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The Cytogenetic Effects Evaluation of Non-thermal Radiofrequency Radiation from Cellular Phones on Rat Peripheral Blood Lymphocytes

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Abstract

The widespread use of mobile phones over the past decade has raised considerable concern public about possible biological effects of electromagnetic field emitted from cell phones and their consequences on human health. The aim of this study was to evaluate whether chronic whole-body exposure to non-thermal radiofrequency (RF) radiation from cellular phones could induce cytogenetic effects on peripheral blood lymphocytes of rats exposed to radiofrequency radiation. Twelve (23) Wistar rats were used for the study and randomly divided into 4 groups according to time of exposure, animals in treatment were exposed respectively for: oh (control), 1h, 2 and 3h daily 7 days a week for up 15 weeks to 900 MHz radiofrequency radiation at an average special absorption rate (SAR) of 0.873-0.352 W/kg, all rats were visually checked daily and were weighed weekly. After the period of exposure, blood samples were collected from all groups and peripheral blood cultures were performed using standard laboratory methods for the extent of genotoxicity, assessed by the cytokinesis-block micronucleus assay. The results indicated that significant increase of micronucleated (MN) cells in the lymphocytes among the study groups in a time-dependent manner compared with the control. The proliferation index (PI), is significantly decreased in all exposed groups in a time-dependent manner compared with the control, however body weight gain was insignificantly changed in all groups exposed comparing to unexposed rats. This study shows that the chronic exposure to 900 MHz radiofrequency radiation from cell phones may induce cytogenetic effects in rat lymphocytes, and this effect is more remarkable for long exposure time.

Keywords

Rat Lymphocytes, Radiofrequency, Micronucleus Test, Proliferation Index, Body Weight Gain

1. Introduction

In environment, the human organism is more and more exposed to non-thermal electromagnetic (EM) pollution emanates from wireless communication devices and many new technology, the massive and daily use of mobile communication system in the world has raised public concern about the biological effects of exposure to radiofrequency (RF) radiation for human health [1], these electromagnetic radiations in frequency range from 0 Hz to 300 GHz are considered as a type of non-ionizing radiation which have a energy unable to break the bonds of the cell tissues of living organisms [2].

The cell phones become an essential communication tool for almost people in modern society, it operates approximately at 300 MHz - 2 GHz and their antenna transmits and receives electromagnetic radiofrequency (RF) radiation.

The increasing use of wireless communication system has prompted growing public attention about a possible biological

thermal or non-thermal effects following exposure of the electromagnetic radiation emitted by mobile phones such as EMR may interact with living matter or a process leading to indirect adverse effects, radiofrequency (RF) radiation exposure can be measured by the amount of energy absorbed by a unit mass of living matter.

In this context, the specific absorption rate (SAR) considered as a parameter assesses the transfer energy from electromagnetic radiofrequency (RF) radiation to organism tissue, it is expressed as with units of W/kg.

Several studies assessing the genotoxicity of RF radiation have been conducted in humans and animals at various frequencies, and the conclusions are controversial.

Diem *et al.* [3] reported that the exposure to RF radiation of 1800 MHz was associated with breakage of DNA in cell cultures.

Hardell *et al*. [4] found an increased risk of brain tumors by a factor of 1.5 after 10 years of use of the digital cellular phone or wireless phone. Zotti-Martelli *et al.* [5] also showed an increase in micronuclei in human cells exposed to a frequency of 1800 MHz. Gandhi *et al*. [6] revealed the genetic damage in individuals residing in the vicinity of a mobile phone base station.

However, lack of genotoxicity of RF radiations have been reported in many studies ([7], [8], [9]; [10]; [11]; [12]; [13]; [14]; [15]; [16]; [17]; [18]; [19]).

The aim of the current study is to investigate the potential cytogenetic effects of chronic exposure to 900 MHz radiofrequency radiation on peripheral blood lymphocytes of rats exposed for: 1h, 2 and 3h a day 7 days a week for up 15 weeks using the micronucleus test (indicator of genotoxic activity, it able to detect both chromosome breakage and chromosome loss) and the cellular proliferation index (which measures the cellular kinetics).

2. Materials and Methods

2.1. Animals

All procedures were performed in compliance according to international laws and regulations on animal welfare.

