Ecotoxicology and Environmental Safety 279 (2024) 116486

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

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

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ARTICLE INFO

Edited by Yong Liang

Keywords:
Radiofrequency electromagnetic fields, RF-EMF
Low dose chronic exposure
Genetic instability
Oxidative stress
DNA damage
Chromosomal aberrations

ABSTRACT

Human exposure to radiofrequency electromagnetic fields (RF-EMF) is restricted to prevent thermal effects in the tissue. However, at very low intensity exposure "non-thermal" biological effects, like oxidative stress, DNA or chromosomal aberrations, etc. collectively termed genomic-instability can occur after few hours. Little is known about chronic (years long) exposure with non-thermal RF-EMF.

We identified two neighboring housing estates in a rural region with residents exposed to either relatively low (control-group) or relatively high (exposed-group) RF-EMF emitted from nearby mobile phone base stations (MPBS). 24 healthy adults that lived in their homes at least for 5 years volunteered. The homes were surveyed for common types of EMF, 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, age, sex, lifestyle and occupational risk factors were similar. 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.

Increased rates of chromosomal aberrations as linked to excess exposure with ionizing radiation may also occur with non-ionizing radiation exposure. Biological endpoints can be informative for designing exposure limitation strategies. Further research is warranted to investigate the dose-effect-relationship between both, exposure intensity and exposure time, to account for endpoint accumulations after years of exposure. As established for ionizing radiation, chromosomal aberrations could contribute to the definition of protection thresholds, as their rate reflects exposure intensity and exposure time.

1. Introduction

From the first use in the 1950s until today, human exposure to radiofrequency electromagnetic field (RF-EMF) has increased drastically with the increase of technological applications utilizing RF-EMF. 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). While a number

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https://doi.org/10.1016/j.ecoenv.2024.116486
Received 8 February 2024; Received in revised form 30 April 2024; Accepted 18 May 2024
Available online 30 May 2024
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of investigators has not found any impact, recent studies may even justify a higher rating, i.e. "2A - probable carcinogen" (ICBE-EMF, 2022). RF-EMF-exposure effects on gene structures could explain a carcinogenic effect as found in animal studies (Falcioni et al., 2018; Wyde et al., 2018). RF-EMF exposure with intensities below current exposure thresholds and short term exposure (in the range of hours) 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 human studies (Akdag et al., 2016; Bektas et al., 2020).

The RF-EMF exposure induced genotoxic effects can be ameliorated by antioxidants (Liu et al., 2013). Only few authors found no exposure related oxidative changes, however the majority of studies reports RF-EMF-exposure induced oxidative stress (Yakymenko et al., 2016). Oxidative stress can damage macromolecules, stimulate inflammation, and can lead to accumulation of mutations (Zhang et al., 2022). After about 16 h, transient DNA damage can occur. Comet assay modifications allow to distinguish between single or double strand DNA breaks (Schwarz et al., 2008), or oxidative 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 biomarker of genetic-toxicology and cancer risk. Whether or not RF-EMF exposure can produce micronuclei is still discussed controversially (Jagetia, 2022). For the assessment of radiation induced genotoxicity, biological dosimetry (e.g. determination of chromosomal aberrations; CAs) is a generally accepted practice.

RF-EMF exposure related chromosomal aberrations, like acentric fragments and dicentric chromosomes after acute in vitro exposure were described, but not confirmed by other studies (Armstrong et al., 2013). However, effects of non-thermal RF-EMF seem to depend on various - sometimes underreported - physical and biological parameters (Armstrong et al., 2013; Belyaev, 2010), which renders study comparison and exposure threshold determination difficult.

In order to avoid heating, current guidelines limit only intensity of RF-EMF exposure (ICNIRP, 2020), which disregards non-thermal RF-EMF effects and duration of exposure, a key factor of "dose". While guidelines 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. While broadcast antennas are typically built outside communities, MPBS are erected close to the mobile phone customers, i.e. in the communities. Potential repercussions of the continuous RF-EMF exposure 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 from a German ecological study (Eger et al., 2004) 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, MO, USA), New England biolabs (NEB, UK), Thermo Fischer Scientific (Waltham, USA) and Merck (Darmstadt, Germany).

