Oxidative stress in electrohypersensitivity self-reporting patients: Results of a prospective *in vivo* investigation with comprehensive molecular analysis

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Abstract. A total of 32 electrohypersensitivity (EHS) self-reporting patients were serially included in the present prospective study for oxidative stress and antioxidative stress response assessment. All thiobarbituric acid-reactive substances (TBARs) were measured in the plasma, particularly malondialdehyde (MDA) for lipid peroxidation; additional measurements included total thiol group molecules, reduced glutathione (GSH), oxidized glutathione (GSSG) for oxidative stress assessment and nitrotyrosine, a marker of peroxynitrite-induced oxidative/nitrosative stress. In addition, the activity of Cu-Zn superoxide dismutase (SOD1) was measured in red blood cells (RBCs) and glutathione reductase (GR) and glutathione peroxidase (GPx) in RBCs and plasma. Depending of the biomarker considered, 30-50% of EHS self-reporting patients presented statistically significantly increased TBARs, MDA, GSSG and NTT mean plasmatic level values in comparison with normal values obtained in healthy controls ($P<0.0001$). By contrast, there were no plasmatic level values above the upper normal limits for GSH, GSH/GSSG ratio, total glutathione (GluT) and GSH/GluT ratio, and values for these GSH-associated biomarkers were statistically significantly decreased in 20-40% of the patients ($P<0.0001$). Furthermore, in RBCs, mean SOD1 and GPx activities were observed to be statistically significantly increased in ~60% and 19% ($P<0.0001$) of the patients, respectively, while increased GR activity in RBCs was observed in only 6% of the patients. The present study reports for the first time, to the best of our knowledge, that overall ~80% of EHS self-reporting patients present with one, two or three detectable oxidative stress biomarkers in their peripheral blood, meaning that these patients-as is the case for cancer, Alzheimer's disease or other pathological conditions-present with a true objective new pathological disorder.

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Abbreviations: CFS, chronic fatigue syndrome; EHS, electrohypersensitivity; EMFIS, electromagnetic fields intolerance syndrome; GluT, total glutathione; GPx, glutathione peroxidase; GSH, reduced glutathione; GR, glutathione reductase; GSSG, oxidized glutathione; MCS, multiple chemical sensitivity; MDA, malondialdehyde; NTT, nitrotyrosine; RBC, red blood cells; SOD1, Cu-Zn superoxide dismutase; TBARs, thiobarbituric acid-reactive substances

Key words: EHS, electromagnetic fields, EMFIS, glutathione, inflammation-associated biomarker, malondialdehyde, nitrotyrosine, oxidative stress, SOD1

Introduction

Electrohypersensitivity (EHS) is a new World Health Organization (WHO)-acknowledged disabling condition occurring in EHS self-reporting patients (1).

Following the WHO-sponsored international workshop on electromagnetic hypersensitivity in 2004 in Prague (Czech Republic) the use of the term 'idiopathic environmental intolerance (IEI) attributed to electromagnetic fields (IEI-EMF)' was proposed to qualify this new EHS-associated detrimental health condition (2).

Using ultrasonic cerebral tomosphygmography (UCTS), it was recently demonstrated that EHS self-reporting patients present with a decrease in mean cerebral tissue pulsation index (PI) in a number of areas of the temporal lobe, particularly in the capsulo-thalamic area, which contains the limbic system and the thalamus; it was additionally suggested that these abnormalities may in fact be associated with a decrease in brain blood flow and/or neuronal dysfunction in these particular brain areas (3-5). EHS self-reporting patients were also objectively identified and characterized by demonstrating potential associations with a number of biological abnormalities, consisting of a degree of inflammation, heat-shock protein-associated cellular stress and autoimmune responses in the peripheral blood, and an abnormal 6-hydroxy-melatonin

sulfate/creatinine ratio in the urine (3). In fact, since it was reported that numerous EHS self-reporting patients present reliable clinical symptoms each time they report exposure to electromagnetic sources, and present with objective UCTS and biological abnormalities (4,5), the authors of the present study proposed the use of the more concise term electromagnetic field intolerance syndrome (EMFIS) to qualify the so-called newly WHO-recognized IEI-EMF pathological condition with which these patients are associated (4).

The present prospective *in vivo* biochemical investigation aimed to determine whether EHS self-reporting patients may also be characterized by oxidative stress abnormalities in the peripheral blood, to further identify and characterize EMFIS.

Materials and methods

Inclusion criteria. According to a previous study (3), EHS, more precisely EMFIS in EHS self-reporting patients, was defined on the basis of the five following clinical criteria: i) Absence of known pathology accounting for the observed clinical symptoms; ii) as reported by the patients, reproducibility of symptoms under the supposed influence of electromagnetic fields (EMFs), regardless of the incriminated source; iii) regression or disappearance of symptoms associated with reported EMF avoidance; iv) clinical symptoms compatible with those previously ascribed to EHS self-reporting patients in the scientific literature; and v) chronic evolution (6-10).

Prior to inclusion, all patients had a face-to-face interview based on a previously validated questionnaire, a complete general and neurological clinical examination and a systematic biological check-up, including currently used peripheral blood tests, to exclude any non-EMFIS-associated pathology. Therefore, to be included in the study, patients had no history of such pathologies as cancer, Alzheimer's disease, diabetes type II and/or cardiovascular disease. Patients also had no associated multiple chemical sensitivity (MCS), and were in an active symptomatic phase of their pathological condition(s), whether or not they had been previously treated. In addition, patients had a normal carotid and vertebral artery echodoppler scan, normal hematological, hepatic, renal and metabolic peripheral blood tests, and, when available, a normal magnetic resonance imaging or computed tomography scan.