Twenty-three Wistar rats involved in this study were purchased from Chouaib Doukkali University animal facility, the animals were approximately 3 months old and weighted 116 and 184 g (males and females) at the beginning of the experiment, These rats were housed in polycarbonate, maintained under a 12h light/12h dark cycle in a temperature (23 ± 1°C), humidity was (40 ± 10%.) with free access to water and food.

Then they are divided into four groups as follows: the control group (n = 8), the 1h exposed group (n = 5), the 2h exposed group (n = 5) and the 3h exposed group (n = 5), respectively, irradiated (0h (control), 1h, 2h and 3h) daily for 15 weeks and weighted every weekend during the treatment.

2.2. Exposure System

The exposure system consisted of two cell phones and a plastic cage (WxLxH) 30x40x40cm, all experimental animals were suited in the same conditions with daylight, in room without near sources of microwave radiation. Each cell phone was positioned and fixed above the side ceiling of plastic cage about 2-3 cm from the body of the rats, its were placed as closely as possible to the whole body of the rats for uniform field distribution of electromagnetic field (EMF) during wave exposure, the cage was constantly aerated and all rats are able to move around freely, the experimental groups were continually exposed to the microwave radiation from two cell phones (900 MHz GSM, electromagnetic field pulsed at 217 Hz) system was used, the peak specific absorption rate (SAR) of the head was 0.873 W/kg and the average SAR of the whole body was 0.352 W/kg), mobile phones were activated by calling each other, all study groups were exposed to radiofrequency radiation at 10:00 a.m daily.

The temperature inside the cage was monitored during the experiments; it was almost unchanged (23 ± 1°C).

2.3. Cellular Culture

At the end of the exposure period (15 weeks), blood samples were collected into the glass tubes (with heparin as anticoagulants) by cardiac puncture under the ether anesthesia, the rats were then decapitated according to the technical conditions employed in laboratory.

Cell cultures were made according to the standard protocol for the micronucleus test as described by Fenech and Morley [20]. Briefly, 0.5 mL of the whole blood were incubated in glass tubes containing 5 mL RPIM 1640 growth medium supplemented with 15% fetal calf serum, 1% phytohemagglutinine and 1% antibiotics (penicillin/streptomycin).

Culture tubes were incubated at 37°C for 72 h. After 44 h of cultivation, 0.1 mL of cytochalasin B was added to stop the cytoplasm division without inhibiting nuclear division.

After 72h incubation at 37°C, total blood cultures were centrifuged at 1000 rpm for 10 minutes and then exposed to a light hypotonic shock with 0.075 M KCl and fixed with a 1/3 acetic-acid/methanol solution, then spread over microscope slides, which were air-dried and stained with 5% Giemsa in phosphate buffer (pH= 6.8) and coded.

2.4. Micronuclei (MN) Analysis

The micronuclei were scored in 500 binucleated lymphocytes per animal, and were counted only in binucleated cells. The criteria for identifying MN are as follows: The MN is nuclear entities independent of the main nucleus, round or oval in shape and has the same staining intensity and texture as the main nucleus, most cells with MN will contain only one MN but it is possible to find cells with two or more MN, the size must be between the sixteenth and the third smallest of the main nucleus [21].

2.5. Proliferation Index

Cell proliferation index (PI) is an indirect measurement of cell cycle duration, and is calculated according to the

following formula [22]:

$$PI = \frac{(1xN1 + 2xN2 + 3xN3 + 4xN4)}{1000 \text{ analyzed cells}}$$

Where N1 is the number of cells in the first division, N2 the number of cells in the second division, N3 the number of cells in the third division and N4 the number of cells in the fourth division.

2.6. Statistical Analysis

Data were expressed as means ± SEM. Statistical analysis were carried out by analysis of variance (ANOVA) ± SEM followed by appropriate post-hoc tests including multiple comparison (LSD), differences were considered significant at 0.05 level.

