Multivariate entropy analysis of oxidative stress biomarkers following GSM phone exposure of human volunteers : a pilot study

Manuscript in preparation

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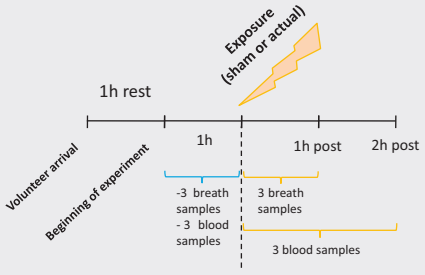
Abstract

Research regarding potential effects of mobile phone emitted electromagnetifc fields (EMF) still shows controversial results as to whether the latter display effects on biological systems. In this context, we conducted a pilot, crossover study on 18 human volunteers who received alternate sham or actual GSM exposure (SAR 0,3W /kg) for 30 minutes on different days. Markers of oxidative attacks of cell membranes or antioxidant defense systems were measured before and after exposure. Results were analysed using an innovative statistical approach based on the global entropic difference of raw data organization. (i.e. the totality of variables and values gathered, assembled in a single dataset). This method computes the degree of organization of the system by calculation of its entropy, and then compares its state before and after exposure. Using this method, we found modulations of the simultaneous expression of all biomarkers, manifested by lower entropy of the dataset, after a single 30 minutes mobile phone exposure. These results will need to be confirmed in larger, future studies.

1 - Exposure and Sampling

The experiment was designed as a crossover. A final number of 18 volunteers among the 24 recruited initially participated in the entire study, namely two one-day sessions, separated by a week, one including an actual EMF exposure (test) and the other one a simulated (sham) pseudo-exposure without any kind of emission; the day of actual exposure has been randomly distributed among volunteers. A dual-band mobile phone Motorola M3688 (900-1800 MHz) was used and located near the right ear using a PVC head holder. During the actual exposure session, participants underwent a 900 MHz radiofrequency field for 30 min, pulsed with a repetition rate of 217 Hz with a pulse width of 0.576 ms at 250 mW mean full power. SAR over 10 g of tissue, calculated and measured as specified on the IEC 85-214 standard, was 0.3 W/kg for the actual emission. The EMF exposure was carried out under double blind conditions. Continuous monitoring of all exposures was performed through 2 PMM 8053 recorders during the experiments. The recorders were hidden, so that volunteers could not see them and be troubled by unknown devices. The electric field was measured every 10 seconds during the 4 hours of one single session for 4 volunteers. Each day, recordings were downloaded on a PC and sent to external collaborators who checked the correct course of the study.

Samples were collected either from the blood by intraveinous catheter or from the exhaled air using special collection cartridges. For each volunteer, 3 blood and breath samples were collected in the hour prior to exposure as controls. After exposure, 3 other breath samples were collected in the span of an hour, and 3 other blood samples within 2 hours. Refer to insert n° 2 for details on the biomarkers.



2 - Biomarkers

All biomarkers followed in this study take part in or reflect the state of the global oxidative balance, whether as antioxidant enzymes or pro-oxidative markers. This equilibrium is precisely regulated, making it a suitable target for investigation of potential cellular EMF effects. Six different biomarkers were followed here, either in the blood (erythrocytes) or the exhaled breath (using a dedicated sampling apparatus) as mentionned in insert n°1:

- Malondialdehyde (MDA) content in erythrocytes, typical cellular membrane oxidative damage marker.
- Exhaled alkanes (BAA), isoprene and aldehydes contents in the breath, also markers of oxidative attacks to biological membranes.
- Superoxide dismutase (SOD) activity in erythrocytes, which is the major superoxide detoxifying enzyme.
- Glutathion peroxidase (GPx) in erythrocytes, responsible for peroxide detoxification and regeneration of reduced glutathion, the major antioxidant molecule in human cells.
- Exhaled halogenated alkanes (BHA) which, in contrast to BAA, manifests the activity of the antioxidant systems.