However, since the majority of clinical symptoms in EHS self-reporting patients are subjective, two biological inclusion criteria were added to objectively identify EMFIS: i) A mean decreased tissue pulsometric index in at least three middle cerebral artery-dependent tissue sections in the temporal lobes, as demonstrated using UCTS, as it has been previously reported that UCTS is able to discriminate between EHS self-reporting patients and healthy subjects using this criterion (11); and ii) an increase in at least one of three inflammation-associated peripheral blood biomarkers that have previously been identified as being possibly detected in EHS self-reporting patients (3): Increased histamine, a mediator of inflammation (12); increased protein S100B, a marker of oxidative stress-associated blood brain barrier opening (13,14); and increased chaperone proteins heat shock protein β1 (HSP27) or heat shock 70 kDa protein 1B (HSP70), markers of heat-shock cell stress-associated inflammation and/or immune response (15,16). References for the

Table I. Inflammation-associated biomarkers investigated in electrohypersensitivity self-reporting patients.

Author, year	Biomarker	Sample type	(Refs.)
Lebel *et al*, 1996	Histamine	Plasma	(17)
Smit *et al*, 2005	Protein S100B	Serum	(18)
De and Roach, 2004	HSP27	Serum	(19)
Pockley *et al*, 1998	HSP70	Serum	(20)

HSP27, heat shock protein β1; HSP70, heat shock 70 kDa protein 1B.

methods used to measure these three inflammation-associated peripheral blood biomarkers are indicated in Table I (17-20).

Oxidative and antioxidative stress-related biomarkers. A battery of biomarkers were used to measure oxidative stress and antioxidative stress responses, in plasma and/or red blood cells (RBCs) (Table II). Measurements were performed following centrifugation (4,000 x g; 10 min; 4˚C) to separate RBCs from plasma.

Oxidative stress biomarkers. For oxidative stress assessment, the following biomarkers were measured in the plasma: All thiobarbituric acid-reactive substances (TBARs), and particularly one of them, malondialdehyde (MDA), which are markers of lipid peroxidation (21); glutathione disulfide (GSSG), which is a marker of reduced glutathione (GSH) oxidation (22); and nitrotyrosine (NTT), which is a marker of peroxynitrite-induced oxidative/nitrosative stress (23).

To measure MDA, the standard method described by Londero and Lo Greco (24) was used. When MDA reacts with TBA, the MDA-TBA complex is separated from interfering substances and specifically identified using reverse-phase high-performance liquid chromatography coupled with UV/visible detection. MDA is quantified on the basis of its strong light-absorbing and fluorescing properties following the reaction with TBA. The results are expressed in μM. For the dosage of lipid peroxidation intermediates, all plasma TBARs were measured, including MDA, using a method similar to that of Ohkawa *et al* (25). The present method was based on the reaction of the aldehyde function of TBARs released by acid hydrolysis at 95˚C with TBA to form a TBAR-TBA colored complex, which is quantified by fluorometry. Results are expressed in μM. Total glutathione (GluT), GSH and oxidized glutathione (GSSG) were determined enzymatically from the acidic protein-free supernatant, according to the method of Akerboom and Sies (26). The assay for GSSG was performed subsequent to masking GSH by adding 2-vinylpyridine to the deproteinized extract. The assay for NTT was performed according to the method of Ischiropoulos *et al* (27), which uses a competitive ELISA test (OxiSelect™ Nitrotyrosine ELISA kit; cat. no. STA-305; Cell Biolabs Inc., San Diego, CA, USA). For the determination of this last marker (NTT), plasma was first added to a nitrated bovine serum albumin (BSA) (OxiSelect™ Nitrotyrosine ELISA kit; cat. no. STA-319) preabsorbed enzyme immunoassay plate.

Following a brief incubation, a specific anti-nitrotyrosine antibody (OxiSelect™ Nitrotyrosine ELISA kit; part no. 230502) was added, followed by the addition of a horseradish peroxidase (HRP)-conjugated secondary antibody [OxiSelect™ Nitrotyrosine ELISA kit; HRP Conjugate (part no. 231009)]. The dilution of the anti-nitrotyrosine antibody was 1:1,000 and that of the secondary antibody was 1:1,000, and the incubation was performed at room temperature for 1 h. The protein NTT content in the plasmatic sample was determined by comparison with a standardized curve that was established from predetermined nitrated BSA standards, the results being expressed in μg/ml.

Antioxidative non-enzymatic proteins. For the non-enzymatic antioxidative response assessment, the total thiol group molecules, which comprise such peptides as glutathione and cysteine- and/or homocysteine-containing proteins, were measured in the plasma. For the dosage of the total SH group molecules, 5,5'-dithio-bis (2-nitrobenzoic acid) was used as reagent and the level of plasmatic SH group molecules was measured spectrophotometrically at 412 nm. The results are expressed in U/l (28). The dosage of GluT, GSH and GSSG in the plasma was calculated using the method of Akerboom and Sies (26). Prior to centrifugation (400 x g; 10 min; 4˚C), 400 μl whole blood was collected in 3.6 ml metaphoric acid. Following centrifugation, GluT and GSH were measured enzymatically in the acidic protein-free-supernatant. The assay of GSSG was performed following masking of GSH by adding 2-vinylpyridine to the deproteinized extract. Similar to GluT and GSH, GSSG was measured enzymatically. Results are expressed in μM.

Antioxidative enzymatic proteins. Measurement of the antioxidative enzymes was performed in RBCs only, or in RBCs and plasma. To measure Cu-Zn superoxide dismutase (SOD1) activity in RBCs, the method described by Marklund and Marklund (29) was used, which consists of a simple and rapid test based on the ability of SOD1 to inhibit the autoxidation of pyrogallol. The principle of this method is based on the competition between pyrogallol autoxidation by the superoxide anion (O_2^-) and the dismutation of this radical by SOD1. In this method, the rate of pyrogallol autooxidation was determined spectrophotometrically from the increase in absorbance at 420 nm; 1 unit of SOD1 activity was defined as the amount of the enzyme required to inhibit the rate of pyrogallol autooxidation by 50%. Results are expressed in U/mg hemoglobin (Hb). For the dosage of glutathione reductase (GR), a standard Randox kit-based colorimetric method was used (cat. no. GR2368; Randox Laboratories, Crumlin, UK). Results are expressed in U/g Hb for GR in RBCs, and U/l for GR in plasma (30). In addition, glutathione peroxidase (GPx) activity was measured in RBCs and plasma, according to a method derived from that of Günzler *et al* (31). The GPx assay was based on the oxidation of reduced nicotinamide dinucleotide phosphate (NADPH) to $NADP^+$, which is associated with a decrease in the absorbance at 340 nm. The rate of this decrease is directly proportional to the GPx activity in the sample. GPx activity was subsequently evaluated in nM NADPH oxidized/min, and the results are expressed in U/g Hb for GPx in RBCs and in U/l for GPx in plasma.