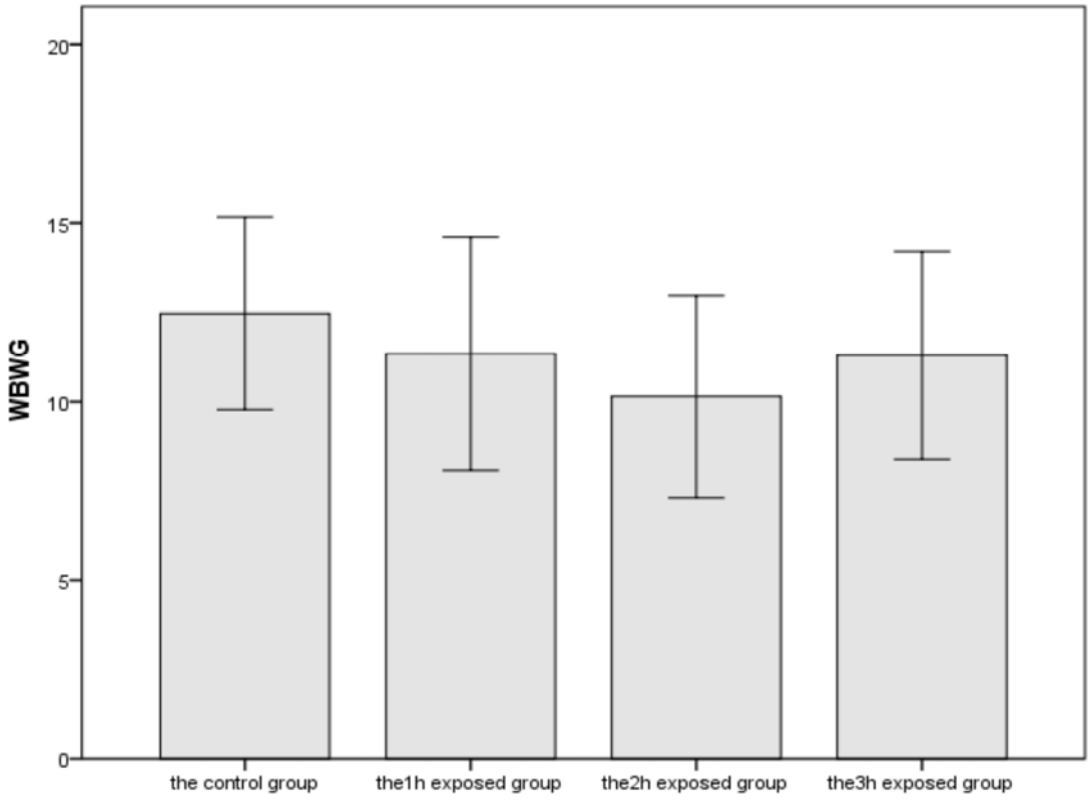
3. Results and Discussion

3.1. The Weekly Body Weight Gain

The weekly body weight gain is defined as: WBWG= the body weight difference of rat after one week.

Table 1. *Weekly body weight gain of all four group rats during the exposure period (15 week).*

WBWG (g) / Week	The group control (n=8)	The 1h exposed group (n=5)	The 2h exposed group (n=5)	The 3h exposed group (n=5)
1	37,38	37,34	35,02	36,26
2	19,56	29,70	14,70	15,38
3	27,25	-3,58	12,38	11,96
4	15,19	14,50	4,30	17,44
5	7,01	20,34	23,50	14,68
6	14,54	5,50	1,22	-4,40
7	2,4	4,78	13,18	19,40
8	9,78	1,24	12,90	10,90
9	10,73	17,52	4,94	-3,86
10	9,63	11,58	14,56	16,66
11	9,07	16,68	-6,10	2,58
12	6,94	-5,66	-2,60	5,30
13	6,15	3,80	7,90	16,80
14	-1,05	5,00	5,96	-1,00



Values are given as the mean ± SEM and differences were considered significant at *P < 0.05

Figure 1. *The values correspond to the mean of the weekly body weight gain of the exposed groups (1h, 2h, 3h) and control.*

The analysis of variance (ANOVA) revealed no significant difference in weekly gain of body weight between all exposed groups and control group (F=0,102; P=0,96).

Figure 1 shows that the average weekly gain of body weight decreases slightly for the study groups compared to the group control and indeed the WBWG of the 1h exposed group is 11,34±2,94g, the WBWG of the 2h exposed group is 10,13±2,94g and the WBWG of the 3h exposed group is 11,29±2,94g while the WBWG of the control group is 12,47±2,94g however, there were no statistically differences when compared each group exposed (1h, 2h, 3h) respectively to the control group (P1=0,79; P2=0,58; P3=0,78).