2.2. Subjects

From the same county in Germany, 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). We documented demographic data (age, sex, etc., Table 1), nutrition style, life style factors, medication intake (Suppl 1), medical procedures involving ionizing radiation exposure (Suppl 2), occupational and environmental exposures, and the participants' subjective judgment on their electro-hyper-sensibility (Suppl 3). 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 exposure assessment in the high-frequency range, we measured the prevalent radio services with a spectrum analyzer. 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-h long-term measurement we used HF59B (27 MHz –

Table 1,
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.

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

To distinguish between participants with "low" and "high" exposures we performed EMF-exposure measurements outdoors, and performed detailed measurements indoors 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 to 30 Hz and 37–2000 Hz, the resolution was 1 nT. After the recording of 5 up to 7 complete days, the entire record was inspected for possible anomalies. The records from 10 p.m. to 6 a.m. of the days 1–5 provided were statistically analyzed. Based on the measured EMF exposure, the participants were assigned to the low (control group) or high exposure group (Table 2).

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, $\mu W/m^2$	1.2±1.6	69.5±108.5	0.040	*
GSM full load RMS, $\mu W/m^2$	4.7±6.4	278.1±434.0	0.040	*
GSM base load PEAK, $\mu W/m^2$	1.5±2.0	87.5±136.6	0.040	*
GSM full load PEAK, $\mu W/m^2$	6.0±8.1	350.1±546.4	0.040	*
LTE base load RMS, $\mu W/m^2$	2.7±2.7	306.7±310.3	0.003	**
LTE full load RMS, $\mu W/m^2$	10.9±10.6	1226.8±1241.1	0.003	**
LTE base load PEAK, $\mu W/m^2$	27.3±26.6	3067.1±3102.7	0.003	**
LTE full load PEAK, $\mu W/m^2$	109.2 ±106.4	122,68.2 ±12,410.7	0.003	**
DECT; PEAK $\mu W/m^2$	61.6±141.7	14.1±27.5	0.266	n.s.
WLAN; PEAK $\mu W/m^2$	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;
*** $p < 0.001$.
* significant, $p < 0.05$;
** significant, $p < 0.01$

2.4. Blood sample analyses

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 6-7 h 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.

2.4.1. Oxidative stress

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 min at 37°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 (Fig. 1) was performed according to the IAEA guidelines (IAEA, 2011). We analyzed 1000 binucleated cells from each participants' blood sample. Binucleated cells were assessed for MN 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 (Fig. 1).

2.4.2.4. DNA double strand breaks (DSB), 53BP1/γH2AX immunostaining. For visualization of DNA-repair foci (53BP1/γH2AX foci), cells were cytospun on microscopic slides, fixed in 3 % paraformaldehyde, and immunostained as previously described (Durdik et al., 2019). For each sample two slides were stained. The scanning was performed by the Metafer Slide Scanning System (Version 3.6;