Statistical analysis. A total of two different statistical tests were used: i) The two-tailed Student's t-test, for comparison between patient values and normal control reference values; and ii) Pearson's correlation test for analyzing the statistical association between the different variables of interest, including oxidative and antioxidative stress-associated biomarkers. All statistical analysis was performed using the XLSTAT software (XLSTAT 2018.1.49725; Addinsoft; https://www.xlstat.com). Considering the fact that the two-tailed Student's t-test was used to perform three comparisons (total EHS patients values, EHS patients with values above upper normal limits and EHS patients with values below the lower normal limits) with the one dataset of normal control reference values, the Bonferroni correction was applied, which sets the α cut-off of significance at 0.05/3, i.e. 0.016. While statistical analysis using the Pearson's correlation test was done with the cut-off value of α=0.05.

Results

Demographic data. A total of 32 EMFIS-bearing patients were included in this prospective study for oxidative and antioxidative stress biomarker analysis. The mean age was 50.6 years, ranging between 32 and 75 years. There were 22 females and 10 males, for an overall female/male sex ratio of 69%.

However, since NTT was measured in only 14 of the 32 cases in this series, the results obtained from a concomitant series of 46 additional EHS self-reporting patients (mean age, 49 years; female/male sex ratio, 71% female), all complying with the inclusion criteria (see above), were added, thus the results presented for NTT are based on the analysis of an overall number of 60 EMFIS-bearing patients. The demographic data are presented in Table III.

Oxidative stress biomarkers. The results are depicted in Fig. 1, and in Tables IV and V. Fig. 1 presents the distribution values of the different oxidative stress biomarkers analyzed in EMFIS-bearing patients in comparison with normal-range values obtained from healthy controls. As indicated in Fig. 1, for a number of cases, TBARs, MDA, GSSG and NTT peripheral blood level values were above the upper normal limits, meaning that these cases were associated with detectable oxidative stress in the peripheral blood. These data are confirmed in Table IV. Overall, in comparison with normal-range values, the mean values (± standard deviation) for all 32 patients analyzed were statistically significantly increased for TBARs (P=0.013), and tended to be increased for MDA and GSSG (P=0.053 and P=0.051 respectively), although not for NTT (P=0.790). However, when restricting the analysis to EMFIS-bearing patients having values above the upper normal limits, relative to the values obtained in normal healthy controls (this concerns 30-50% of the patients, depending on the biomarker considered), a statistically significant difference was evident for TBARs, in addition to MDA, GSSG and NTT (P<0.0001); that is, for all the oxidative stress biomarkers analyzed thus far.

Non-enzymatic protein-associated biomarkers. By contrast, as indicated in Fig. 1, considering the overall series of patients investigated, all values for the total protein thiol

Table II. Methods of measurement of oxidative stress-associated biomarkers, antioxidative non-enzymatic proteins and antioxidative enzymes in the plasma and/or red blood cells in electrohypersensitivity self-reporting patients, with electromagnetic field intolerance syndrome.

Author, year	Biomarkers oxidative stress	Sample type	(Refs.)
Londero and Lo Greco, 1996	MDA	Plasma	(24)
Okhawa *et al*, 1979	TBARS	Plasma	(25)
Akerboom and Sies, 1981	GSSG	Plasma	(26)
Ischiropoulos *et al*, 1992	NTT	Plasma	(27)
	Antioxidative non-enzymatic proteins		
Jocelyn, 1987	Total thiol	Plasma	(28)
Akerboom and Sies, 1981	GSH	Plasma	(26)
Akerboom and Sies, 1981	GluT[a]	Plasma	(26)
	Antioxidative enzymes		
Marklund and Marklund, 1974	SOD	RBC	(29)
Mannervik, 2001	GR	Plasma/RBC	(30)
Günzler *et al*, 1974	GPx	Plasma/RBC	(31)

[a]GluT includes GSH and GSSG. MDA, malondialdehyde; TBARs, thiobarbituric acid reactive substances; NTT, nitrotyrosine; GSSG, oxidized glutathione; GSH, reduced glutathione; GluT, total glutathione; SOD, superoxide dismutase; GR, glutathione reductase; GPx, glutathione peroxidase; RBC, red blood cell.

Table III. Demographic data.

No. cases	Mean age, years	Age range, years	Sex ratio, F/M (%F)
32[a]	50.6	32-75	22/10 (69)
46[b]	49	19-79	33/13 (71)
123[c]	44	18-65	61/62 (50)

[a]Measurement of all markers in 32 EHS self-reporting patients except for NTT which was measured in 14 patients. [b]Measurement of NTT in 46 additional patients from a concomitant series of EHS self-reporting patients. NTT, nitrotyrosine; EHS, electrohypersensitivity. [c]These historical apparently-normal controls were selected on the basis of a lack of clinical symptoms and medical history of diseases.

group were within the limits of normal-range values. There were also no blood level values above the upper normal limits for GSH, the GSH/GSSG ratio, GluT and the GSH/GluT ratio; however, in a number of cases, for these GSH-related biomarkers and for NTT, blood level values were below the normal-range values. These data are detailed in Table V. When analyzing the overall series of patients, all investigated biomarkers, with the exception of GluT and NTT, were observed to be statistically significantly below the lower normal limit values. However, when considering the 20-40% of patients with values below the lower normal limit values, this finding was confirmed for these biomarkers and also for GluT and NTT ($P<0.0001$), suggesting that certain oxidative stress-associated biomolecular processes resulting in a decrease in GSH, GluT and NTT may have occurred in these particular cases.