Body weight is considered an important parameter reflecting the healthy state of living organisms but also an early indicator of the possibility of developing certain diseases or health outcome.

This study indicates that the body weight gain of rats exposed for 15 weeks decreased slightly over the exposure time, but there was no significant difference in the body weight gain between the groups.

So, the study groups were exposed respectively for: 1h, 2 and 3h a day 7 days a week for up 15 weeks to 900 MHz radiofrequency radiation at an average special absorption rate (SAR) of 0.873- 0.352 W/kg) did not induce a significant effect on body weight gain when compared each group exposed or all groups exposed to the control group (P1= 0, 79; P2=0,58; P3 = 0,78); (F=0,102; P=0,96).

The present study is in agreement with data reported by Jin *et al*. [23] how exposed two groups of rats to 849 MHz CDMA (code division multiple access) and 1.95 GHz WCDMA (Wideband Code Division Multiple) radiofrequency (RF) radiations simultaneously at 2.0 W/kg (total 4.0 W/kg) for 45 min/day, 5 days per week for one year, no significant alteration in body weight was found with the simultaneous combined RF exposure.

Similarly, Sonmez *et al.* [24] showed that the exposure three groups of rats to 900 MHz electromagnetic field (EMF) for 1 hour/day, during 28 days, with a SAR of 0.016-2 W/kg, did not induce a change significantly in the body weight after EMF exposure.

Grafstrom *et al*. [25] did not record any changes in body weight after exposing rats to (GSM-900) radiofrequency radiation for 2 hours per week about 55 weeks with a DAS (average) of 0.6 and 60 mW / kg.

However, Ray and Bihari [26] concluded that the exposure of Young albino rats (both sexes) to 7.5 GHz microwaves (1.0 KHz square wave modulation, average power 0.6 mW/cm^2) for 3 h daily for up 60 days causes a reduction in body weight gain of exposed animals.

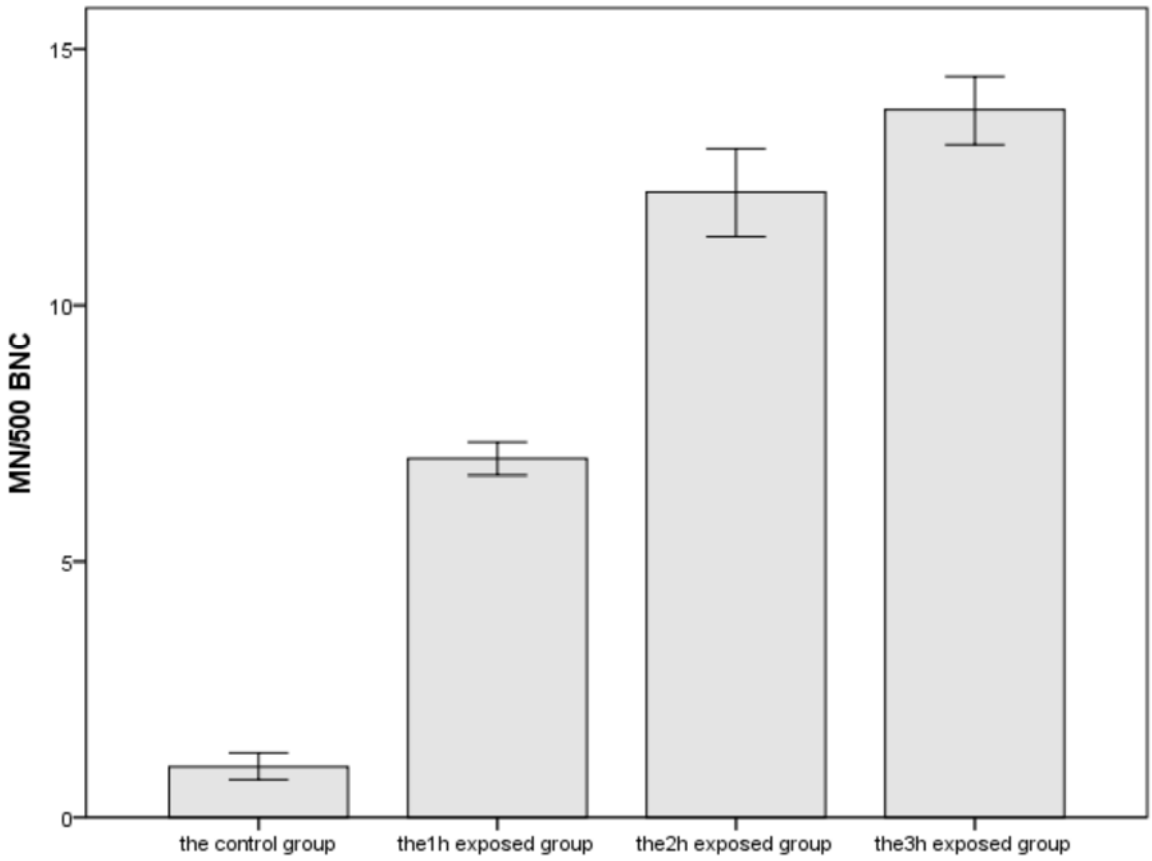
The same findings were reported by Krstic *et al* [27] showing the irradiation a group of mice to GSM 900 MHz for 30 minutes per day for 2 months.)