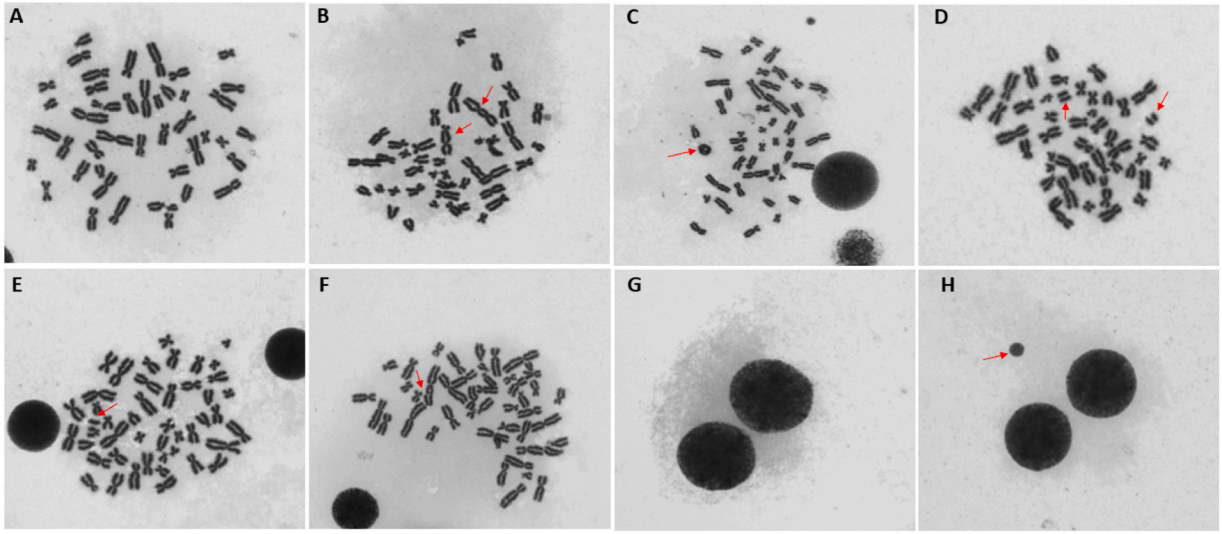

Fig. 1. Metaphase chromosomal spreads to observe chromosomal aberrations: normal metaphase without signs of damage (A); examples of different chromosomal aberrations like dicentric chromosomes (B), ring chromosome (C), acentric chromosome (D), fragments (E) and chromatid gap (F). Micronucleus assay for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

MetaSystems, Altlussheim, Germany). The data from at least 400 cells (200 from each slide) were subjected to statistical analysis.

2.4.3. Specific gene analyses

2.4.3.1. Preleukemic gene rearrangements, FISH. To analyze for 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). Fig. 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.

Normal cells contain two MLL-genes, with red and green signals co-localized (A). Translocation is represented by: one intact co-localized green, one red signal and one green signal separated from each other (B). Whole gene deletion is represented by only one co-localized green and red signal (C). A whole gene duplication is represented by an additional site of colocalised signals (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 as 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 2.2×10^6 – 6×10^6 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 2×10^6 cells, was reversely transcribed to

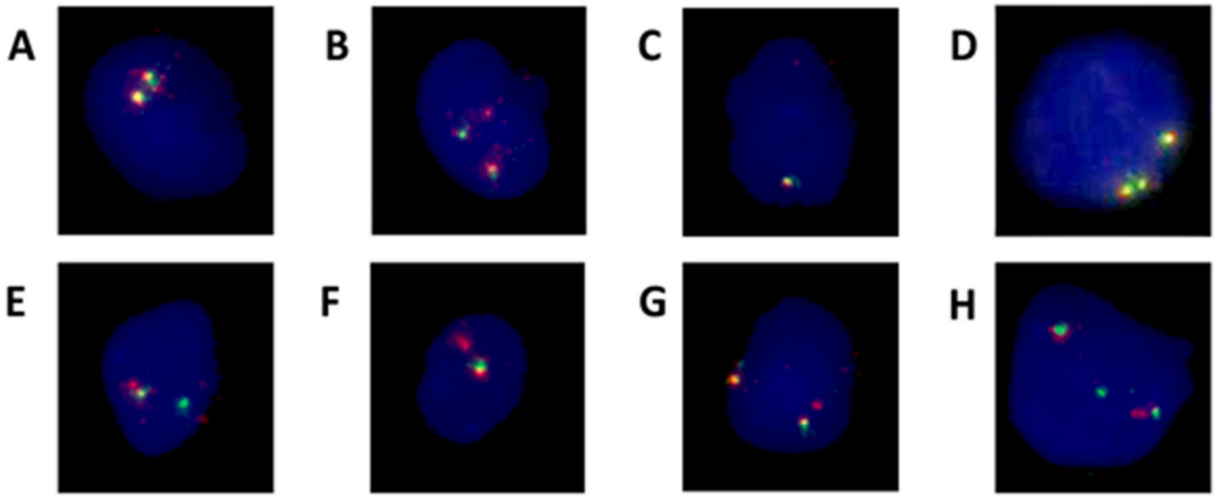

Fig. 2,. Fluorescent In Situ Hybridization (FISH) using MLL break apart probes. Cell nuclei are blue (DAPI-staining). The red signal represents the gene segment closer to telomere, the green signal stains the opposite site beyond the breaking point (i.e. closer to the centromere).

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 manufactureŕs protocol, details are shown in Suppl. 4.

2.4.4.2. R-T qPCR and sequencing. 2 μl of cDNA (1/10 vol 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 s, 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 Suppl 6.