Antioxidative stress enzymes. The previous oxidative stress data were confirmed by measuring a number of antioxidative stress-associated key enzymes in RBCs and plasma. The results are depicted in Fig. 2 and Table VI. An important observation indicated in Fig. 2 was that SOD1 activity measured in RBCs was associated with values above the upper normal limits in ~60% of the patients, suggesting that this antioxidative stress-inducible enzyme is primarily involved in the oxidative stress detoxification process occurring in EMFIS-bearing patients. Furthermore Table VI indicates that when considering all included cases, there was statistically significantly increased activity in RBCs of SOD1, although not GPx ($P=0.002$ and $P=0.044$, respectively), and of GPx in the plasma. Likewise, in comparison with normal-range values, the mean values (± standard deviation) obtained in the ~60% of EMFIS-bearing patients having increased SOD1 activity, were revealed to be statistically significantly increased ($P<0.0001$). However, when restricting the analysis to the patients with GPx and CG increases, as indicated in Table VI, a statistically significant difference in comparison with normal control reference values in RBCs and plasma was identified in 19 and 10% of the patients respectively, and for GR in RBCs in ~6% ($P<0.0001$), meaning that EMFIS may be characterized by increased antioxidative stress-associated enzymatic activity in RBCs, primarily involving SOD1.

Overall oxidative stress occurrence in EMFIS-bearing patients. Table VII reports the overall results obtained with the

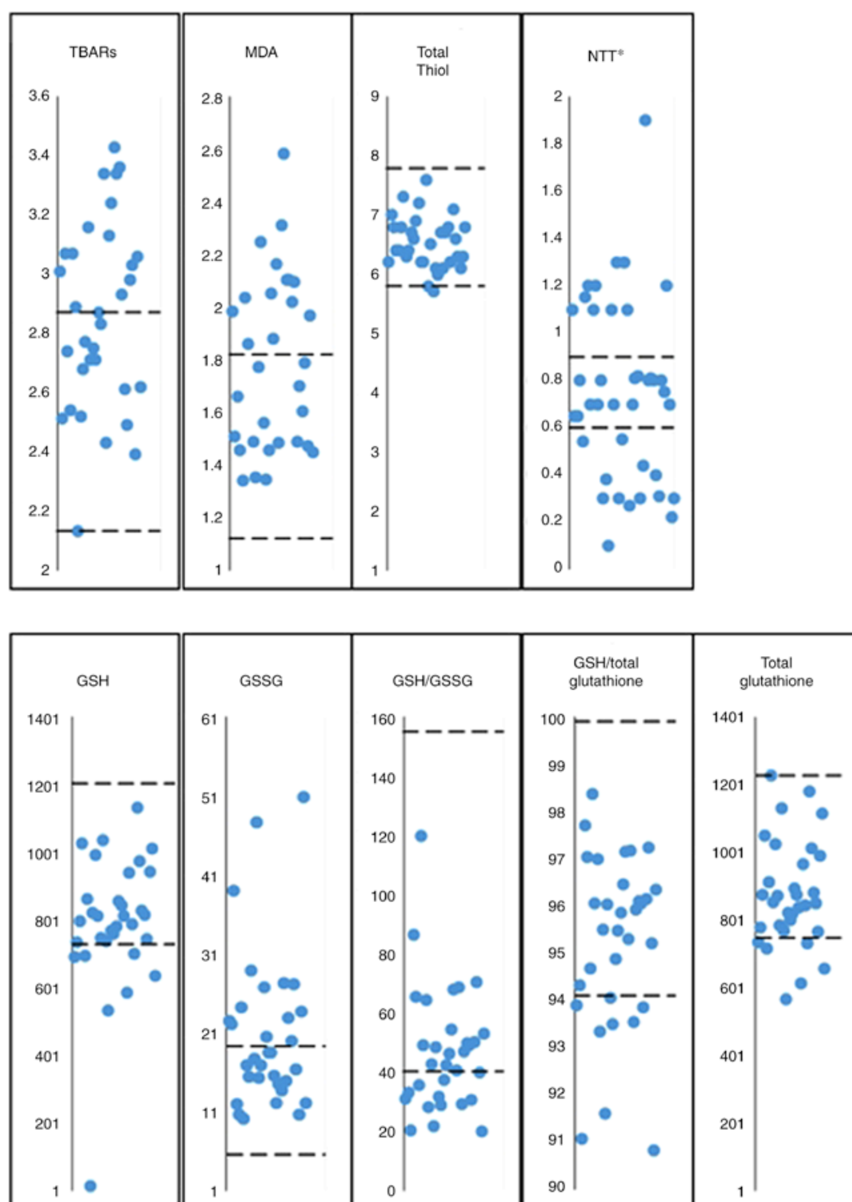

Figure 1. Values of oxidative stress biomarkers in the plasma of EHS self-reporting patients (electromagnetic fields intolerance syndrome-bearing patients) in comparison with normal range values. *Data were obtained from an overall series of 60 EHS self-reporting patients. TBARs, thiobarbituric acid-reactive substances; MDA, malondialdehyde; NTT, nitrotyrosine; GSH, reduced glutathione; GSSG, oxidized glutathione; EHS, electrohypersensitivity.

three principal categories of oxidative stress biomarkers used in this study: TBARs/MDA, GSSG and NTT. Fig. 3 summarizes the results: 42.85% of EHS self-reporting patients had one positive detectable oxidative stress biomarker, and 21.43 and 14.28% had 2 or 3 positive detectable oxidative stress biomarkers, respectively, meaning that overall, 80-90% of the cases were associated with at least one detectable oxidative stress biomarker in the peripheral blood. However, in order to provide a comprehensive interpretation of the findings characterizing EMFIS, the present study included a provisional molecular bioanalysis of the different results obtained, as presented in Figs. 4-6.