3.2. Cytogenetic Study

Table 2. *Cytogenetic results for the all groups.*

the exposure time (h)	sex	MN (total)	Cells with 1 MN	Cells with 2 MN	Cells with 3 MN	PI
1h	M	6	2	2	0	1,399
1h	M	7	4	0	1	1,264
1h	M	7	3	2	0	1,342
1h	F	8	3	1	1	1,298
1h	F	7	2	1	1	1,387
2h	M	12	8	2	0	1,235
2h	M	9	7	1	0	1,209
2h	M	14	8	3	0	1,186
2h	F	13	7	3	0	1,217
2h	F	13	4	3	1	1,281
3h	M	16	10	0	2	1,251
3h	M	12	10	1	0	1,188
3h	M	13	9	2	0	1,173
3h	M	14	8	3	0	1,25
3h	F	14	10	2	0	1,253
0h (control)	M	1	1	0	0	1,552
0h	M	2	2	0	0	1,611
0h	M	1	1	0	0	1,553
0h	M	0	0	0	0	1,542
0h	M	1	1	0	0	1,582
0h	F	1	1	0	0	1,672
0h	F	2	2	0	0	1,542
0h	F	0	0	0	0	1,602

3.3. MN Test



*Values are given as the mean ±± SEM and differences were considered significant *P < 0.05*

Figure 2. *The values correspond to the mean of MN in the peripheral blood of the four groups: 0h (control), 1h, 2h and 3h.*

The analysis of variance (ANOVA) showed statistically significant difference in micronucleus frequency (F = 138.68, P <0.001) between all exposed groups and control group.

Figure 2 revealed that all exposed groups showed an increased MN formation with an increase in the exposure time.

in fact the average number of MN in binucleated cells (BNC) among all exposed groups (7,00±0,56; 12,20±0,56; 13,80±0,56 respectively for the 1h exposed group, the 2h exposed group and the 3h exposed group) far exceeding that observed in control group (MN=1,00 ± 0,44), this difference was highly significant (P<0.001).

Similarly, the average number of MN in BNC among the 2 and 3h exposed groups (12.20 ± 0.56; 13.80 ± 0.56 respectively for the 2h exposed group and the 3h exposed group) exceeds that observed in the 1h exposed group (7.00 ± 0.56), this difference is significant (p <0.001).

