Evaluation of oxidative stress and genetic instability among residents near mobile phone base stations in Germany

Sachin Gulati[1], Wilhelm Mosgoeller[2], Dietrich Moldan[3], Pavol Kosik[1], Matus Durdik[1], Lukas Jakl[1], Milan Skorvaga[1], Eva Markova[1], Dominika Kochanova[1], Katarina Vigasova[1], Igor Belyaev[1,*]

[1)] Department of Radiobiology, Cancer Research Institute, Biomedical Research Centre, Slovak Academy of Sciences, Bratislava, 845 05, Slovak Republic
[2)] Cancer Research Centre, Medical University Vienna, 1090 Vienna, Austria
[3)] Dr. Moldan Umweltanalytik, 97346 Iphofen, Germany

***Correspondence:** Prof. Igor Belyaev, Dr.Sc., Head, Department of Radiobiology, Cancer Research Institute, Biomedical Research Centre, Slovak Academy of Sciences, Bratislava, Slovak Republic; Tel: +421 2 32295119 E-mail: igor.beliaev@savba.sk

Abstract

Human exposure to radiofrequency electromagnetic fields (RF-EMF) is usually restricted by guidelines to prevent thermal effects in the tissue. However, at very low intensities "non-thermal" biological effects, like oxidative stress, DNA or chromosomal aberrations, etc. collectively termed genomic-instability are possible. Little is known about chronic (years long) exposure with non-thermal RF-EMF. We identified two adjacent housing estates in a rural region with residents exposed to either relatively low (control-group) or relatively high (exposed-group) RF-EMF GSM-900 and LTE-signals emitted from nearby mobile phone base stations. 24 healthy adults that lived in their homes at least for 5 years volunteered. Their homes were surveyed for common types of EMF and their blood samples were tested for oxidative status, transient DNA alterations, permanent chromosomal damage, and specific cancer related genetic markers like MLL gene rearrangements. We documented possible confounders, like age, sex, nutrition, life-exposure to ionizing radiation (X-rays), occupational exposures, etc. The groups matched well in terms of age, sex and occupational risk factors. The years long exposure had no measurable effect on MLL gene rearrangements and c-Abl-gene transcription modification. Associated with higher exposure, we found higher levels of lipid oxidation and oxidative DNA-lesions, though not statistically significant. DNA double strand breaks, micronuclei, ring chromosomes, and acentric chromosomes were not significantly different between the groups. Chromosomal aberrations like dicentric chromosomes (p=0,007), chromatid gaps (p=0,019), chromosomal fragments (p<0,001) and the total of

chromosomal aberrations (p<0,001) were significantly higher in the exposed group. No potential confounder interfered with these findings. The most likely cause for this higher rate of chromosomal aberrations in the exposed group appear to be the chronic exposure to the RF-EMF signals GSM 900 and LTE.

Keywords: Radiofrequency electromagnetic fields, RF-EMF; low dose chronic exposure; genetic instability; oxidative stress; DNA damage; chromosomal aberrations.

1. Introduction

From the first use in the 1950s until today, technological applications based on radiofrequency electromagnetic field (RF-EMF) and the human exposure to RF-EMF increased. Since about 1996, the introduction of mobile telephony and the installation of a mobile phone base station (MPBS) network has fueled the discussion about possible biological effects related to RF-EMF exposure. In 2011 the International Agency for Research on Cancer (IARC), categorized RF-EMF as "2B - possible carcinogen" (Baan et al., 2011). Since then, additional animal studies and epidemiologic data on long term exposure more likely than not to justify a higher rating, i.e. "2A - probable carcinogen" (ICBE-EMF, 2022). While a number of investigators found no impact of the exposure to RF-EMF on gene structures that would explain a carcinogenic effect, many studies did find a genotoxic effect. RF-EMF exposure can increase DNA damage (Garaj-Vrhovac et al., 2011), and can disturb the blood-brain barrier (Nittby et al., 2009). RF-EMF induced DNA damage was observed in animal models as well as in humans (Akdag et al., 2016; Bektas et al., 2020).

After RF-EMF exposure, the induced genotoxic effects can be ameliorated by antioxidants (Liu et al., 2013). Only few studies found no effect of RF-EMF exposure on the oxidative level while the majority of studies describes that RF-EMF-exposure can induce oxidative stress (Yakymenko et al., 2016), which - in some cases - is followed by the damage of macromolecules, inflammation and accumulation of mutations (Zhang et al., 2022), a biomarker of elevated cancer risk. Transient DNA damage can be visualized with modifications of comet assays, which allow distinguishing between single or double strand DNA damage (Schwarz et al., 2008), or oxidated DNA lesions (Al-Serori et al.,

2018). A sensitive method to investigate DNA double strand breaks is the staining of γH2AX/53BP1 DNA repair foci (Rothkamm et al., 2015).

Micronuclei assessed by the cytokinesis blocked micronucleus assay (CBMN) constitute a cytogenetic biomarker of genetic-toxicology and cancer risk. Whether or not RF-EMF exposure can produce micronuclei is still discussed controversially (Jagetia, 2022).To assess radiation induced genotoxicity, biological dosimetry utilizes chromosomal aberrations (CAs), which is a generally accepted practice. While some studies described RF-EMF exposure related specific chromosomal aberrations, like acentric fragments and dicentric chromosomes after acute exposure in vitro, other studies did not confirm these findings (Armstrong et al., 2013). However, the published data are difficult to compare because non-thermal RF-EMF effects are strongly dependent on multiple physical and biological parameters (Armstrong et al., 2013; Belyaev, 2010). If reported, they differ significantly between available studies.

The intensity of acute exposure to thermal RF-EMF is limited by widely accepted exposure guidelines (ICNIRP, 2020). While recommendations for long-term exposure to non-thermal RF-EMF has been suggested by the European Academy for Environmental Medicine (EUROPAEM) (Belyaev et al., 2016), they are not internationally recognized.

A main public concern is over harmful effects of RF-EMF exposure from MPBS. In contrast to broadcast antennas that are typically built outside communities, MPBS are erected close to the mobile phone customers, i.e. in the communities. The potential biological, environmental and health repercussions associated with the emission of RF-EMF from MPBS have remained largely unexamined. A recent case report describes that mobile phone stations can have short term effects (Nilsson and Hardell, 2023). An early hint to the carcinogenic potential of long term exposure comes from a German ecological study (Eger et al., 2004), but was not reproduced in another region (Meyer et al., 2006). Also in a Brazilian study the cancer risk was higher in the vicinity of MPBS (Dodê et al., 2011). Wolf and Wolf (Wolf and Wolf, 2004) found an association, which was not confirmed by another study in Israel (Atzmon et al., 2012).

To investigate whether or not long term exposure to environmental RF-EMF from MPBS yields a cancer risk, we investigated human blood cells for oxidative stress, transient and

permanent DNA damage, cytogenetic endpoints, and leukemia specific MLL (KMT2A-Histone-lysine N-methyltransferase 2A) gene alterations (Harper and Aplan, 2008).

2. Materials and methods

2.1 Chemicals

All chemicals were from Sigma (St. Louis, MI, USA), New England biolabs (NEB, UK), Thermo Fischer Scientific (Waltham, USA) and Merck (Darmstadt, Germany).

2.2 Subjects

We enrolled twenty-four individuals residing more than five years either close to or distant from MPBS. The participants provided written informed consent to the study procedures. The Ethics commission of the medical physicians' board Nordrhein (Düsseldorf, Germany) approved the study protocol (Az-2021403). Recruited participants provided data on demographics (age, sex, etc., Table 1). We documented nutrition habits and food preferences, individual life style factors, medication intake (**Error! Reference source not found.**), experienced medical procedures with ionizing radiation exposure (**Error! Reference source not found.**), occupational and environmental exposures, and the participants' subjective judgment on their electro-hyper-sensibility (**Error! Reference source not found.**). Exclusion criteria were acute and chronic medical conditions (e.g. fever, autoimmune diseases, cancer, dementia, etc.), and any condition that required medical intervention within 3 months before blood sampling.

2.3 Environmental electromagnetic fields documentation

2.3.1 Measuring Equipment

The measurements of low frequency alternating electric fields (LFEF) were carried out with a three-dimensional, potential-free E-field probe and the basic device EFA 3 (Narda Safety Test Solutions GmbH, Pfullingen, Germany; Serial number SN D 0117). Three-dimensional measurements of the low frequency alternating magnetic fields (LFMF) were carried out using MLog3D (Merkel Messtechnik, Maintal, Germany) and EMLog2 (ESTEC, Schwäbisch Hall, Germany) data loggers. For the documentation of the high-frequency range, we performed frequency-selective measurements of the individual radio services using a radiofrequency spectrum analyzer with biconical antennas. The spectrum analyzer was FSL 6, 9 kHz - 6 GHz, Serial number 10 04 23, (Rohde & Schwarz

Messgerätebau GmbH, Memmingen, Germany). Measuring biconical antennas were EFS 9218 9 kHz - 300 MHz, serial number 102 and SBA 9113-B 80 MHz - 3 GHz, serial number 362 and log-per antenna USLP 9143 250 MHz – 6 GHz, serial number 198, all from Schwarzbeck Mess-Elektronik OHG (Schönau, Germany). The antenna cable was from Schwarzbeck, type AK 9513 with 3 m length (serial number 161030). For high frequency broadband measuring at the participants sleeping areas for a 24-hour long-term measurement we used HF59B (27 MHz – 2,5 GHz) with an UBB antenna (27 MHz – 3,3 GHz; Gigahertz-Solutions, Langenzenn, Germany) with a ASB3-Adapter to the data logger (EMLog2 from ESTEC, Schwäbisch Hall, Germany).