Search for statistical correlations. Using Pearson's statistical correlation test, the present study sought to identify a correlation between the different biological parameters so far investigated.

Table VIII reports the results. It was observed that the plasma level of MDA (a well-known TBAR) was positively correlated with the TBAR plasma level, and that the GSSG plasma level was positively correlated with GSH and GluT plasma levels, and with GSH/GluT and GSH/GSSG ratios.

Furthermore, it was observed that the GSH/GSSG ratio was positively correlated with SOD1 activity in RBC, as tended to be the case for the GSH/GluT ratio (P=0.06). It was additionally identified that the GSH plasma level was positively correlated with the GPx activity level in plasma, although not with GPx activity in RBCs (P=0.371).

Discussion

It is well established that oxidative stress may cause profound alterations in biomolecules, including lipids, proteins and nucleic acids, and consequently may alter various cellular

Table IV. Electromagnetic field intolerance syndrome-associated oxidative stress biomarkers measured in the peripheral blood of EHS self-reporting patients, including mean values (± SD) for all patients, and mean values (± SD), numbers and percentages of patients with mean values above the upper normal limits.

				Patients with EHS with values above upper normal limits			
Oxidative stress biomarkers	Normal values (range)	Patients with EHS Mean ± SD	P-value[a]	No. of cases	% of total cases	Mean ± SD	P-value[b]
TBARS	2.5±0.18 (2.13-2.86) μM	2.85±0.06	0.013	15/32	48.88	3.14±0.17	<0.0001
MDA	1.46±0.17 (1.12-1.81) μM	1.76±0.06	0.053	14/32	43.75	2.10±0.19	<0.0001
GSSG	12.4±3.4 (5.5-19.3) μM	20.74±1.74	0.051	13/32	40.63	29.46±9.95	<0.0001
NTT	0.75±0.08 (0.6-0.9) μg/ml	0.78±0.35	0.790	20/60	33.33	1.19±0.21	<0.0001

[a]P-values obtained for comparisons between the patients with EHS and the controls. The Bonferroni correction sets the α cut-off for significance at 0.016. [b]P-values obtained for comparison between the patients with EHS with values above the upper normal limits and the control group. The Bonferroni correction sets the α cut-off for significance at 0.016. MDA, malondialdehyde; TBARs, thiobarbituric acid reactive substances; NTT, nitrotyrosine; GSSG, oxidized glutathione; EHS, electrohypersensitivity; SD, standard deviation.

Table V. Electromagnetic field intolerance syndrome-associated non-enzymatic protein biomarkers measured in the peripheral blood of EHS self-reporting patients, including mean values (± SD) for all patients, and mean values (± SD), numbers and percentages of patients with mean values above the upper normal limits.

				Patients with EHS with values above upper normal limits			
Oxidative stress biomarkers	Normal values (range)	Patients with EHS Mean ± SD	P-value[a]	No. of cases	% of total cases	Mean ± SD	P-value[b]
GSH	965±118 (729-1203) μM	794.62±34.74	0.012	6/32	18.75	639.47±69.27	<0.0001
GSH/GSSG ratio	84.15±29.35 (40.1-155) μM/μM	46.92±3.68	<0.0001	13/32	40.63	29.77±4.72	<0.0001
GluT	989±120 (749-1228) μM	873.47±27.85	0.041	6/32	18.75	669.83±9.67	<0.0001
GSH/GluT ratio	99±0.19 (94.1-99.9) %	95.25±0.33	0.0009	9/32	29.13	92.86±1.29	<0.0001
NTT	0.75±0.08 (0.6-0.9) μg/ml	0.78±0.35	0.790	20/60	33.33	0.41±0.14	<0.0001

[a]P-values obtained for comparisons between the patients with EHS and the controls. The Bonferroni correction sets the α cut-off for significance at 0.016. [b]P-values obtained for comparison between the patients with EHS with values above the upper normal limits and the control group. The Bonferroni correction sets the α cut-off for significance at 0.016. NTT, nitrotyrosine; GSSG, oxidized glutathione; GSH, reduced glutathione; GluT, total glutathione; EHS, electrohypersensitivity; SD, standard deviation.

functions and structures (32,33). This explains why oxidative stress has been implicated in ageing and in a number of age-associated pathologies, including cancer, Alzheimer's disease, diabetes and cardiovascular diseases through genetic and/or epigenetic mechanisms (34). Concerning more particularly the role of oxidative stress in tumorigenesis, a general free radical theory was recently proposed linking oxidative stress to direct genetic toxicity and DNA mutagenesis, and indirectly to epigenetic alterations through free radical-induced protein epimutations (35). The present study reports for the first time, to the best of our knowledge, that ~80% of so-called EHS self-reporting patients present with oxidative stress, and thus may be considered to be bearing a truly objective pathological disorder, as is the case for cancer, Alzheimer's disease, or other diseases or pathological conditions. In the present study, the term EMFIS was preferred to EHS since, according to the clinical criteria used, it was not possible to clearly assess whether the patients exhibited a decreased tolerance threshold when exposed to EMFs. Furthermore, the term EMFIS was used preferentially to IEI-EMF, which has been proposed by the WHO, as all patients included in the present study clearly reported EMF-associated clinical symptoms (4).

During oxidative stress, among the reactive oxygen species (ROS) are the superoxide anion ($O_2^{\circ-}$), hydrogen peroxide (H_2O_2), the hydroxyl radical (OH°) and the hydroperoxyl radical ($O_2H°$). Further, in redox cycling, transition metals including Fe, Cu, Ni and Co serve an important role in ROS formation (36).