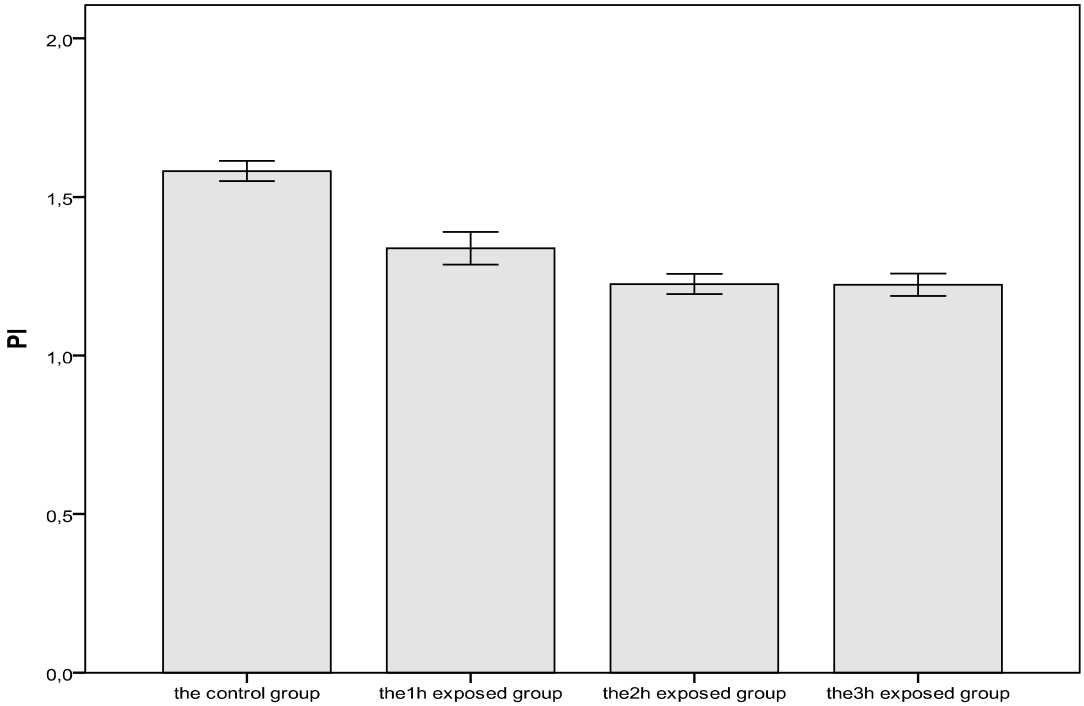
However, the average number of MN in BNC among the 3 exposed group (13.80 ± 0.56) exceeds marginally that of the 2h exposed group (12.20 ± 0.56), this difference was not significant (P = 0.06).

3.4. PI Test

Statistical analysis (ANOVA) showed a significant difference in Proliferation indices (PI) between all exposed groups and unexposed group (control) (F = 94.3561, P <0.0001). All exposed groups showed a decrease in PI with an increase in duration of exposure.

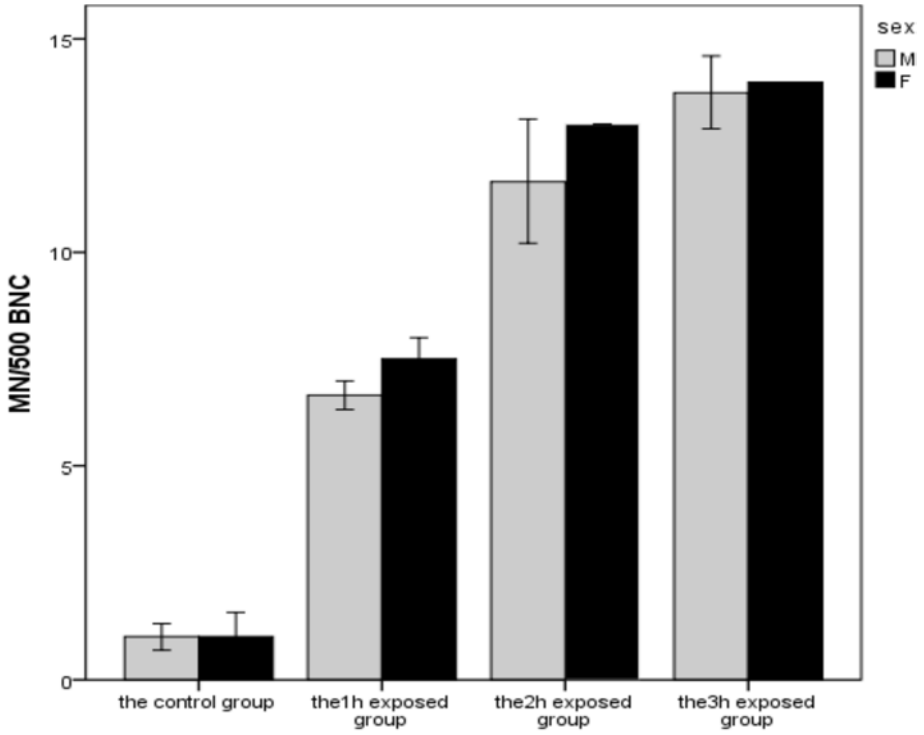
As shown in Figure 3 the average values of PI in the exposed groups (PI=1, 338±0,020; PI=1,223±0,020; PI=1,226± 0,020 respectively for the 1h exposed group, the 2h exposed group and the 3h exposed group) is much lower compared to that observed in the control (PI=1,582± 0,016), the difference was highly significant (p<0.001).

