	2.00 ± 3.23
> 10 (n = 13)	7.08 ± 4.63	3.31 ± 4.03	3.23 ± 2.86
Ocular disease			
Present (n = 9)	7.67 ± 4.18	$5.78 \pm 3.83^*$	3.89 ± 3.14
Absent (n = 11)	5.00 ± 4.47	$1.45 \pm 2.72^*$	1.91 ± 1.45
Skin disease			
Present (n = 13)	6.23 ± 4.83	2.85 ± 2.48	3.69 ± 3.99
Absent (n = 7)	6.14 ± 3.98	2.71 ± 2.75	2.86 ± 3.93
Central nervous system disease			
Present (n = 10)	6.60 ± 4.30	$5.10 \pm 4.15^*$	3.80 ± 2.97
Absent (n = 10)	5.80 ± 4.78	$1.70 \pm 2.87^*$	1.80 ± 1.48
Sex			
Females (n = 10)	$8.40 \pm 4.77^*$	$6.30 \pm 3.56^{***}$	$4.40 \pm 2.32^{**}$
Males (n = 10)	$4.00 \pm 2.91^*$	$0.50 \pm 0.71^{***}$	$1.20 \pm 1.48^{**}$

*Significant at $p < 0.05$ by the Mann-Whitney U test.
 **Significant at $p < 0.01$ by the Mann-Whitney U test.
 ***Significant at $p < 0.001$ by the Mann-Whitney U test.

and control groups, the frequencies of MNC and nuclear abnormalities were significantly higher in females. Among the exposed group, females showed at least twice more MNC, BNC and BEC than males ($p < 0.05$), (Table 3). The Spearman correlation coefficient between MNC and sex was positive ($p = 0.029$).

Among the females and males, the exposed group showed more MNC than the control group ($p < 0.01$) (Table 4).

Based on the p-values estimated by Spearman rank coefficients, in our analysis sex was the main factor affecting MNC frequency in exfoliated cells.

Discussion

For many years scientists and engineers believed that electromagnetic fields of low frequency could not cause alterations in human cells. This hypothesis was based on the idea that EMF could not generate a sufficient amount of heat to increase the body temperature, not causing damage at the dna level (Lechter, 1991). However, data available in