Fe is the most commonly involved transition metal, and there are three classical reaction types. In the first step, namely the Haber-Weiss reaction, the superoxide anion reduces ferric ion into ferrous ion: $Fe^{3+} + O_2^{\circ-} \rightarrow Fe^{2+} + O_2$ [A]; while in a second step, namely the Fenton reaction, ferrous ion reacts

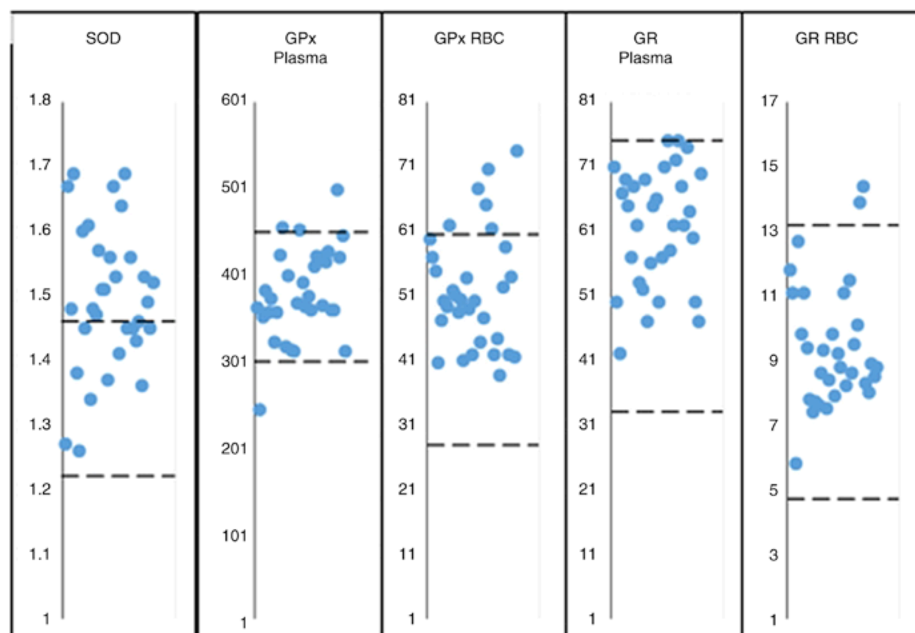

Figure 2. Values of specific activity of antioxidative detoxification enzymes measured in the plasma and RBCs of electrohypersensitivity self-reporting patients (electromagnetic fields intolerance syndrome-bearing patients) in comparison with normal range values. For GPx and GR, the patients with elevated plasma levels were different from those with elevated RBC levels. SOD, superoxide dismutase; GPx, glutathione peroxidase; GR, glutathione reductase; RBC, red blood cell.

with hydrogen peroxide to generate hydroxyl radicals and hydroxide ions: $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH° + OH^-$ [B].

Finally, in a third reaction, ferric ion is reduced to ferrous ion by reacting with a second hydrogen peroxide molecule, to recycle ferrous ion and form a hydroperoxyl radical and a proton: $Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + O_2H° + H^+$ [C].

The net effect of [B] and [C] is the generation of the two ROS, OH° and $O_2H°$, with H_2O as a byproduct.

ROS are, however, difficult to measure directly due to their very short half-life. This explains why measurement of the products resulting from the molecular damage induced by ROS is the usual way to assess and measure oxidative stress. To that end, different oxidative stress and antioxidative response biomarkers were selected in the present study, representative of the different biochemical pathways and biological structural alterations that may occur when the organism is subjected to environmental stressors (37).

TBARs, which reflect the overall damage induced by non-enzymatic ROS-associated lipid peroxidation, among which MDA is the most prevalent byproduct (38), are commonly used biomarkers of lipoxidative stress (21). The reactive aldehyde MDA is a major indicator of the tissue damage resulting from the peroxidation of polyunsaturated fatty acids (PUFAs) induced by the two most prevalent ROS involved in lipoxidative stress: OH° and $O_2H°$ (39).

In fact, lipid peroxidation leads to the formation of numerous aldehydes, among which certain of them are highly reactive and may be considered as secondary messengers, which disseminate and amplify the initial oxidative stress. This is particularly the case for MDA, which is a bi-functional electrophile that is able to react strongly with nucleophiles, including amino acid residues in proteins (39). MDA adducts are thus biologically highly toxic, since they induce profound alterations in the structure and function of biomolecules by creating intramolecular or intermolecular protein/DNA cross-links (40,41).

This may explain why the majority of assays that have been developed to measure MDA on the basis of its derivatization with TBA have been challenged for their relative lack of specificity (42). This is due to the fact that TBA (in addition to MDA) is able to react spontaneously with numerous other molecules present in the test tube; by using a high temperature (90-100˚C) to obtain the TBA/MDA spectrophotometrically-measurable condensation product, the process is able to generate *in vitro* further oxidation (43). In the present study, the method developed by Londero and Lo Greco (24) was used, which is considered to minimize the biases due to the procedure itself, and thus may increase specificity. Moreover, TBARs and MDA were measured simultaneously in the same sample and the values obtained for these two biomarkers were compared with the normal-range values obtained in healthy controls. Using this procedure, it was demonstrated that 40-50% of the patients had statistically significantly increased TBAR and MDA mean plasmatic values relative to normal values, a finding which strongly suggests that these patients present with an increased lipid peroxidation state detectable in their peripheral blood. In addition, these data were confirmed in the overall sample of 32 patients studied for TBARs, and tended to be significant for MDA.

Indeed these data may not be restricted to the peripheral blood, since cellular and nuclear membranes are primarily composed of fatty acids, including PUFAs. In the past 20 years MDA has been recognized as a reliable lipid peroxidation marker in a number of diseases, including cancer (44-47), type 2 diabetes (48), cardiovascular diseases (49,50) and Alzheimer's disease (51). On the basis of the present data, this is also the case for EMFIS, and this result is unsurprising since oxidative stress, including lipid peroxidation, has also been evidenced in

Table VI. Electromagnetic field intolerance syndrome-associated antioxidative detoxification enzymatic activity measured in red blood cells and the plasma of EHS self-reporting patients, including mean values (± SD) for all patients, and mean values (± SD), number and percentage of patients with mean values above the upper normal limits.