2.3.2 Exposure Documentation

All measurements were in the house in the sleeping area. In a first step, we searched for stationary field sources in the area to locate devices with transformers (electric alarm clocks, watches, ventilators, radio, TV, WLAN, etc.), and found no static magnetic field sources. Starting on the next day, recordings of up to 7 days were carried out with data loggers. The two main frequencies of 16.7 Hz railroad power supply and 50 Hz public power grid were recorded separately. The sampling rate was every two seconds. The magnetic flux densities were recorded in the frequency ranges 16.7 Hz and 50-2000 Hz, the resolution was 10 nT. Alternatively, we employed EMlog2 devices with a sampling rate of one per second. In this case the recorded magnetic flux densities were from 5-30 Hz and 37-2000 Hz, and the resolution was 1 nT. After the recording of 5 up to 7 complete days, the entire record was inspected to find possible anomalies. The records from 10 p.m. to 6 a.m. of the days 1-5 provided were statistically analyzed.

2.4 Blood sample analyses

2.4.1 Blood sampling and oxidative stress

Each participant contributed 12 ml blood for the biological tests. The blood was taken by venipuncture in the office of a local family doctor in the morning hours. The vials (disposable pre-sterilized vacutainers tubes coated with EDTA/Heparin anticoagulant) were anonymized by numbering, then, transported in temperature-insulated boxes at 20°C (± 2°C) within 6 to 7 hours to the laboratory in Bratislava (Slovakia). The samples of each delivery were prepared on the same day. To warrant double-blind conditions, the courier and the laboratory team members had no information on the donor except for the

sample ID number. The key to assign the sample ID to the participant was kept at the German partners' office, who had organized the blood sampling in a local physicians' office. Upon arrival in the partner laboratory the viability of the lymphocytes was above 95% throughout. To obtain an index of the level of oxidative stress in the volunteers blood samples the TBARS assay (thiobarbituric acid reactive substance assay) was applied immediately after the sample arrival as described before (Buege and Aust, 1978).

2.4.2 DNA related Analyses

2.4.2.1 Alkaline comet assay

DNA damage like single-strand breaks (SSB) and alkali-labile sites was assayed using alkaline comet method according to Singh et al. (Singh et al., 1988) with minor modifications. After staining the slides with ethidium bromide (5 µg/ml), hundred cells selected randomly from each of the two slides per sample were examined on a Zeiss Axioscope 2 epifluorescence microscope (Carl Zeiss Microscopy, Jena, Germany). Using the Metafer software (Metasystems, Altlussheim, Germany), comets were analyzed as tail moment.

2.4.2.2 FPG enzyme based (modified) comet assay

Oxidatively generated DNA damage was identified as formamidopyrimidine glycosylase (FPG)-sensitive sites by employing the modified comet assay according to Collins et al. (Collins et al., 1993). The method was the same as for the alkaline comet assay except for treatment of slides after lysis with the FPG buffer (0.1 M KCl, 0.5 mM Na2EDTA,40 mM HEPES-KOH, 0.2 mg/mL bovine serum albumin, pH 8). The slides were incubated with FPG enzyme (New England BioLabs Ltd. Massachusetts, US) at 1 mg/ml in the FPG buffer for 30 minutes at 37^{O} C. For each sample, slides were prepared in duplicate. Comets were analyzed as tail moment (TM) using the Metafer software (Metasystems, Altlussheim, Germany).

2.4.2.3 Micronuclei (MN) and chromosomal aberrations (CA)

The MN assay (Figure 1) was performed according to the IAEA guidelines (IAEA, 2011). We analyzed 1000 binucleated cells from each participants blood sample. Cells were scored with the Metafer software (Metasystems, Altlussheim, Germany).

CA investigation was carried out by short term peripheral blood lymphocytes (PBL) cultures using the techniques of Moorhead et al. (Moorhead et al., 1960) with minor

modifications. From each sample whole blood cultures were set up in duplicates. For the analysis, one thousand well spread metaphases were analyzed using the Metafer software (Metasystems, Altlussheim, Germany). According to generally accepted criteria we identified CA like chromatid gaps, fragments, acentrics, dicentrics and ring chromosomes (Figure 1).

Figure 1

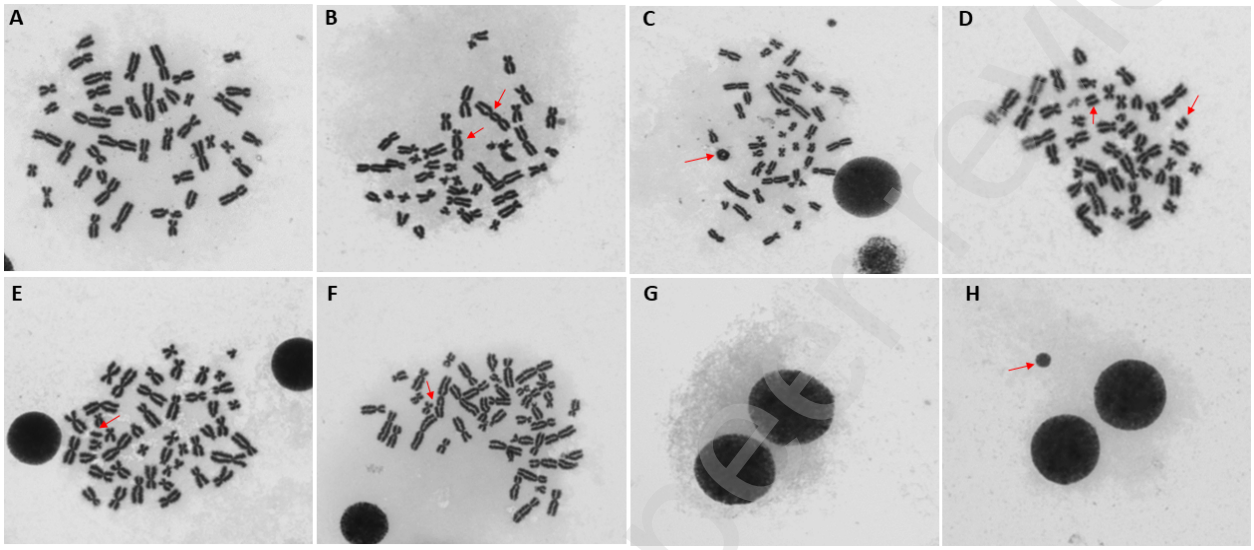

Figure 1, Metaphase chromosomal spreads to observe chromosomal aberrations; normal metaphase without signs of damage (A); examples of different chromosomal aberrations like dicentric chromosomes (B), ring chromosome (C), acentric chromosome (D), fragments (E) and chromatid gap (F). Micronucleus assay for visualization of permanent DNA damage; the Cytokinesis Block produces binucleated cells (G), prevalent chromosomal fragments appear as micronucleus (H).

2.4.2.4 DNA double strand breaks (DSB), 53BP/γH2AX immunostaining

For visualization of DNA-repair foci, cells were cytospun on microscopic slides, fixed in 3 % paraformaldehyde, and immunostained as previously described (Durdik et al., 2019). For each sample, two stained slides were scanned by the Metafer Slide Scanning System Version 3.6 (MetaSystems, Altlussheim, Germany) and the 53BP/γH2AX foci were enumerated by semi-automatic classifier. At least 200 cells were analyzed from each slide. The pooled results from the two slides per blood sample were statistically analyzed.

2.4.3 Specific Gene Analyses

2.4.3.1 Preleukemic gene rearrangements, FISH

To analyze the presence of leukemia specific rearrangements in MLL (KMT2A) gene (Harper and Aplan, 2008), we applied DNA FISH methods with break apart DNA FISH probe (HPL013, Cytocell, Cambridge, UK). Figure 2 illustrates the test principle. Smears

from 200 µl fresh blood on frosted slides (Manzel-Glaeser, Thermo Scientific, Waltham, Massachusetts, USA) were dried and processed as previously (Jakl et al., 2020). Briefly, the stained slides were analyzed with a fluorescent microscopy (Olympus BX51, Shinjuku, Japan) with a 100x lens, at the appropriate spectrum, i.e. blue (nucleus), green and red (FISH probe). Within the cell nucleus the red signal corresponds to the MLL-gene segment between breaking point and the gene PHLDB1 (Pleckstrin homology like domain family B member 1) lying closer to the telomere. The green signal represents the MLL gene segment between UBE4A gene (Ubiquitination factor E4A) and breaking point closer to the centromere. Depending on the yield of stained nuclei 740 – 1340 cells were analyzed.