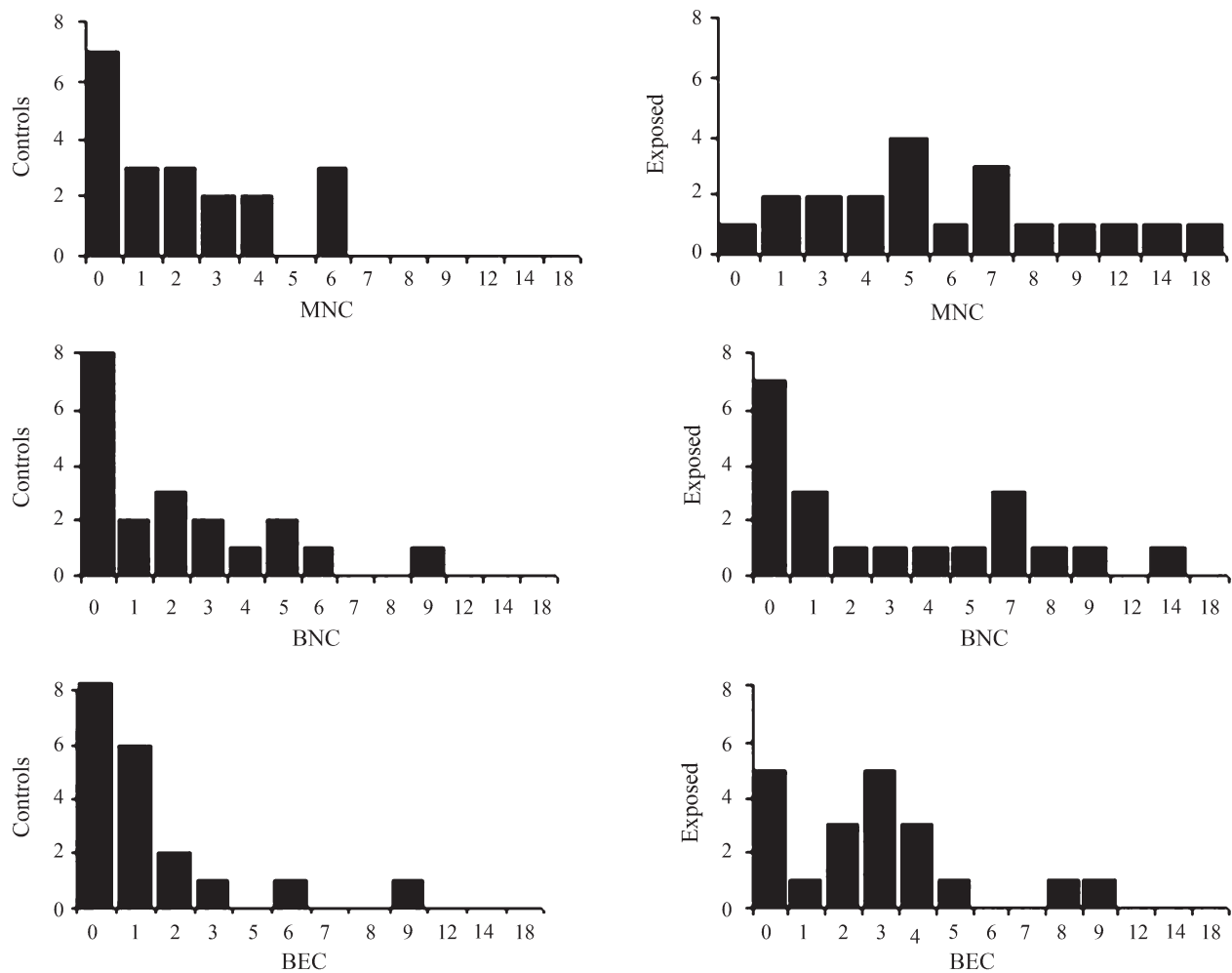


Figure 1 - Distribution of number of cells with micronuclei (MNC), binucleated (BNC) and broken egg (BEG) cells (2,000 cells per individual).

Table 4 - Cytological observations made on 10 males and 10 females occupationally exposed to cathode ray tube computer video display monitors (VDMs) as compared to unexposed control individuals matched for age and sex. We assessed 2,000 cells for each individual.

Individuals	Cytological observations		
	Micronucleated cells (Mean \pm SD)	Binucleated cells (Mean \pm SD)	Broken egg cells (Mean \pm SD)
Females			
Exposed (n = 10)	8.40 \pm 4.74*	6.30 \pm 3.56	4.40 \pm 2.32**
Unexposed (n = 10)	3.60 \pm 1.90*	4.10 \pm 2.23	1.90 \pm 1.66**
Males			
Exposed (n = 10)	4.00 \pm 2.91**	0.50 \pm 0.71	1.20 \pm 1.48
Unexposed (n = 10)	0.50 \pm 0.97**	0.20 \pm 0.42	1.0 \pm 2.83

*Significant at $p < 0.05$ by the Mann-Whitney U test.

**Significant at $p < 0.01$ by the Mann-Whitney test.

the literature suggest that exposure to elf-emf directly causes genetic changes in biological systems (McCan *et al.*, 1993, 1998; Murphy *et al.*, 1993; Simko *et al.*, 1998a, b;

Repacholi and Greenebaum, 1999; Walleczek *et al.*, 1999). Furthermore, some studies suggest the hypothesis that EMF is not involved on the onset of the tumoral growth, but may be acting as a promoter or co-promoter in this process (Goodman and Shirley-Henderson, 1990).

The evidence that EMF could cause genotoxic effects in human cells can reinforce the relation between exposure to EMF and increased incidence of cancer in exposed individuals. Gangi and Johansson (1997) detected an increased incidence of skin and CNS alterations among microcomputer's workers. In their study, the workers reported to feel skin - and mucosa - related symptoms, such as pain, itch, heat sensation, erythema, papules, and pustules. The CNS symptoms were, *e.g.* dizziness, tiredness, and headache, the same symptoms caused by sunburn. Kisner and Fedeman (1998) reported that female workers exposed to VDM presented obstetric complications, besides skin, ocular, and CNS diseases. However, the authors conclude that the data reviewed were inconsistent or methodically flawed, and believed that continued research had to be carried out to further define and elucidate the risk of EMF produced by

video display terminals. In our study we also detected skin, ocular and CNS diseases among approximately 50% of the exposed group, maybe as a result of continual exposure to EMF emitted by microcomputer monitor. However, no association was found between these diseases and frequencies of MNC and BEC, and only frequency of BNC was related with ocular and CNS diseases within the exposed individuals

Significantly our results obtained from the investigation of micronucleus in exfoliated cells indicate that exposed individuals have a significant increased number of MNC. Exposed individuals showed three times more cells with micronucleus than control individuals. In the same way, the frequency of BEC among exposed individuals was two times higher than in the control group. Estécio and Silva (2002) also evaluated possible nuclear alterations in microcomputer's workers and found that exposed individuals had two times more chromosomal aberrations in cultured lymphocytes than control individuals, reinforcing the data obtained in our study.