				Patients with EHS with values above upper normal limits			
Anti-oxidative stress enzymes	Normal values (range)	Patients with EHS Mean ± SD	P-value[a]	No. of cases	% of total cases	Mean ± SD	P-value[b]
SOD (RBC)	1.34±0.06 (1.22-1.46) U/mg Hb	1.50±0.02	0.002	19/32	59.38	1.57±0.08	<0.0001
GPx (RBC)	44.1±8.2 (27.8-60.5) U/g Hb	51.92±1.62	0.044	6/32	18.75	66.70±4.76	<0.0001
GPx (plasma)	375±37.5 (300-450) U/l	379.28±9.30	0.83	3/32	9.38	469.67±26.31	<0.0001
GR (RBC)	8.9±2.1 (4.7-13.2) U/g Hb	9.42±0.34	0.56	2/32	6.25	14.15±0.35	<0.0001
GR (plasma)	54±9 (33-75) U/l	61.69±9.17	0.16	0	0	-	-

[a]P-values obtained for comparisons between the patients with EHS and the controls. The Bonferroni correction sets the α cut-off for significance at 0.016. [b]P-values obtained for comparison between the patients with EHS with values above the upper normal limits and the control group. The Bonferroni correction sets the α cut-off for significance at 0.016. SOD, superoxide dismutase; GR, glutathione reductase; GPx, glutathione peroxidase; RBC, red blood cell.

Table VII. Percentage of electrohypersensitivity self-reporting patients (electromagnetic field intolerance syndrome-bearing patients) having positive TBARs, GSSG and/or NTT oxidative stress biomarkers measured in the peripheral blood.

No. of positive biomarkers	Markers	Percentage of patients (%)
1	NTT	14.28
	GSSG	7.14
	TBARs	21.43
	NTT or GSSG or TBARs	42.85
2	TBARs and GSSG	7.14
	NTT and TBARs	7.14
	NTT and GSSG	7.14
	TBARs and GSSG, or NTT and TBARs, or NTT and GSSG	21.42
3	NTT and TBARs and GSSG	14.28

TBARs, thiobarbituric acid reactive substances; NTT, nitrotyrosine; GSSG, oxidized glutathione.

similar recognized pathological conditions, including chronic fatigue syndrome (CFS) (52-56) and MCS (57).

However, as previously outlined, oxidative stress is an extremely complex redox cycling process resulting in various oxidizing/nitrosating free radical and molecular species attacks that exceed natural defense mechanisms; thus, it may not be measured by only one biomarker. In addition to TBARs and MDA, the present study measured GSH and, more specifically, GSSG and NTT as oxidative stress biomarkers. Glutathione is the primary compound that determines the redox state of a cell. It is a prototype antioxidant involved in cellular protection from the noxious effects of oxidative stress, directly and as cofactor of GPx. This thiol-containing tripeptide exists in an oxidized (GSSG) and reduced (GSH) form, and thus is a nucleophile and a reducing agent that is able to react with electrophilic and oxidizing species, allowing cells to escape the interaction of ROS with critical molecular targets, including proteins or nucleic acids (58). The ratio of GSH to the GSSG is a well-known marker of the redox state of a cell (59). Consequently GSH and GSSG were measured and their ratio (GSH/GSSG) and sum (GluT) were determined for the analysis of oxidative stress and the antioxidative stress response.

During oxidative stress, GSSG results from the oxidation of two GSH molecules by one hydrogen peroxide molecule, according to the following formula: $2GSH + H_2O_2$ $GSSG + 2H_2O$ [D]; while the two GSH molecules are usually recycled from the reduction of GSSG, according to a reaction involving the coenzyme NADPH: $GSSG + NADPH$ $2GSH + NADP^+$ [E].

It is notable that the oxidation of GSH according to [D] is catalyzed by GPx, while the reduction of GSSG according to [E] is catalyzed by GR; thus, according to [D] and [E], the activity of these two key enzymes in RBCs and plasma was measured.

In the present study, it was demonstrated that in comparison with normal-range values, the GSSG plasmatic mean value was statistically significantly increased in 40% of the patients, meaning that these patients presented with an oxidative redox state detectable in their peripheral blood. However, these data were not confirmed for the overall 32-patient sample for which the mean GSSG plasmatic mean value was not statistically significantly increased.

According to [D], it is assumed that increased GSSG may result in GSH depletion and consequently contribute to a decrease in antioxidant defenses. This may explain the result that in the overall patient sample studied, mean plasmatic values of GSH, the GSH/GSSG ratio and the GSH/GluT ratio were all statistically significantly decreased in comparison with normal control values; and that 20-40% of the patients

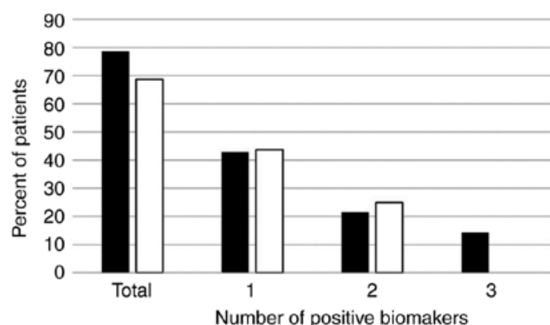

Figure 3. Percentage of electrohypersensitivity self-reporting patients (electromagnetic fields intolerance syndrome-bearing patients) having positive TBARs, GSSG and/or NTT oxidative stress biomarkers measured in the peripheral blood. Positive biomarkers correspond to marker levels above the upper normal limit; 'total' corresponds to the patients with one or more positive biomarkers. Black bars indicate the percentage of patients with one, two or three of the three positive biomarkers (TBARS, GSSG and NTT), detected in 14 of the 32 included patients; white bars indicate the percentage of patients with one or two out of TBARs and GSSG in the total 32 included patients. TBARs, thiobarbituric acid-reactive substances; GSSG, oxidized glutathione; NTT, nitrotyrosine.