Figure 2

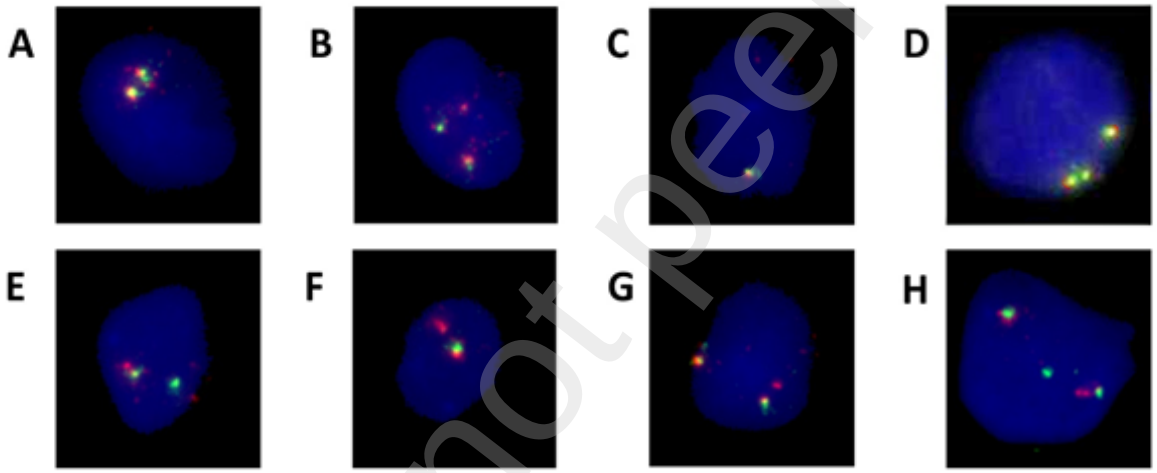

Figure 2, Fluorescent In Situ Hybridization (FISH) using MLL break apart probe. Cell nuclei are stained in blue by DAPI. The red signal represents the gene segment closer to telomere, the green signal stains the opposite site beyond the breaking point (i.e. closer to the centromere). Normal cells contain two MLL-genes, with red and green signals co-localized (A). Translocation is represented as a one intact co-localized green and red signal and one separated green and separated red signal (B). Whole gene deletion is represented only one co-localized green and red signal (C). An additional colocalized signal represents a whole gene duplication (D). The loss of either the red (E) or the green (F) signal indicates a partial deletion. A loss of both signals may indicate a whole gene deletion on one chromosome. Any additional signal (either red (G) or green (H)) is considered a partial duplication.

2.4.4 PFG analysis

2.4.4.1 RNA isolation and cDNA synthesis

Total RNA was isolated with innuPREP DNA/RNA Mini Kit (Analytik Jena) from $2.2x10^6$ – $6x10^6$ mononuclear cells according to the manufacturer´s instructions. The concentration and purity of isolated RNA were determined by NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, U.S.A). Subsequently, the RNA yield and pg/cell were calculated.

The RNA extracted from $2x10^6$ cells, was reversely transcribed to cDNA using RevertAidTM H- M/MuLV reverse transcriptase (Thermo Fisher Scientific), the reaction mix contained 5 µMol random hexamers and oligo $(dT)_{18}$, The procedure followed the manufacturer´s protocol, details are shown in Suppl. 4.

2.4.4.2 R-T qPCR and Sequencing

2 µl of cDNA (1/10 volume of RT-reaction) *per* each real-time quantitative PCR was used. Each sample was run in triplicate. The R-T qPCR was performed according to a standardized protocol (Gabert et al., 2003) with following modifications introduced to achieve optimal activity of master mix: (i) final volume: 20 µl, (ii) template: 2 µl undiluted cDNA, (iii) PCR cycling conditions: 1 cycle 95°C 12 min, 45 cycles 95°C 15 sec, 60°C 1 min.

Both MLL-AF4 and MLL-AF9 R-T qPCR analyses were done in two separate R-T qPCRs:

MLL-AF4: (i) MLL-F1 + AF4-R + AF4-Pr, (ii) MLL-F2 + AF4-R + AF4-Pr

MLL-AF9: (i) MLL1-F + AF9-R1 + MLL-T1-Pr, (2) MLL1-F + AF9-R2/3 + MLL-T1-Pr.

Validation of positivity of samples for studied PFG was accomplished by sequencing. The R-T qPCR product was subcloned into pUC18 vector and subsequently, resultant recombinant plasmid DNA verified by colony PCR was used as a template in sequencing reaction with universal M13/pUC reverse primer, enabling the sequencing of entire DNA insert. The sequencing was performed by a standard procedure using BigDye® Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Thermo Scientific).

The quality of RNA was assessed based on the expression level of the control (housekeeping) c-Abl gene. The RNA is regarded as suitable for RT-qPCR when > 10,000 copies of control gene per 10^5 cells is present. Suppl. 5 shows that expression level of c-Abl is ranging from 12,000 up to 48,000 copies suggesting that the RNA isolated from PB

lymphocytes of studied participants is undegraded, i.e. suitable for further analyses by RT-qPCR. The data illustrate that efficiency, coefficient of correlation and slope of all five RT-qPCR assays used in this study fall within acceptable values. The sequences of primers and probes and the sequencing data analyses are shown in **Error! Reference source not found.**.

2.5 Statistical analysis

To screen for possible associations between physical measurements and biological variables we performed a Spearman correlation analysis. Then we computed the means and standard deviations (SD) and compared the data from the two participant groups by univariate ANOVA or students t-test. The impact of possible confounding factors was tested with bifactorial ANOVA (SPSS, V. 28.0). To avoid alpha error accumulation, we applied Bonferoni corrections to the p-values. For the analyses of frequency data, we used Fishers exact test. The level of significance was set as $p<0.05$.

3. Results

3.1 Participants

The participants were allocated to two groups based on the distance to the next MPBS (Table 1). The distance to the nearest MPBS antennas was significantly different ($p<0,001$), while sex, age, body weight and body size, and the length of stay at their respective home was similar between the groups (Table 1).

Table 1, Study participants

Basic characteristics of study participants. The participant groups living close to (Group E) or distant from (Group C) MPBSs were comparable in sex, age, body weight and size, and duration of residency in their homes. Information, like nutritional preferences, lifestyle factors, health status and medications, X-ray exposure history, specific environmental expositions, including self-rated hyper-electromagnetic-hypersensitivity are summarized in the supplemental tables (Suppl. 1 to Suppl. 3).

	Group C (Control) Relatively low exposure	Group E (Exposed) Relatively high exposure	Total
Number of participants N	12	12	24
Sex (M/F)	6 / 6	5 / 7	11 / 13

	Range from – to	AVG ±SD	Range from – to	AVG ±SD	Significance (ANOVA), P
Distance to the nearest MPBS (meters)	490 – 1 020	767 ±241	75 – 160	125 ±35	< 0.001
Age (years)	24 – 63	47.8 ±13.6	39 – 62	52.8 ±7.3	0.266
Body weight (kg)	56 – 96	80.3 ±14.3	56 – 113	82.2 ±16.4	0.775
Body size (cm)	158 – 187	173.8 ±7.6	152 – 185	169.8 ± 8.5	0.238
Live there for (years)	5 – 54	26 14	12 – 34	23 ±9	0.476

Legend: AVG – mean value; SD – standard deviation; ANOVA – Analysis of Variance.

The documented nutrition styles and food preferences, i.e. the consumption of meat, grilled stuff and cheese were similar, and life style factors like alcohol and nicotine consumption matched well between the groups. Most participants indicated to take “no medication”. None of the participants had ever received therapies interfering with their DNA (e.g. cytostatic drugs). Participants who took antibiotics within three months before participation and the intake of iodine or L-thyroxine (to maintain thyroid function) were evenly distributed between the groups (**Error! Reference source not found.**).

There was no significant difference between the groups in any of the respective exposures to ionizing radiation throughout their life (X-rays, CTs, etc., **Error! Reference source not found.**). Four participants reported an occupation related risk linked to genomic (DNA) instability, the distribution between the groups was even (**Error! Reference source not found.**).

The participants’ general self-assessment on their electro-hyper-sensitivity revealed a higher - though statistically not significant - score in the exposed group, symptoms related to electrohypersensitivity were not significantly different (**Error! Reference source not found.**). Although the project was rolled out during the Covid pandemic, none of the participants had Covid prior or during the study. Twelve (50%) participants were vaccinated, the others were tested for Corona, and were found negative.