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 Bonferroni corrections, which are known to be overconservative. 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 preliminary assigned 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).

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 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 (Suppl 1).

There was no significant difference between the groups considering exposure to ionizing radiation during their life (X-rays, CTs, etc., Suppl 2). Four participants reported an occupation related risk linked to genomic (DNA) instability, their assignment to the groups was even (Suppl 3).

The participants' general self-assessment on their electro-hypersensitivity revealed a higher - though statistically not significant - score in the exposed group, and symptoms related to electro-hypersensitivity were not significantly different (Suppl 3). 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 group: 490 – 1 020 m; range Exposed: 75–160 m), nor with the GSM (range C: 0.0 – 4.5 μW/m^2; range E: 7.1-295.8 μW/m^2) or LTE signals (range C: 0.1 – 7.7 μW/m^2; range E: 54.0 – 804.0 μW/m^2). The range of exposure to GSM/LTE signals showed no overlap, the group difference was highly significant (Table 2). We did not find signals above 2.5 GHz.

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 revealed very low or insignificant correlations 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.

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 group-E. FISH analysis of the participantś samples showed no increase of deletions, duplications, gain, breaks or total gene rearrangements in MLL gene in the group-E (Fig. 2, Table 3). 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. Biomarkers of double DNA strand repair (γH2AX, 53BP1) and the micronucleus

Table 3,
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 and 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
Biologic. Endpoint Line		age	Fpg Comet Assay	Alk Comet Assay	TBARS	Micro-Nuclei %	γH2AX	53BP1	γH2AX/ 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	γH2AX	.482*	-0.010	0.081	-0.014	0.062											
6	53BP1	0.247	-0.159	0.162	0.300	0.064	.769**										
7	γH2AX/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	0257	0.130	0192	0.017	-0341	-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: Spearman correlation, two-sided significance:
* $p<0.05$;
** $p<0.01$;

Table 4
Biological markers in the 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)	ANOVA P	Bonferoni P (N=19)	ANOVA 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,	Micronucleus assay	2.4±0.5	2.3±0.4	0.928	> 0.999	n.s.
not	% dicentric chromosomes	0.4±0.2	0.7±0.2	0.007	0.142	*
repairable	% ring chromosomes	0.1±0.1	0.1±0.2	0.375	> 0.999	n.s.
DNA	% acentric chromosomes	0.9±0.5	1.3±0.7	0.100	> 0.999	n.s.
damage	% 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	0.324	> 0.999	n.s.

Legend:
* $p < 0.05$;
** $p < 0.001$; n.s. – not significant;

assay (Fig. 1) showed no particular differences. The rate of chromosomal aberrations (Fig. 1), like 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).

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. 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.

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 (Table 5), 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 (Table 5).

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. Bonferroni p-value correction for multiple testing (19 parameters from each blood sample) did not change the outcome (Table 4). We found that the rate of chromosomal aberrations (non-repairable, permanent indicators of genotoxic effects) was significantly 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 another mechanism that could explain ecologic and epidemiologic study data on elevated cancer risk 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 (Fig. 1) 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 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, 53BP1, 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.

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
Confounding factors				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/ESex	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/ESmoking	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/EAlcohol	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/EX-ray	P =	0.916	0.956	0.374	0.747	0.310	0.049*	0.584
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/ EFluoroscopy	P =	0.819	0.900	0.608	0.387	0.626	0.352	0.248
CT/ 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/ECT	P =	0.616	0.114	0.092	0.407	0.400	0.316	0.839

Legend:
* significant difference between the variable strata, ANOVA

(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 electro-hypersensitivity, 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 fields related to electricity power supply were low, and also not significantly different between the study groups (Table 1), which renders 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 of the blood samples was in an isolated box at steady temperatures. To exclude the possible impact of transport related events, upon arrival in the lab the cell viability was checked and found sufficient.