Similarly, the mean values of PI in the 2 and 3h exposed groups (PI=1,226±0,020; PI=1,223±0,020 respectively for the 2h exposed group and the 3h exposed group) is lower than that observed in the 1h exposed group (PI=1,338 ± 0,020), these differences were statistically significant (p= 0.001). Whereas, the mean of PI in the 3h exposed group (PI=1,223±0,020) is lower slightly than that observed in the 2h exposed group (PI= 1,226± 0,020), the difference was not significant (P= 0, 9284).



Values are given as the mean ±± SEM and differences were considered significant at *P < 0.05.

Figure 3. *The values correspond to the mean of Proliferation indices (PI) in all groups: 0h (control), 1h, 2h and 3h.*



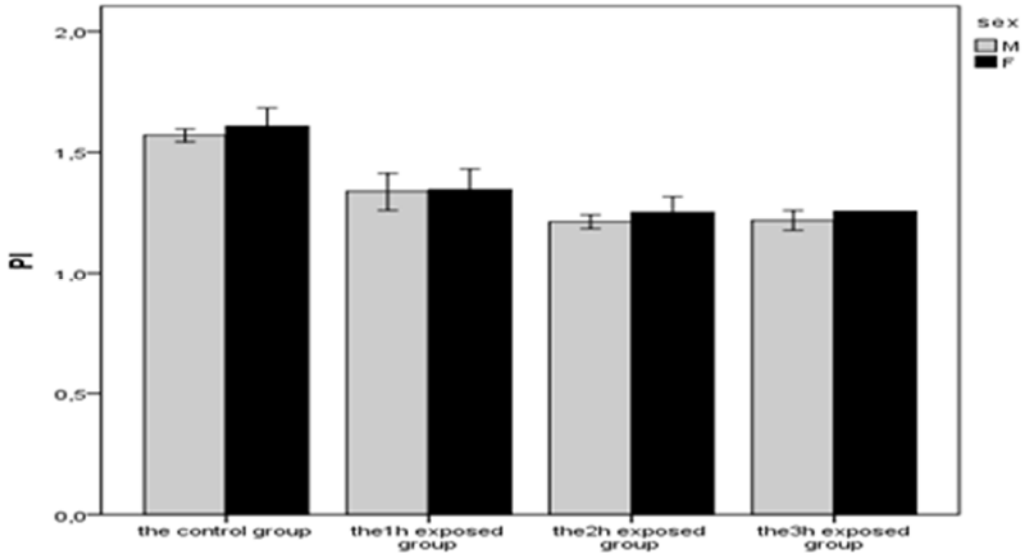
Values are given as the mean ±± SEM and differences were considered significant at 0.05 level**.**

Figure 4. *MN frequency according to gender in all groups: 0h (control), 1h, 2h and 3h (M=males, F=females).*

Figure 4 showed that the average number of MN in BNC in female exposed groups (7.5±0,941; 13±0,941; 14±1,331 respectively for the 1h exposed group, the 2h exposed group and the 3h exposed group) is higher slightly than that of males in the exposed groups (6.667±0,769; 11.667± 0,769; 13.750±0,666 respectively for the 1h exposed group, the 2h exposed group and the 3h exposed group).

Statistical analysis (ANOVA) revealed that difference was not significant (F=0,2768; P =0,8412) while the number of MN remains unchanged for males and females belonging to

the control group



Values are given as the mean ±± SEM and differences were considered significant at 0.05 level.

Figure 5. *Proliferation indices (PI) according to gender in all groups: 0h (control), 1h, 2h and 3h (M=males, F=females).*

As shown in Figure 5, the average values of PI in female exposed groups (PI=1.343±0.033; PI=1.249±0.033; PI=1.253±0.047 respectively for the 1h exposed group, the 2h exposed group and the 3h exposed group) is higher marginally than that of males in the exposed groups (PI=1.335±0,027; PI=1.21±0.027; PI=1.215±0,023 respectively for the 1h exposed group, the 2h exposed group and the 3h exposed group), the same for control (female: PI=1.605±0.027; male: PI=1.568±0.021), the statistical analysis (ANOVA) confirmed that the increase was not significant (F=0,1282; P = 0,9419).