3.2 Exposure measurements

Table 2 compares the various EMF exposures between Group-E and Group-C. While the exposure to electric power fields and "homemade" RF-EMF (DECT, WLAN) was not statistically different between the groups, the statistical analysis of the RF-EMF fields coming from MPBS substantiated the volunteers group assignment to the exposed group (E) and the control group (C) based on the distance from the nearest MPBS (Table 1). There was no overlap between the groups, neither in the distance (range Control: 490 – 1 020 m; range Exposed: 75 - 160 m), nor with the GSM (range C: 0,0 – 4,5 μW/m²; range E: 7,1- 295,8 μW/m²) or LTE signals (range C: 0,1 – 7,7 μW/m²; range E: 54,0 – 804,0 μW/m²). Without overlap of the data range the difference in exposure to GSM/LTE exposure was highly significant between the groups (Table 2). We did not find signals above 2,5 GHz.

Table 2, Physical parameters in control and exposed groups

EMF related physical parameters in the sleeping area of the participants living close or distant to the next MPBS. Extremely low frequency fields (16,7 Hz, 50 Hz) were generally low. The group difference to MPBS signal exposure (GSM, LTE) was significant between the groups, while exposure to indoor RF-EMF fields (DECT, WLAN) was not.

Physical parameters	Control-Group N=12 (Mean ± SD)	Exposed-Group N=12 (Mean ± SD)	ANOVA Group difference	
			P	Significance
LFEF; V/m; max	20.4±21.8	27.8±33.4	0.530	n.s.
LFMF 22-6; nT; 16,7 Hz; Max	18.8±16.8	29.2±20.7	0.190	n.s.
LFMF 22-6; nT; 16,7 Hz; AVG	0.8±1.9	3.5±3.2	0.021	*
LFMF 22-6; nT; 50 Hz; Max	61.5±45.2	46.9±22.6	0.326	n.s.
LFMF 22-6; nT; 50 Hz; AVG	18.4±11.5	13.9±9.6	0.312	n.s.
GSM base load RMS, μW/m²	1.2±1.6	69.5±108.5	0.040	*
GSM full load RMS, μW/m²	4.7±6.4	278.1±434.0	0.040	*
GSM base load PEAK, μW/m²	1.5±2.0	87.5±136.6	0.040	*
GSM full load PEAK, μW/m²	6.0±8.1	350.1±546.4	0.040	*

LTE base load RMS, µW/m²	2.7±2.7	306.7±310.3	0.003	**
LTE full load RMS, µW/m²	10.9±10.6	1226.8±1241.1	0.003	**
LTE base load PEAK, µW/m²	27.3±26.6	3067.1±3102.7	0.003	**
LTE full load PEAK, µW/m²	109.2±106.4	12268.2±12410.7	0.003	**
DECT; PEAK µW/m²	61.6±141.7	14.1±27.5	0.266	n.s.
WLAN; PEAK µW/m²	98.8±187.4	130.4±239.0	0.722	n.s.

Legend: LFEF - Low frequency electric alternating fields; LFMF – Low frequency magnetic alternating field; GSM, LTE– MPBS signals; DECT – Cordless telephone signals; WLAN – Wireless Local Area Network (WLAN-Router signal), 22-6 – nighttime, PEAK and RMS – detector for RF-spectrum analysis. n.s. – not significant; * - significant, $p < 0,05$; ** significant, $p < 0,01$; or *** $p < 0,001$.

3.3 Explorative correlation EMF-exposure/biological endpoint

We explored the correlations between specific biological variables and the specific environmental exposure via Spearmen rank correlation. Table 3 reports the correlations between oxidation status, transient and permanent DNA lesions, or specific gene alterations and the various environmental physical measures. The highest correlations coefficients were between chromosomal aberrations and the exposure with the MPBS signals (GSM and LTE) as assessed by both the distance from MPBS and RF-EMF measurements. Other physical parameters either showed were very low correlation or did not significantly correlate with the biological endpoints. Of note, housekeeping c-Abl gene expression positively correlated with exposure to DECT, WLAN, and LFEF at high statistical level. In line with this data, RNA content per cell positively correlated, although not always statistically significantly, with exposure to DECT, WLAN, and LFEF and also with c-Abl gene expression. This data may indicate that these exposure types could affect gene expression while being not genotoxic.

Table 3, Explorative Spearman rank correlations biomarkers and EMF

Explorative Spearman rank correlation between biomarkers and specific EMF exposures. Significant correlations are highlighted (* $p < 0.05$; ** $p < 0.01$). Significance levels are not corrected for multiple testing an isolated single significance could possibly constitute a by-chance result. However, there are arrays of significant correlations, which were further analyzed. The most noticeable correlation was among the different chromosomal aberrations (column: I, n), which positively correlate with the GSM and LTE measurements.

In line with this data CAs negatively correlate with the distance to the MPBSs, again specific chromosomal aberrations were statistically significant (column: i, l, m, n). None of the low-frequency magnetic fields (public and train magnetic fields, line 26–29) shows significant associations with any biological parameter.

	Column	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p
Line	Biologic. Endpoint	age	FPG Comet Assay	Alk Comet Assay	TBA RS	Micro-Nuclei %	gH2AX	53BP1	yH2AX /53BPI	% Di-centric	% Ring Chromosomes	% Acentric	% Gap	% Fragments	total % Aberration	RNA (pg/cell)	C-ABL
1	FPG Comet Assay	0,203															
2	Alk Comet Assay	0,248	0,383														
3	TBARS	0,141	-0,093	,648**													
4	Micro Nuclei %	-0,404	0,003	-0,316	-0,351												
5	gH2AX	,482*	-0,010	0,081	-0,014	0,062											
6	53BP1	0,247	-0,159	0,162	0,300	0,064	,769**										
7	yH2AX/53BPI	0,393	-0,055	0,100	0,045	-0,007	,924**	,768**									
8	% Di-centric	0,259	-0,086	0,101	,413*	0,087	-0,124	-0,118	-0,176								
9	% Ring	-0,007	-0,201	-0,144	0,013	-0,013	0,044	-0,066	0,049	0,040							
10	% Acentric	-0,197	-0,268	-0,177	0,029	,457*	-0,142	-0,092	-0,076	0,325	0,078						
11	% Gap	0,113	0,268	,585**	,633**	-0,122	-0,062	0,048	-0,118	,491*	0,067	0,224					
12	% Fragments	0,079	0,121	0,028	0,067	0,081	-0,273	-0,392	-0,237	,526**	0,053	0,250	0,083				
13	total % Aberration	0,151	0,106	0,200	0,327	0,080	-0,231	-0,259	-0,261	,786**	0,087	,546**	,642**	,708**			
14	RNA (pg/cell)	0,209	0,150	-0,361	-,452*	0,050	0,143	-0,105	-0,029	0,121	0,335	-0,158	0,015	0,048	0,092		
15	C-ABL	0,080	0,074	-0,321	-0,337	0,339	0,011	-0,130	-0,047	0,077	0,315	-0,044	-0,162	0,243	0,077	,406*	
16	GSM base load RMS	0,207	0,172	0,191	0,261	-0,092	-0,300	-0,319	-0,337	,598**	0,227	0,205	0,372	,598**	,677**	0,051	0,131
17	GSM full load RMS	0,207	0,172	0,191	0,261	-0,092	-0,300	-0,319	-0,337	,598**	0,227	0,205	0,372	,598**	,677**	0,051	0,131
18	GSM base load PEAK	0,207	0,172	0,191	0,261	-0,092	-0,300	-0,319	-0,337	,598**	0,227	0,205	0,372	,598**	,677**	0,051	0,131
19	GSM full load PEAK	0,207	0,172	0,191	0,261	-0,092	-0,300	-0,319	-0,337	,598**	0,227	0,205	0,372	,598**	,677**	0,051	0,131
20	LTE base load RMS	0,084	0,257	0,130	0,192	0,017	-0,341	-0,323	-0,352	,529**	0,267	0,241	0,365	,639**	,668**	0,119	0,163
21	LTE full load RMS	0,084	0,257	0,130	0,192	0,017	-0,341	-0,323	-0,352	,529**	0,267	0,241	0,365	,639**	,668**	0,119	0,163
22	LTE base load PEAK	0,084	0,257	0,130	0,192	0,017	-0,341	-0,323	-0,352	,529**	0,267	0,241	0,365	,639**	,668**	0,119	0,163
23	LTE full load PEAK	0,084	0,257	0,130	0,192	0,017	-0,341	-0,323	-0,352	,529**	0,267	0,241	0,365	,639**	,668**	0,119	0,163
24	DECT; PEAK	-0,027	0,192	-0,235	-0,108	,434*	-0,189	-0,066	-0,353	0,135	-0,043	0,062	-0,037	0,291	0,175	0,209	,554**
25	WLAN; PEAK	0,076	0,273	-0,278	-0,221	0,365	0,053	0,117	-0,061	0,110	0,238	-0,133	-0,070	0,240	0,061	,485*	,657**
26	LFMF 22-6; 16,7 Hz; Max	0,134	0,156	-0,151	-0,255	0,172	0,168	0,216	0,047	0,015	0,056	-0,007	-0,047	0,002	0,102	0,326	0,129
27	LFMF 22-6; 16,7 Hz; AVG	0,210	0,057	-0,200	-0,187	-0,036	-0,139	-0,188	-0,251	0,272	0,169	0,154	0,077	0,266	0,390	0,397	0,139
28	LFMF 22-6; 50 Hz; Max	0,012	0,079	0,095	-0,022	0,028	0,080	0,052	0,040	-0,179	-0,176	-0,245	-0,235	-0,193	-0,254	-0,307	-0,150