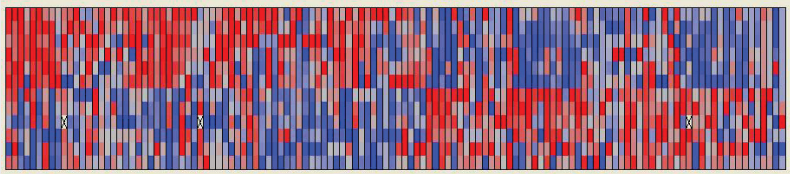
The following table illustrates the mean levels of these biomarkers, before and after either sham or actual exposure. Note that average values and standard deviations 1) are consistent with the known levels and interindividual variability described for these markers in humans and 2) appear to show no signs of an effect of EMF exposure in this form.

		GPX (U/g Hb*)	SOD (U/g Hb*)	MDA (nmol/g Hb*)	BAA (index)	BHA (index)	Isoprene (ng)	Aldehyde (ng)
Sham	T0	57,5 ± 15	1198 ± 86	25,5 ± 6	6,3 ± 3,1	6,3 ± 7,2	1302 ± 973	439 ± 213
	T	57,2 ± 16	1186 ± 87	24,9 ± 5	6,1 ± 3,2	4,0 ± 4,1	1421 ± 1020	417 ± 212
EMF	T0	58,1 ± 15	1200 ± 73	25,4 ± 6	5,2 ± 2,3	3,3 ± 3,5	1093 ± 812	424 ± 202
	T	56,9 ± 15	1188 ± 77	24,9 ± 6	6,3 ± 2,4	3,7 ± 3,1	1512 ± 789	486 ± 207

Values are means ± S.E.M * Hemoglobin

3 – Results and Conclusion

1 The table presented in insert n°2 is not suited for actual statistical analysis, as it lacks crucial computations such as subject effect and confidence interval. Hence, we applied the innovative analytical method "non-negative matrix factorization" (NMF) to the dataset comprising every single data collected during the study: a table of 126 columns (18 volunteers x 7 biomarkers) by 12 rows (6 samples x 2 days). After disclosure of groups assignments, the first six rows of non-exposure (sham) were labeled "A", while the last six rows of EMF treatments were noted "B". Exposure was randomized to distribute the actual or sham exposure on different days for the different volunteers. NMF was then used to compute right and left factoring vectors. NMF basically points to the best possible ordering of columns and rows thereby making trends and contrasts clear, giving an informative visualization of the data table. The rows and columns of the matrix, ordered by the elements of the left and right vectors, give the image below:



Row	Sample	Group
1	A-T01	A
2	A-T02	A
3	A-T03	A
4	A-T3	A
5	A-T2	A
6	A-T1	A
7	B-T1	B
8	B-T2	B
9	B-T3	B
10	B-T03	B
11	B-T01	B
12	B-T02	B

2 As a result of this process, OFF (rows A) and ON (rows B) samples appeared clearly separated. Since this could be partially attributed to a difference in baseline signals (OFF and ON samples were measured on two separate days), ON and OFF days have been distributed as homogeneously as possible during the process. In order to make a quantitative assessment of the level of contrast in sub-image ON (last six B rows), we calculated its entropy, a process widely used in image analysis.

3 Entropy of the sub-image ON was compared with the entropies of sub-images obtained through random selection of 8 individuals and permutation of the 56 associated columns across the entire original dataset. 8 corresponded to the initial randomization, in which only 8 volunteers were exposed to the actual GSM emission on day 1. *In fine*, there were 43,758 possible permutations, one of them resulting in the particular sub-image ON of the image above. We then counted the number of the random sub-images generated which entropies proved lower than the tested sub-image. Their ratio constituted the significance (P-value) of the entropy of the tested sub-image, which is shown in the table on the right. Note that reduction of entropy proved significant only in data corresponding to the actual GSM exposure.

	Sub-image ON	Sub-image OFF
Raw data	0.0202 *	ns
Raw data without outliers [a]	0.0169 *	ns

* significant ($P < 0.05$). ns: not significant

[a] 7 outliers out of 864 values.

Data shown after stringent multiplicity corrections.

In conclusion, the innovative NMF/entropy calculation method applied to the biomarker measurements enabled us to evidence global modulations after a 30 min exposure of the volunteers to a GSM mobile phone emission, even when classical methods based on data averaging completely or partially failed to (refer to coming article for additional material). This vouches for the superior relevance of the NMF/entropy calculation strategy in the context of extended, wildly varying datasets and support studies pointing to at least some kinds of interactions of GSM EMF with biological systems.

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