On the other hand, there is an inverse correlation between the frequency of MN and the PI among the exposed groups in the range of -0.924 and very significant ($p < 0.0001$).

In this study, we used the micronucleus test to investigate the genotoxicity, that detects the activity of clastogenic and aneugenic of genotoxic agents so acentric chromosome fragments (i.e. lacking a centromere) or whole chromosomes are unable to migrate to the poles during the anaphase stage of cell division that results in micronucleus formation in telophase and also, the proliferation index related to cell cycle duration considered as detector the toxic activity is evaluated.

The present study clearly demonstrated that a Chronic Exposure to 900 MHz of rats respectively for three times (1, 2 and 3h) at a maximum SAR (0.873- 0.352 W/kg) induce formation micronucleus in the blood cells and decrease cell proliferation value among the exposed rats, the statistical analysis (ANOVA) confirms that changes in those parameters were respectively significant ($F = 138.68$, $P < 0.001$) et ($F = 94.3561$; $P < 0.0001$).

Indeed, the number of MN and PI in the exposed groups was affected after exposure to radiofrequency radiation, the effects are much stronger with increasing time of exposure Consequently, these radiations increased the frequency of micronucleus formation and decreased PI level in a time-dependent manner, the obtained results show that a correlation between the frequency of MN and IP level has been calculated and found to be negatively correlated. The increased formation of micronuclei, delays the normal progression through the cell cycle, it is probably due to checkpoint activation in response to DNA damage. That is, radiofrequency considered as non-ionising radiations characterized by low energy and neither sufficient directly to break a chemical bond nor for the induction of heat in tissues leading to elevation of temperature in a subject exposed, that genotoxic effects are probably mediated by indirect mechanisms as microthermal processes, generation of oxygen radicals (ROS), or a disturbance of DNA-repair process [28].

These results are in agreement with data published by Sekeroglu *et al.* [29] showing that exposure of the immature and mature rats for 2 h a day for up 45 days to 1800 MHz RF radiation with a SAR of 0.37 W/kg and 0.49 W/kg led to a significant differences in chromosomal aberrations (CA), micronuclei frequency (MN), mitotic index (MI) and ratio of polychromatic erythrocytes (PCEs) for all exposed groups, the immature rats more affected by cytogenotoxic damage, even after the recovery period.

Similarly Trosic *et al*. [30] have exposed male rats for 2 h a day, 7 days a week for up to 2, 8, 15 and 30 days to continuous 2450MHz radiofrequency microwave (rf/MW) at DAS 1.25+ / - 0.36 W / kg, the frequency of micronucleated was significantly increased on experimental day 15 and the polychromatic erythrocytes were showed also a significant differences after 8 and 15 days of exposure.

A significant Micronucleated erythrocyte frequency in peripheral blood of female Latvian Brown cows from a farm close to and in front of the Skrunda Radar was found by Balode [31] in vivo animal study.

However, Vijayalaxmi, *et al*. [32] found the frequency of micronuclei polychromatic erythrocytes was not significant compared with the control after the exposure of rats and their nursing offspring for 2h/day, 7 days/week to a 1.6 GHz at whole-body average SARs of 0.036 – 0.077 W/kg until weaning then followed by chronic, head-only exposure (SAR 0.16 or 1.6 W/kg in the brain) of the offspring to a near-field 1.6 GHz signal for 2h/day, 5 days/week, over 2 years.

An in vitro study was conducted by Scarfi *et al*. [33] how demonstrated that the exposure of cultured human peripheral blood lymphocytes by 900 MHz exposure (GSM signal) at SAR of 0, 1, 5 and 10 W/kg for 24 hours did not cause any further increase in MN frequency nor variation in PI.

4. Conclusion

In conclusion, the present findings indicate that the in vivo exposure of mobile phone GSM-900 MHz radiofrequencies at 873-0.352 W/kg for 1, 2 and 3h /day for up 15 weeks to rat lymphocyte cell leads to cytogenetic alterations by significant differences in the number of MN and PI in all treatment groups and that this effect is more important for long exposure time, in this context more studies are necessary to clarify the mechanisms responsible for the damage of EMF.

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