29	LFMF 22-6; 50 Hz; AVG	0,290	-0,114	0,004	-0,059	-0,398	-0,016	-0,207	-0,118	-0,177	-0,164	-0,284	-0,118	-0,283	-0,229	-0,080	-0,245
30	Distance (meters)	-0,142	-0,283	-0,312	-0,288	-0,064	0,044	-0,026	0,031	-,478*	-0,202	-0,302	-,496*	-,646**	-,758**	0,013	-0,087
31	LFEF; V/m; max.	-,459*	0,169	-0,371	-,474*	0,278	-0,271	-0,248	-0,322	-0,315	0,113	-0,101	-0,256	0,080	-0,125	0,152	,544**

Legend: two-sided significance: * p<0,05; **p<0,01;

3.4 Biological endpoints, group comparison (C-control, E-exposed)

Table 4 compares the outcome of the laboratory investigations. Lipid peroxidation in the blood samples scored higher in the group-E, though not statistically significant. The mean rate of oxidative DNA lesions (FPG comet assay) was higher, single strand DNA lesions (alkaline Comet assay) was significantly higher in the group-E. FISH analysis of the participants´ samples has shown no increase of any deletion, duplication, gain, break or total gene rearrangement in MLL gene in the exposed group. Suppl. 4 shows results of R-T qPCR analysis of participants for the presence of MLL-AF4 and MLL-AF9 PFGs known to be associated with leukemia. Out of the two PFGs studied, only MLL-AF4 fusion transcripts of very low copy number were identified in two control and two exposed persons. Three of these positive samples were validated by sequencing. The group comparison analysis showed no statistically significant differences between control and exposed group. While biomarkers of double DNA strand repair (γH2AX, 53BP1) and the micronucleus assay showed no particular differences, some of chromosomal aberrations, i.e. dicentric chromosomes, chromatid gaps, and fragments were significantly higher in the group-E. Also, the total of all chromosomal aberrations was significantly higher in the group-E ($p = 0.001$, Table 4).

Table 4: Biological markers in control (C) and exposed (E) group

Blood sample analyses; group comparison by univariate ANOVA. Significant differences between the groups were seen with chromosomal aberrations (CAs). i.e. dicentric chromosomes, chromatid gaps, fragments, and the total of the CAs.

Biological Marker	Parameter	Control-N=12 (Mean ± SD)	Exposed N=12 (Mean ± SD)	P	Bonferoni P (N=19)	Difference
Oxidation	Lipid peroxidation assay (nmoles/mg protein)	8.9±8.1	13.8±9.6	0.184	> 0.999	n.s.
Transient DNA damage	FPG Comet assay Tail moment (µM)	30.1±9.6	33.4±6.3	0.332	> 0.999	n.s.
	Alkaline Comet assay Tail moment (µM)	9.1±4.2	13.5±5.8	**0.045**	0,856	*

DNA double strand break repair foci	γH2AX	0.9±0.3	0.8±0.2	0.445	> 0.999	n.s.
	53BP1	1.4±0.3	1.2±0.3	0.247	> 0.999	n.s.
	γH2AX/53BP1	0.7±0.2	0.6±0.2	0.328	> 0.999	n.s.
Permanent, not repairable DNA damage	Micronucleus assay	2.4±0.5	2.3±0.4	0.928	> 0.999	n.s.
	% dicentric chromosomes	0.4±0.2	0.7±0.2	**0.007**	0,142	*
	% ring chromosomes	0.1±0.1	0.1±0.2	0.375	> 0.999	n.s.
	% acentric chromosomes	0.9±0.5	1.3±0.7	0.100	> 0.999	n.s.
	% chromatid gaps	1.2±0.5	2.3±1.5	**0.019**	0,377	*
	% fragments	1.3±0.6	2.6±0.9	**<0.001**	**0,009**	*
	% of total Chromosomal Aberration	3.9±1,1	7.1±1.3	**<0.001**	**< 0,001**	*
Specific gene activity	RNA yield in pg/cell	1.7±0.4	1.7±0.6	0.892	> 0.999	n.s.
	c-ABL copy number	28841.7 ±9000.1	31244.2 ±12504.9	0.594	> 0.999	n.s.
Specific gene damage	MLL Deletion, FISH analyses	1.09±0.90	1.96±1.58	0.174	> 0.999	n.s.
	MLL Duplication, FISH analyses	0.07±0.13	0.06±0.09	0.934	> 0.999	n.s.
	MLL break apart, FISH analyses	0.18±0.29	0.02±0.04	0.069	> 0.999	n.s.
	Total MLL gene rearrangements	1.57±0.86	2.26±1.77	0.3235	> 0.999	n.s.

Legend: * - $p < 0,05$; n.s. – not significant;

3.5 Confounder analyses

Besides our observation that single strand DNA lesions and various chromosomal aberrations are different between the groups (Table 4), we also observed confounding factors, which could bias the observed difference between the groups. With the aim to estimate the possible influence of confounding factors, we performed bi-factorial univariate ANOVA. The first factor was group difference throughout; the second factor were the biomarkers that could constitute a confounder. Table 5 summarizes the outcome of these analyses.

Table 5, Confounder (bias) analyses

Bi-factorial, univariate ANOVA to identify a possible confounder bias. None of the possible confounding factors was un-evenly distributed between the groups. Only the outcome of alk. Comet assay showed significant potential for bias (alcohol consumption). Double strand repair foci (γH2AX, 35BP1, γH2AX/35BP1) revealed some dependencies on alcohol consumption and X-ray exposures, however, the bi-factorial analyses identified none of them as possible confounder that might bias the group comparison (Control/Exposed) shown in Table 4.

Confounding factors \ Endpoints Variables		Lipid peroxidation assay (nmoles/mg protein)	Alk, Comet assay Tail moment (μM)	DNA double strand repair foci			Micronuclei (per 1000 cells)	% chromosomal Aberrations/ cells
				γH2AX	53BP1	γH2AX/53BP1		
Sex	Male (n=11)	10.6±10.15	10.5±5.27	0.8±0.22	1.2±0.22	0.7±0.23	2.2±0.33	5.0±2.06
	Female (n=13)	12.0±8.37	11.9±5.67	0.8±0.28	1.3±0.30	0.70±.22	2.5±0.42 *	5.9±1.96
bi-factorial Group C/E * Sex	P =	0.757	0.744	0.757	0.644	0.991	0.249	0.706
Smoking	Non-smokers (n=22)	10.9±8.79	11.5±5.59	0.8±0.25	1.3±0.26	0.7±0.22	2.3±0.43	5.4±1.92
	Smokers (n=2)	16.0±14.50	8.5±1.34	0.7±0.22	1.3±0.45	0.6±0.13	2.4±0.36	6.1±3.85
bi-factorial Group C/E * Smoking	P =	0.208	0.726	0.268	0.036	0.370	0.386	0.181
Alcohol Consumption	Rare (n=19)	12.8±9.53	11.2±4.91	0.8±0.18	1.3±0.27	0.6±0.16	2.4±0.43	5.8±2.06
	Regular (n=5)	5.8±3.57	11.3±7.78	1.1±0.33 *	1.4±0.25	0.9±0.26 *	2.2±0.32	4.5±1.62
bi-factorial Group C/E * Alcohol	P =	0.960	0.024*	0.019*	0.079	0.132	0.466	0.287
X-rays / Life	<= 5 times (n=9)	8.3±4.78	10.3±4.13	0.9±0.25	1.2±0.24	0.8±0.21	2.3±0.38	5.6±2.28
	> 5 times (n=15)	13.16±10.59	11.9±6.13	0.9±0.26	1.1±0.21 *	0.6±0.16 *	2.4±0.43	5.4±1.92
bi-factorial Group C/E * X-ray	P =	0.916	0.956	0.374	0.747	0.310	0.049	0.584
X-ray fluoroscopy / Life	None (n=8)	9.3±5.66	10.0±3.56	0.8±0.22	1.2±0.24	0.7±0.21	2.4±0.49	5.1±1.4
	>= 1 (n=9)	16.5±11.87	11.8±5.61	0.7±0.07	1.3±0.20	0.6±0.08	2.3±0.38	6.8±2.23
bi-factorial Group°C/E*Fluoroscopy	P =	0.819	0.900	0.608	0.387	0.626	0.352	0.248
CT-tomography / Life	None (7)	8.7±5.37	11.66±2.10	0.7±0.17	1.1±0.21	0.6±0.15	2.3±0.47	5.2±1.56
	1-2 times (n=10)	14.9±10.17	10.9±6.20	0.8±0.21	1.31±0.26	0.64±0.17	2.4±0.38	6.7±1.93 *
	>= 3 times (n=5)	9.7±11.30	11.8±7.82	1.1±0.32 *	1.5±0.19 *	0.95±0.25 *	2.3±0.55	4.0±1.38
bi-factorial Group C/E * Ct-tomography	P =	0.616	0.114	0.092	0.407	0.400	0.316	0.839

Legend: *significant difference between the variable strata, ANOVA

Although micronuclei were more frequent in women (p = 0.035), in view of the almost equal number of females in the groups (C/E) and the outcome of the bi-factorial analysis, sex cannot be considered a confounder. There were only two smokers among the participants. Therefore, in this study we can exclude bias related to smoking. In the five persons who regularly consume alcohol – compared to those who rarely or never drink alcohol (n = 19) - we found increased DNA damage measured by enumeration of γH2AX (p = 0.011) and γH2AX/53BP1 co-localized foci (p = 0.002). The statistical comparison revealed a higher amount of DNA damage measured by alkaline comet assay in the exposed group E with borderline significance (Table 4, p = 0.045).