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 (Suppl 1). From the list of possible confounders, we could exclude life style factors such as alcohol and nicotine consumption (Suppl 1, Table 5). We could rule out possible bias due to the participants' health status, or the individuals' exposure to previous exposure to ionizing radiation (Suppl 1, Suppl. 2). As occupation related risk factors were rare and were evenly distributed between the groups (Suppl 3), our findings are not related to these factors. Also, the subjective electro-hypersensitivity (EHS), which might raise bias issues, played no significant role in our group comparison (Suppl 3). 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 (Fig. 1), 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 "real result" or 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 (Fig. 1, 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, 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 double strand breaks (DSB) can be visualized by 53BP1 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 (Fig. 1) arise from lagging chromosomes or acentric chromosome fragments that do not incorporate into daughter nuclei. While disruption of many aspects of spindle assembly have long been known to produce MN, additional molecular players and mechanisms have been recently implicated in the formation of MN (Krupina et al., 2021). 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. The different cellular mechanism involved in the generation of MN or CA may explain their different prevalence in the samples analyzed here (Krupina et al., 2021). CAs (Figure1) are key markers of genomic damages by excess exposure to ionizing radiation. CA 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 the 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 absorbed doses varied from 0.0 to 194.9 mSv, the equally effective mean ± Std absorbed dose was 76.4±19.8 mSv for the group-E. Safety limit for whole body irradiation of general public is 1 mSv per year (IAEA, 2018). Assuming 10 or 20 years of exposure in the residents, the obtained equally effective absorbed dose significantly exceeds the safety limit of the IAEA. The finding, that RF-EMF can increase the rate of dicentrics to a level that exceed safety limits designed for ionizing radiation should be treated with care, due to the proposed different nature of ionizing and non-ionizing radiations and exposure conditions.

Because our data suggest that after years of low dose exposure to RF-EMF the frequency of CAs is still higher compared to low-exposed controls (Table 4, group-C), 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).

The study's outcomes could be influenced by the variability in individual exposure. However, because our preliminary measurements were outdoor, and the group assignment was based on the indoor exposure during night hours, we excluded a possible bias by factors such as indoor shielding. Excessive RF-EMF life exposures, or confounders like ionizing radiation exposure were documented (Suppl. 1–3) and considered in the analysis (Table 5).

The not-significant results, especially regarding the MLL gene rearrangements and cAbl-gene transcription modification, may be additional evidence, that the observed signs of genetic instability hit DNA and various chromosomes rather stochastically, than affect specific genes or DNA sequences. This finding corroborates the role of excess oxidative stress as underlying pathogenetic mechanism, it can explain the accumulated chromosomal aberrations after years of exposure.

Summing up, the highly significant differences between the controls and exposed group (Table 4) along with correlation between specific RF-EMF signals (GSM, LTE) and the various CAs (Table 3) after chronic (years long) exposure point to the MPBS signals (GSM, LTE) as cause of the observed genetic instability. Thus, our findings on chromosomal aberrations may provide a biologically plausible mechanism for the data on significantly increased risk of cancer among persons exposed to MPBS signals (Li et al., 2012; Eger et al., 2004; Wolf and Wolf, 2004; Rodrigues et al., 2021).

5. Conclusion

In this study, 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.

CRediT authorship contribution statement

Eva Markova: Project administration. **Lukas Jakl:** Visualization,

Investigation. **Milan Skorvaga:** Validation, Investigation. **Igor Belyaev:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Conceptualization. **Sachin Gulati:** Writing – original draft, Visualization, Investigation. **Dominika Kochanova:** Visualization, Investigation. **Katarina Vigasova:** Investigation. **Pavol Kosik:** Investigation. **Matus Durdik:** Investigation. **Wilhelm Mosgoeller:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Dietrich Moldan:** Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2024.116486.