Similarly, Ivancsits *et al.* (2002) showed that intermittent exposure to a 50Hz magnetic field causes a reproducible increase in DNA strand breaks in cultured human diploid fibroblasts. They evaluated the genotoxic effect of cultured fibroblasts from skin biopsy of healthy donors that were continuously exposed to ELF-EMF, through the comet assay, and obtained significant results. Although these findings suggest that the exposure to EMF increase the frequency of chromatic and/or chromosomal breaks, it is not clear yet the exactly molecular mechanism affected by this type of radiation.

Several hypotheses like induction of electric currents, increased free radical activity or acceleration of electron transfer in different enzymes and proteins have been proposed, but all of these hypotheses have been mostly speculative. Recent observations have shown that DNA can transfer electrons within its base pairs. These studies suggest that EMF may initiate transcription by generating repulsive forces causing chain separation at specific DNA sequences. As in the state of transcription the DNA presents itself as a vulnerable target to genotoxic influences, the on/off switching at intermittent ELF-EMF exposure may lead to DNA disruption, causing DNA strand breaks (Ivancsits *et al.*, 2002).

According to Estécio and Silva (2002) the chromatic aberrations could be a result of malfunction of enzymes that duplicate and repair DNA, leading to alterations on the DNA strand recently polymerized. Despite of the molecular mechanism the EMF leads to chromosomal destabilization. Thus, individuals who have this destabilization could present cells with high frequency of mutations, chromosomal aberrations, micronucleus, and increased risk of cancer growth than individuals with stable genome.

Several reports have described an association between chronological aging and aneuploidy. The chromosomes most frequently lost are the X chromosome in females and Y chromosome in males. The autosomes may be lost at the same frequency as the sex chromosomes, but these events should lead to death as they contain genes required for cell survival. As lagging chromosomes and fragments can be incorporated into a MN and the increase in aneuploidy occur according to the age, this factor should be correlated with the increase in MN formation (Burkvic *et al.*, 2001). In contrast, our results suggest that there is no association between the individual's age and the frequency of MNC, BNC, and BEC among microcomputer's workers. However, when this factor was analyzed among male workers, it was observed a slight increase of MNC and nuclear abnormalities in older workers, although this result was not statistically significant.

In the same way, analysis of MNC, BNC and BEC frequencies after different working times showed a small, not significant increase among the exposed group on the longest working time (< 10 years).

Particularly evident is the increment observed in women with respect to men. In females it was observed significant higher frequencies of MNC, BNC, and BEC than in males, and the Spearman correlation coefficients between MNC and sex were positive ($p = 0,029$). In agreement with our data, some reports in this area have been shown similar results. Burkvic *et al.*, 2001 demonstrated that there are 22% more sexual chromosomes lost in females than in males, confirming that females cells are more sensitive to action of genotoxic agents. Both the preferential involvement of sex chromosomes in somatic aneuploidy in women and the relation between malsegregation of X chromosomes and age are well documented. However, the molecular mechanisms underlying instability of sex chromosomes in females are not fully elucidated yet. Studies have demonstrated the high susceptibility of sex chromosomes to be lost at mitosis, as revealed by the high incidence of micronucleated X-positive lymphocytes in female donors. Further investigation by *in situ* hybridization of cytokinesis-blocked lymphocytes using a X chromosome's centromere probe, demonstrated that both, chromosomal loss and non-disjunction, contribute to the malsegregation of sex chromosomes of the females (Zijno *et al.*, 1996).

Different physiological parameters, such as menstrual cycle, estrogen levels, and oral contraceptives may affect the frequency of sister chromatid exchange (SCE) (Ghosh and Ghosh, 1988; Joseph-Lerner *et al.*, 1993), but in our investigation we did not take in consideration neither estrogen levels nor oral contraceptive assumption.

To conclude, our preliminar results strong suggest that microcomputer's workers, exposed to EMF presented an increased frequency of cells with micronuclei, probably due to the genotoxic action of EMF, which they are exposed to at work, and these findings are straightly related to the

sex of the individuals. Finally, extensive studies and standardized tests to evaluate biological damage at different levels are recommended to public agencies concerned with environmental quality and public health. Genotoxic evaluation is a necessary measure to ensure environmental quality and occupational health, as is the workers orientation to increase awareness and decrease the risk for serious disease caused by computer cathode ray tube video display monitors radiation.

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