Fifteen participants who had undergone X-ray imaging more than 5 times - compared to those with ≤ 5 times - had a higher level of 53BP1 repair foci (p = 0.006) and co-localized γH2AX/53BP1 DNA repair foci (p = 0.013). As participants with high X-ray experience were distributed evenly between both groups, and the bi-factorial analyses revealed insignificant dependencies (**Error! Reference source not found.**), a high X-ray-exposure rate does not bias the outcome of the group comparison in this study. The same applies for Computer tomography (CT). While a high number of CTs (≥ 3) relates to an increased rate of DNA repair foci, the equal distribution of these participants between the groups renders the bi-factorial confounder analyses insignificant (**Error! Reference source not found.**).

Neither of the possible confounder affected any type of chromosomal aberrations (Table 5). The statistical group difference was highly significant for the observed chromosomal aberrations. Bonferoni p-value correction for multiple testing (19 parameters from each blood sample) did not change the outcome (Table 4). We found that chromosomal aberrations, which are non-repairable (permanent) indicators of genotoxic effects, were higher in the blood cells of exposed participants (Group-E).

4. Discussion

The first German study that linked "living in proximity to a cell tower" to an elevated risk for cancer dates back to 2004 (Eger et al., 2004). Independently from this, an increased incidence of cancer and living in proximity to a cell-phone transmitter station was described in Israel (Wolf and Wolf, 2004). Our observation on CAs provides mechanism

that can explain the findings of Eger et al. (Eger et al., 2004) and Wolf and Wolf (Wolf and Wolf, 2004). A recent study to describe significant genomic instability after exposure to RF-EMF from MPBSs was in mice (Zosangzuali et al., 2021). Already before that Zothansiama et al. had investigated various genetic instability related endpoints in peripheral human lymphocytes and found biological effects in residents living close to a MPBS (Zothansiama et al., 2017). The findings were a significantly higher frequency of micronuclei and altered antioxidant status with increasing RF power density, which can be considered as another mechanism that could explain ecologic and epidemiologic study data on elevated cancer risk living in those living in proximity to MPBS. Rodrigues et al. investigated the rate of death and the RF-EMF exposure from MPBSs and conclude that exposure to radiofrequency electromagnetic fields from MPBS increases the rate of death for all types of cancer (Rodrigues et al., 2021).

Our analyses revealed chromosomal aberrations as possible long-term result of the residents' year-long exposure to RF-EMF signals from MPBS. The preliminary group assignment based on the distance (Table 1) to the next MPBS was validated by the measures of the respective RF-EMF. The control group-C lived distant with low exposure, while the exposed group-E lived close with a high exposure to the RF-EMF from the MPBS (Table 2).

To prevent any investigator bias or bias related to electrohypersensitivity, the blood sampling and the analyses were strictly under double blind conditions. The blinding code was broken only after the completion of the laboratory analyses. Because RF-EMF emissions can be highly variable, and because the sleeping area is the place with the longest duration of stay in the house, we consider the measures between the night hours as the most representative in a pragmatic study setting. The various fields related to electricity power supply were not only low, but also not significantly different between the study groups (Table 1), rendering their impact on the study outcome negligible. Also, the indoor RF-EMF (DECT, WLAN) exposure was below average household levels and was not different between the study groups (Table 2).

To standardize pre-laboratory procedures between the samples and sampling days, the transport was in an isolated box at steady temperatures. To exclude the possible impact of transport related events, the cell viability was controlled upon arrival in the lab.

Many factors other than the RF exposure affect genomic integrity, or may cause genomic instability. Factors like age, sex, diet, lifestyle, etc. may significantly influence the MN frequency in peripheral blood lymphocytes (Fenech and Bonassi, 2011). In our study, possible nutrition styles and food preferences was evenly matched between the groups (**Error! Reference source not found.**). From the list of possible confounders, we could exclude life style factors such as alcohol and nicotine consumption (**Error! Reference source not found.**, Table 5). We considered the participants' health status, and including the individuals' exposure to medically indicated exposure to ionizing radiation (**Error! Reference source not found.**, **Error! Reference source not found.**). As occupation related risk factors were rare and were evenly distributed between the groups (**Error! Reference source not found.**), our findings are not related to these factors. Also, the subjective electrohypersensitivity (EHS), which might raise bias issues, played no significant role in our group comparison (**Error! Reference source not found.**). None of these person specific factors and no EHS related information revealed significant potential for bias of the found difference between the study groups. None of the possible confounding factors interfered with chromosomal aberrations, which corroborates that long term (years long) exposure to GSM and LTE signals at intensities measured in the homes of the volunteers of group-E increases the rate of chromosomal aberrations.

Transient DNA damages (alkaline comet assay) were higher in the group-E (Table 4), although the confounder analysis yielded a significant dependence of this specific variable with a higher alcohol consumption (Table 5). This somehow isolated result may be a statistical error type one, however, we excluded transient DNA damage related parameters from our final conclusions. The MN frequency was higher in females (Table 5). However, both sexes were represented in both groups at similar rates (Table 1). Therefore, the bi-factorial ANOVA did not yield the observed higher MN frequency in females as confounder. Exposure to a physical factor like RF-EMF exposure, or exposure to chemical mutagens can lead to excessive production of ROS and result in oxidative stress which increases the risk for chronic disease (Sies et al., 2022). It has been suggested that oxidative stress and DNA damage could be a key factor for RF-related incidence of brain tumors and childhood leukemias (De Iuliis et al., 2009). There are many studies that describe non-thermal effects of RF-EMF exposure like oxidative stress

(Yakymenko et al., 2016). In our study, we found a slightly higher lipid peroxidation rate in the exposed group although not statistically significant ($p > 0.05$). Also oxidized DNA lesions were slightly higher in the group-E, but again not to the extent of statistical significance (Table 4). Summing up, our observations on oxidative changes due to RF-EMF exposure fit into the overall picture that RF-EMF exposure can cause oxidative stress (Yakymenko et al., 2016).

Comet assay for the assessment of DNA strand break is a widely used sensitive technique. Gandhi et al. used comet assays and described a significant elevation of SSBs in residents living closer to MPBS than 300 m (Gandhi et al., 2014). In our sample, comet assays revealed a higher amount of DNA damage ($p = 0.045$) in the exposed group (Table 4). Under laboratory conditions short term RF-EMF exposure for few hours was repeatedly associated with transient DNA damage (Franzellitti et al., 2010; Lai and Singh, 1996; Schwarz et al., 2008). Compared to laboratory conditions the exposure in our participants was weak even in the exposed group. The low dose and years long exposure time may have activated adaptive response mechanism in our participants, a reaction described for lymphocytes (Sannino et al., 2013).

DNA DSB can be visualized by 53BP or γH2AX staining. In our participants, an increase of DSB repair foci was found associated with repeated exposure to ionizing radiation (e.g. multiple X-rays, fluoroscopies, computer tomograms), but not with higher environmental exposure to RF-EMF. Again, the intensity of the exposure may have been too low in the exposed group, alternatively adaptive responses may contribute to this finding.

We found no studies to test whether or not specific mutations related to brain tumors and childhood leukemias are caused by exposure to RF-EMF signals from MPBS. MLL-AF4 and MLL-AF9 anomalies are most frequent in pediatric acute myeloid leukemia. Thus, we analyzed induction of the aforementioned PFG by the RT-qPCR and FISH. We didn't observe any deletions, duplications, breaks or total gene rearrangements in MLL gene by FISH ($P > 0.05$) (Table 2). PFG genes, namely MLL-AF4 and MLL-AF9 that are responsible for leukemogenesis by gene rearrangements were identified by RT-qPCR method and results were validated by sequencing. However, their abundance was not different between the groups of participants.

Micronuclei are biological markers, which indicate unsuccessful DNA repair and damage of a mitotic spindle. Along with chromosomal aberrations, MN are indicators for an increased risk in the context of carcinogenesis. In our study, we did not observe any association of chronic RF-EMF exposure with the frequency of micronuclei ($p > 0.05$, Table 4). This finding may serve as hint to the existence of an exposure threshold for micronuclei.