References

Akdag, M.Z., et al., 2016. Does prolonged radiofrequency radiation emitted from Wi-Fi devices induce DNA damage in various tissues of rats? J. Chem. Neuroanat. 75, 116–122.
Al-Serori, H., et al., 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., et al., 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., et al., 2012. Cancer risks in the Druze Isifya village: reasons and RF/MW antennas. Pathophysiology 19, 21–28.
Baan, R., et al., 2011. Carcinogenicity of radiofrequency electromagnetic fields. Lancet Oncol. 12, 624–626.
Bektas, H., et al., 2020. Comparison of effects of 2.4 GHz Wi-Fi and mobile phone exposure on human placenta and cord blood. Biotechnol. Biotechnol. Equip. 34, 154–162.
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. Mattioli 1885, 187–217.
Belyaev, I., et al., 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., 2019. Main Regularities and Health Risks from Exposure to Non-Thermal Microwaves of Mobile Communication. IEEE, New York.
Buege, J.A., Aust, S.D., [30] Microsomal lipid peroxidation. In: S. Fleischer, L. Packer, Eds.), Methods in Enzymology. Academic Press, 1978, pp. 302-310.
Collins, A.R., et al., 1993. Direct enzymic detection of endogenous oxidative base damage in human lymphocyte DNA. Carcinogenesis 14, 1733–1735.
De Iuliis, G.N., et al., 2009. Mobile phone radiation induces reactive oxygen species production and DNA damage in human spermatozoa in vitro. PLoS One 4, e6446.
Dodê, A., et al., 2011. Mortality by neoplasia and cellular telephone base stations in the Belo Horizonte municipality, Minas Gerais state, Brazil. Sci. Total Environ. 409, 3649–3665.
Durdik, M., et al., 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., et al., 2004. The influence of being physically near to a cell phone transmission mast on the incidence of cancer. Umw. Med. Ges. 17, 326–332.
Falcioni, L., et al., 2018. Report of final results regarding brain and heart tumors in Sprague-Dawley rats exposed from prenatal life until natural death to mobile phone radiofrequency field representative of a 1.8 GHz GSM base station environmental emission. Environ. Res. 165, 496–503.
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., et al., 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., et al., 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., et al., 2014. DNA and chromosomal damage in residents near a mobile phone base station. Int. J. Hum. Genet. 14, 107–118.
Garaj-Vrhovac, V., et al., 2011. Assessment of cytogenetic damage and oxidative stress in personnel occupationally exposed to the pulsed microwave radiation of marine radar equipment. Int. J. Hyg. Environ. 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., et al., 2020. BCR/ABL preleukemic fusion gene in subpopulations of hematopoietic stem and progenitor cells from human UCB. Neoplasma 67, 158–163.
Krupina, K., et al., 2021. Causes and consequences of micronuclei. Curr. Opin. Cell Biol. 70, 91–99.
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., et al., 2012. A population-based case-control study of radiofrequency exposure in relation to childhood neoplasm. Sci. Total Environ. 435-436, 472–478.
Liu, C., et al., 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., et al., 2006. Mobilfunkbasisstationen und Krebshäufigkeit in Bayern. Umw. Forsch. Prax. 11, 89–97.
Moorhead, P.S., et al., 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., et al., 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., et al., 2021. The effect of continuous low-intensity exposure to electromagnetic fields from radio base stations to cancer mortality in Brazil. Int. J. Environ. Res. Public Health Vol. 18.
Rothkamm, K., et al., 2015. DNA damage foci: meaning and significance. Environ. Mol. Mutagen. 56, 491–504.
Sannino, A., et al., 2013. Adaptive response in human blood lymphocytes exposed to non-ionizing radiofrequency fields: resistance to ionizing radiation-induced damage. J. Radiat. Res. 55, 210–217.
Schwarz, C., et al., 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., et al., 2022. Defining roles of specific reactive oxygen species (ROS) in cell biology and physiology. Nat. Rev. Mol. Cell Biol. 23, 499–515.
Singh, N.P., et al., 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.
Wyde, M.E., et al., 2018. Effect of cell phone radiofrequency radiation on body temperature in rodents: Pilot studies of the National Toxicology Program's reverberation chamber exposure system. Bioelectromagnetics 39, 190–199.
Yakymenko, I., et al., 2016. Oxidative mechanisms of biological activity of low-intensity radiofrequency radiation. Electromagn. Biol. Med. 35, 186–202.
Yildirim, M.S., et al., 2010. Effect of mobile phone station on micronucleus frequency and chromosomal aberrations in human blood cells. Genet. Couns. 21, 243–251.
Zhang, Z.H., et al., 2022. Are reactive oxygen species (ROS) a suitable metric to predict toxicity of carbonaceous aerosol particles? Atmos. Chem. Phys. 22, 1793–1809.
Zosangzuali, M., et al., 2021. Effects of radiofrequency electromagnetic radiation emitted from a mobile phone base station on the redox homeostasis in different organs of Swiss albino mice. Electro Biol. Med. 40, 393–407.
Zothansiama, et al., 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. Electromagn. Biol. Med. 36, 295–305.