CA are key markers of damaged genomes, which are typically investigated to document adverse effects related to ionizing radiation. They are key for the screening of the mutagenic potential of environmental exposures, be it in vitro, in vivo, or in human studies. As far as dicentrics are considered to be a gold standard for biodosimetry, we assessed the equally effective absorbed doses for the exposed individuals using the obtained data on dicentrics according to the equation recommended by the IAEA for protractive exposure to low dose ionizing radiation (IAEA, 2011). While the estimated equally effective individual absorbed doses varied from 0.0 to 19.49 cSv, the equally effective mean absorbed dose was found to be 7.64 cSv with standard error 1.98, for the exposed group-E. Safety limit for whole body irradiation of general public is 1 mSv per year (IAEA, 2018). While the obtained equally effective mean absorbed dose significantly exceeds the safety limit, this circumstance should be treated with care due to different nature of ionizing and non-ionizing radiations and exposure conditions, which induce the same level of dicentrics.

Because our data suggest that after years of low dose exposure to RF-EMF the frequency of CAs is still higher compared to un-exposed controls (Table 4), this indicates that possible adaptive response do not effectively prevent the generation of new CAs when the low-level RF-EMF exposure lasts over years. Negative reports show, that the relation between RF-EMF-exposure and genetic instability is yet not sufficiently understood. Thus, Yildirim et al. didn’t find any increase of chromosomal aberrations or micronuclei in individuals residing near MPBS (Yildirim et al., 2010). Several factors as duration of exposure and type of signal (frequency, modulation, intermittence et cetera) may account for eventual inconsistency (Armstrong et al., 2013; Belyaev, 2019).

Summing up, the highly significant differences between the controls and exposed group along with correlation between specific RF-EMF signals (GSM, LTE) and the various CAs

(Table 3) after chronic (over years) exposure point to the MPBS signals (GSM, LTE) as cause of the observed genetic instability. Thus, our findings on chromosomal aberrations may provide biologically plausible mechanism for the data on significantly increased risk of cancer in persons exposed to MPBSs (Li et al., 2012) (Eger et al., 2004) (Wolf and Wolf, 2004). (Rodrigues et al., 2021).

5. Conclusion

In this study, even though the means of some biological endpoints were higher in the group with higher exposure, with the exception of chromosomal aberrations we found no statistically significant DNA damages and/or oxidative stress attributable to residency nearby mobile phone base stations (MPBS). We did not find any statistically significant effects related to specific gene parameters either. The cytogenetic damage, i.e. chromosomal aberrations was significantly increased in the residents with higher exposure to RF-EMF. It negatively correlated with the distance from MPBS and positively correlated with LTE and GSM signals of MPBS.

6. Acknowledgements

The study was sponsored by Kompetenzinitiative zum Schutz von Mensch, Umwelt und Demokratie e.V., 66133 Saarbrücken-Scheidt, Germany. The Sponsor had no role in the design of the project design.

7. Author contributions

Conceptualization, I.B. and W.M.; methodology, I.B. and W.M; validation, M.S.; investigation, S.G., D.M., P.K., M.D., L.J., M.S., D.K., and K.V.; writing original draft preparation, S.G., I.B. and W.M.; writing-review and editing W.M. and I.B.; visualization, S.G., D.K. and L.J.; supervision, I.B. and W.M.; project administration E.M., I.B. and W.M.; funding acquisition, W.M. All authors have read and agreed to the published version of the manuscript

8. Competing interests

S.G., D.M., P.K., M.D., L.J., M.S., E.M., D.K., K.V. report no conflict of interest. As experts, W.M. and I.B. took part in a Cell Phone Litigation in the USA on the association between

exposure to mobile phones and brain tumors and provided expert opinion on the association between RF-EMF exposure and health.

References

Akdag, M.Z., Dasdag, S., Canturk, F., Karabulut, D., Caner, Y., Adalier, N., 2016. Does prolonged radiofrequency radiation emitted from Wi-Fi devices induce DNA damage in various tissues of rats? Journal of Chemical Neuroanatomy 75, 116-122.

Al-Serori, H., Ferk, F., Kundi, M., Bileck, A., Gerner, C., Misik, M., Nersesyan, A., Waldherr, M., Murbach, M., Lah, T.T., Herold-Mende, C., Collins, A.R., Knasmuller, S., 2018. Mobile phone specific electromagnetic fields induce transient DNA damage and nucleotide excision repair in serum-deprived human glioblastoma cells. PLoS One 13, e0193677.

Armstrong, B., Baan, R., Belyaev, I., Benbrahim-Tallaa, L., Blackman, C., Blettner, M., Bontoux, L., Bouvard. V, Bromen, K., Byrnes, G., Cardis, E., Hardell, L., Saracci, R., Schüz, J., de Seze, R., Shirai, T., Siemiatycki, J., Sim, M., Straif, K., Szmigielski, S., Verschaeve, L., 2013. IARC monographs on the evaluation of carcinogenic risks to humans. Non-ionizing radiation, Part 2: Radiofrequency electromagnetic fields. World Health Organization, International Agency for Research on Cancer.

Atzmon, I., Linn, S., Richter, E., Portnov, B.A., 2012. Cancer risks in the Druze Isifya Village: Reasons and RF/MW antennas. Pathophysiology 19, 21-28.

Baan, R., Grosse, Y., Lauby-Secretan, B., El Ghissassi, F., Bouvard, V., Benbrahim-Tallaa, L., Guha, N., Islami, F., Galichet, L., Straif, K., 2011. Carcinogenicity of radiofrequency electromagnetic fields. The Lancet Oncology 12, 624-626.

Bektas, H., Dasdag, S., Bektas, M.S., 2020. Comparison of effects of 2.4 GHz Wi-Fi and mobile phone exposure on human placenta and cord blood. Biotechnology & Biotechnological Equipment 34, 154-162.

Belyaev, I., 2019. Main Regularities and Health Risks from Exposure to Non-Thermal Microwaves of Mobile Communication. IEEE, New York.

Belyaev, I., Dean, A., Eger, H., Hubmann, G., Jandrisovits, R., Kern, M., Kundi, M., Moshammer, H., Lercher, P., Muller, K., Oberfeld, G., Ohnsorge, P., Pelzmann, P., Scheingraber, C., Thill, R., 2016. EUROPAEM EMF Guideline 2016 for the prevention, diagnosis and treatment of EMF-related health problems and illnesses. Rev Environ Health 31, 363-397.

Belyaev, I.Y., 2010. Dependence of non-thermal biological effects of microwaves on physical and biological variables: implications for reproducibility and safety standards, Non-thermal effects and mechanisms of interaction between electromagnetic fields and living matter, 1 ed. Mattioli 1885, pp. 187-217.

Buege, J.A., Aust, S.D., 1978. [30] Microsomal lipid peroxidation, in: Fleischer, S., Packer, L. (Eds.), Methods Enzymol. Academic Press, pp. 302-310.

Collins, A.R., Duthie, S.J., Dobson, V.L., 1993. Direct enzymic detection of endogenous oxidative base damage in human lymphocyte DNA. Carcinogenesis 14, 1733-1735.

De Iuliis, G.N., Newey, R.J., King, B.V., Aitken, R.J., 2009. Mobile phone radiation induces reactive oxygen species production and DNA damage in human spermatozoa in vitro. PLoS One 4, e6446.

Dodê, A., Leão, M.M.D., Tejo, F., Gomes, A.C.R., Dode, D.C., Dode, M.C., Moreira, C.W., Condessa, V.A., Albinatti, C., Caiaffa, W.T., 2011. Mortality by neoplasia and cellular telephone base stations in the Belo Horizonte municipality, Minas Gerais state, Brazil. Science of the Total Environment 409, 3649-3665.

Durdik, M., Kosik, P., Markova, E., Somsedikova, A., Gajdosechova, B., Nikitina, E., Horvathova, E., Kozics, K., Davis, D., Belyaev, I., 2019. Microwaves from mobile phone induce reactive oxygen species but not DNA damage, preleukemic fusion genes and apoptosis in hematopoietic stem/progenitor cells. Sci Rep 9, 16182.

Eger, H., Hagen, K., Lucas, B., Vogel, P., Voit, H., 2004. The Influence of Being Physically Near to a Cell Phone Transmission Mast on the Incidence of Cancer. Umwelt Medizin Gesellschaft 17, 326-332

Fenech, M., Bonassi, S., 2011. The effect of age, gender, diet and lifestyle on DNA damage measured using micronucleus frequency in human peripheral blood lymphocytes. Mutagenesis 26, 43-49.

Franzellitti, S., Valbonesi, P., Ciancaglini, N., Biondi, C., Contin, A., Bersani, F., Fabbri, E., 2010. Transient DNA damage induced by high-frequency electromagnetic fields (GSM 1.8 GHz) in the human trophoblast HTR-8/SVneo cell line evaluated with the alkaline comet assay. Mutat Res 683, 35-42.

Gabert, J., Beillard, E., van der Velden, V.H., Bi, W., Grimwade, D., Pallisgaard, N., Barbany, G., Cazzaniga, G., Cayuela, J.M., Cavé, H., Pane, F., Aerts, J.L., De Micheli, D., Thirion, X., Pradel, V., González, M., Viehmann, S., Malec, M., Saglio, G., van Dongen, J.J., 2003. Standardization and quality control studies of 'real-time' quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia - a Europe Against Cancer program. Leukemia 17, 2318-2357.

Gandhi, G., Naru, J., Kaur, M., Kaur, G., 2014. DNA and Chromosomal Damage in Residents Near a Mobile Phone Base Station. International Journal of Human Genetics 14, 107-118.

Garaj-Vrhovac, V., Gajski, G., Pažanin, S., Šarolić, A., Domijan, A.-M., Flajs, D., Peraica, M., 2011. Assessment of cytogenetic damage and oxidative stress in personnel occupationally exposed to the pulsed microwave radiation of marine radar equipment. International Journal of Hygiene and Environmental Health 214, 59-65.

Harper, D.P., Aplan, P.D., 2008. Chromosomal rearrangements leading to MLL gene fusions: clinical and biological aspects. Cancer Res 68, 10024-10027.

IAEA, 2011. Cytogenetic Dosimetry: Applications in Preparedness for and Response to Radiation Emergencies. INTERNATIONAL ATOMIC ENERGY AGENCY, Vienna.

IAEA, 2018. Occupational Radiation Protection. INTERNATIONAL ATOMIC ENERGY AGENCY, Vienna.

ICBE-EMF, I.C.o.t.B.E.o.E.F., 2022. Scientific evidence invalidates health assumptions underlying the FCC and ICNIRP exposure limit determinations for radiofrequency radiation: implications for 5G. Environ Health 21, 92.

ICNIRP, 2020. International Commission on Non-Ionizing Radiation Protection; Guidelines for Limiting Exposure to Electromagnetic Fields (100 kHz to 300 GHz). Health Phys 118, 483-524.

Jagetia, G.C., 2022. Genotoxic effects of electromagnetic field radiations from mobile phones. Environ Res 212, 113321.

Jakl, L., Skorvaga, M., Beresova, K., Kosik, P., Durdik, M., Jakubikova, J., Holop, M., Kubes, M., Zastko, L., Markova, E., Beliaev, I., 2020. BCR/ABL preleukemic fusion gene in subpopulations of hematopoietic stem and progenitor cells from human UCB. Neoplasma 67, 158-163.

Lai, H., Singh, N.P., 1996. Single- and double-strand DNA breaks in rat brain cells after acute exposure to radiofrequency electromagnetic radiation. Int J Radiat Biol 69, 513-521.

Li, C.Y., Liu, C.C., Chang, Y.H., Chou, L.P., Ko, M.C., 2012. A population-based case-control study of radiofrequency exposure in relation to childhood neoplasm. Sci Total Environ 435-436, 472-478.

Liu, C., Gao, P., Xu, S.C., Wang, Y., Chen, C.H., He, M.D., Yu, Z.P., Zhang, L., Zhou, Z., 2013. Mobile phone radiation induces mode-dependent DNA damage in a mouse spermatocyte-derived cell line: a protective role of melatonin. Int J Radiat Biol 89, 993-1001.

Meyer, M., Gärtig-Daugs, A., Radespiel-Tröger, M., 2006. Mobilfunkbasisstationen und Krebshäufigkeit in Bayern. Umweltmed Forsch Prax 11, 89-97.

Moorhead, P.S., Nowell, P.C., Mellman, W.J., Battips, D.M., Hungerford, D.A., 1960. Chromosome preparations of leukocytes cultured from human peripheral blood. Exp Cell Res 20, 613-616.

Nilsson, M., Hardell, L., 2023. A 49-Year-Old Man Developed Severe Microwave Syndrome after Activation of 5G Base Station 20 Meters from his Apartment. J Community Med Public Health 7, 382.

Nittby, H., Brun, A., Eberhardt, J., Malmgren, L., Persson, B.R.R., Salford, L.G., 2009. Increased blood-brain barrier permeability in mammalian brain 7 days after exposure to the radiation from a GSM-900 mobile phone. Pathophysiology 16, 103-112.

Rodrigues, N.C., Dode, A.C., de Noronha Andrade, M.K., O'Dwyer, G., Monteiro, D.L., Reis, I.N., Rodrigues, R.P., Frossard, V., Lino, V.T., 2021. The Effect of Continuous Low-Intensity Exposure to Electromagnetic Fields from Radio Base Stations to Cancer Mortality in Brazil, International Journal of Environmental Research and Public Health.

Rothkamm, K., Barnard, S., Moquet, J., Ellender, M., Rana, Z., Burdak-Rothkamm, S., 2015. DNA damage foci: Meaning and significance. Environ Mol Mutagen 56, 491-504.

Sannino, A., Zeni, O., Romeo, S., Massa, R., Gialanella, G., Grossi, G., Manti, L., Vijayalaxmi, Scarfì, M.R., 2013. Adaptive response in human blood lymphocytes exposed to non-ionizing radiofrequency fields: resistance to ionizing radiation-induced damage. Journal of Radiation Research 55, 210-217.

Schwarz, C., Kratochvil, E., Pilger, A., Kuster, N., Adlkofer, F., Rudiger, H.W., 2008. Radiofrequency electromagnetic fields (UMTS, 1,950 MHz) induce genotoxic effects in vitro in human fibroblasts but not in lymphocytes. Int Arch Occup Environ Health 81, 755-767.

Sies, H., Belousov, V.V., Chandel, N.S., Davies, M.J., Jones, D.P., Mann, G.E., Murphy, M.P., Yamamoto, M., Winterbourn, C., 2022. Defining roles of specific reactive oxygen species (ROS) in cell biology and physiology. Nature Reviews Molecular Cell Biology 23, 499-515.

Singh, N.P., McCoy, M.T., Tice, R.R., Schneider, E.L., 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res 175, 184-191.

Wolf, R., Wolf, D., 2004. Increased incidence of cancer near a cell-phone transmitter station. Int J Canc Prev 1, 123-128.

Yakymenko, I., Tsybulin, O., Sidorik, E., Henshel, D., Kyrylenko, O., Kyrylenko, S., 2016. Oxidative mechanisms of biological activity of low-intensity radiofrequency radiation. Electromagnetic Biology and Medicine 35, 186-202.

Yildirim, M.S., Yildirim, A., Zamani, A.G., Okudan, N., 2010. Effect of mobile phone station on micronucleus frequency and chromosomal aberrations in human blood cells. Genet Couns 21, 243-251.

Zhang, Z.H., Hartner, E., Utinger, B., Gfeller, B., Paul, A., Sklorz, M., Czech, H., Yang, B.X., Su, X.Y., Jakobi, G., Orasche, J., Schnelle-Kreis, J., Jeong, S., Gröger, T., Pardo, M., Hohaus, T., Adam, T., Kiendler-Scharr, A., Rudich, Y., Zimmermann, R., Kalberer, M., 2022. Are reactive oxygen species (ROS) a suitable metric to predict toxicity of carbonaceous aerosol particles? Atmos. Chem. Phys. 22, 1793-1809.

Zosangzuali, M., Lalremruati, M., Lalmuansangi, C., Nghakliana, F., Pachuau, L., Bandara, P., Zothan, S., 2021. Effects of radiofrequency electromagnetic radiation emitted from a mobile phone base station on the redox homeostasis in different organs of Swiss albino mice. Electromagn Biol Med 40, 393-407.

Zothansiama, Zosangzuali, M., Lalramdinpuii, M., Jagetia, G.C., 2017. Impact of radiofrequency radiation on DNA damage and antioxidants in peripheral blood lymphocytes of humans residing in the vicinity of mobile phone base stations. Electromagnetic Biology and Medicine 36, 295-305.

Evaluation of oxidative stress and genetic instability among residents near mobile phone base stations in Germany

Sachin Gulati[1], Wilhelm Mosgoeller[2], Dietrich Moldan[3], Pavol Kosik[1], Matus Durdik[1], Lukas Jakl[1], Milan Skorvaga[1], Eva Markova[1], Dominika Kochanova[1], Katarina Vigasova[1], Igor Belyaev[1,*]

[1)] Department of Radiobiology, Cancer Research Institute, Biomedical Research Centre, Slovak Academy of Sciences, Bratislava, 845 05, Slovak Republic
[2)] Cancer Research Centre, Medical University Vienna, 1090 Vienna, Austria
[3)] Dr. Moldan Umweltanalytik, 97346 Iphofen, Germany

***Correspondence:** Prof. Igor Belyaev, Dr.Sc., Head, Department of Radiobiology, Cancer Research Institute, Biomedical Research Centre, Slovak Academy of Sciences, Bratislava, Slovak Republic; Tel: +421 2 32295119 E-mail: igor.beliaev@savba.sk

Keywords: Radiofrequency electromagnetic fields, RF-EMF; low dose chronic exposure; genetic instability; oxidative stress; DNA damage; chromosomal aberrations.