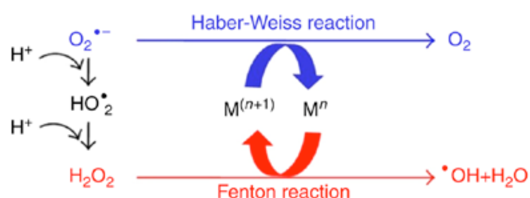

Figure 4. Fenton and Haber-Weiss reactions. Reduced form of transition-metals (M n) is oxidized by hydrogen peroxide to oxidized form of transition metals [M(n+1)], forming hydroxyl radical and water as byproducts. Superoxide radical ($O_2\bullet^-$) can also react with oxidized form of transition metals [M(n+1)] in the Haber-Weiss reaction leading to the production of reduced form of transition-metals (M n).

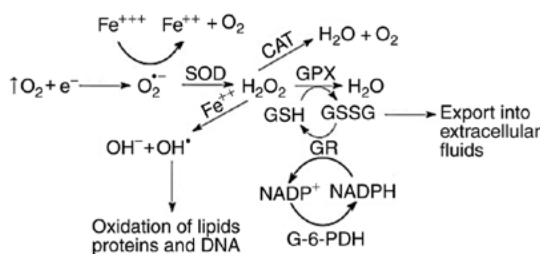

Figure 5. Schematic diagram showing the detoxification role of GSH, SOD1, GPx, GR and Cat during oxidative stress. Superoxide radical can be generated through the activation of specialized enzymes or be generated as by-product of abnormal cellular metabolism, occurring particularly from the mitochondrial electron transport chain. Superoxide dismutase then converts superoxide to hydrogen peroxide which has to be rapidly removed from the system. This is generally achieved by catalases or peroxidases, such as the glutathione peroxidases which use reduced glutathione (GSH) as electron donor. Alternatively Iron(II) (present in the system) is oxidized by hydrogen peroxide to iron(III), forming hydroxyl radical and hydroxide ion. GSH, reduced glutathione; SOD1, Cu-Zn superoxide dismutase; GPx, glutathione peroxidase; GR, glutathione reductase; Cat, catalase; NADPH, reduced nicotinamide adenine dinucleotide phosphate.

with values of these biomarkers below the lower normal limits presented with statistically significantly decreased mean values in comparison with normal values, a finding which

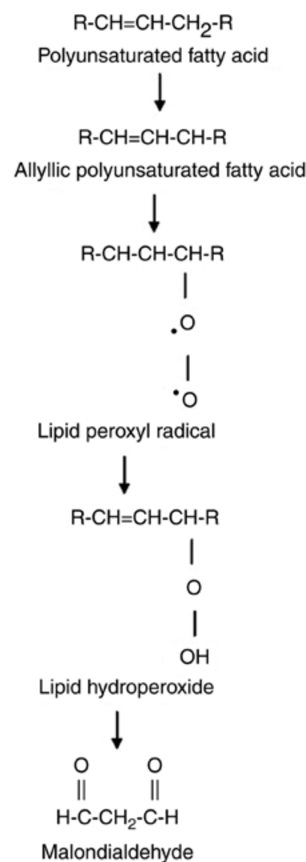

Figure 6. Schematic representation of the lipid peroxidative chain reaction leading to malondialdehyde. Malondialdehyde may be generated *in vivo* as a side product of PUFA decomposition by enzymatic processes during the biosynthesis of allylic PUFA, lipid peroxyl radical and lipid hydroperoxyde. PUFA, polyunsaturated fatty acid.

also confirmed that EHS self-reporting patients present with oxidative stress. Similar data were obtained in RBCs for GSH, although not for the GSSG/GluT ratio, in the De Luca *et al* (57) study, suggesting that the plasmatic measurement of GSSG, the GSH/GSSG ratio or the GSH/GluT ratio may be more informative compared with the measurement of the GSSG/GluT ratio in RBCs for the assessment of oxidative stress in EHS self-reporting patients.

Since the GSSG increase may be caused by an increase in GPx activity and/or by a decrease in GR activity, as indicated above, the present study measured the activity of these two key enzymes in RBCs and plasma. The overall mean GPx activity in the all 32 cases studied was not statistically significantly increased in RBCs and in plasma; with the exception of two cases, mean GR activity in RBCs and plasma was normal in all sample cases studied. However when considering the 18.75 and 9.28% of patients with increased GPx activity in RBCs and in plasma, respectively, a statistically significant difference was identified. Accordingly, it was suggested that the GSSG mean level increase in the peripheral blood may be associated with the increased GPx activity in ~19% of the patients, and/or by lower or normal GR activity; according to [E], the activity of this latter inducible enzyme is insufficient to recycle GSH from GSSG.

In the redox process GPx is an important enzyme as, by acting as a peroxynitrite reductase, it is able to efficiently

Table VIII. Analysis of statistically significant correlations between oxidative stress biomarkers, enzymatic and non-enzymatic antioxidative stress proteins, using the Pearson's correlation test.

	P-values											
Variables	TBARS	MDA	GSSG	GSH	GSH/GluT	GSH/GSSG	GluT	SOD1	GPx Plasma	GPx RBC	GR Plasma	GR RBC
TBARS	-	<0.001	0.391	0.931	0.417	0.775	0.884	0.655	0.189	0.352	0.736	0.838
MDA	<0.001	-	0.373	0.923	0.551	0.736	0.540	0.811	0.157	0.581	0.432	0.542
GSSG	0.391	0.373	-	0.023	<0.001	<0.001	0.043	0.105	0.249	0.603	0.145	0.388
GSH	0.931	0.923	0.023	-	0.201	0.030	0.070	0.611	0.014	0.371	0.625	0.339
GSH/GluT	0.417	0.551	<0.001	0.201	-	<0.001	0.963	0.062	0.725	0.870	0.102	0.180
GSH/GSSG	0.775	0.736	<0.001	0.030	<0.001	-	0.112	0.030	0.775	0.543	0.322	0.294
GluT	0.884	0.540	0.043	0.070	0.963	0.112	-	0.695	0.100	0.112	0.802	0.284
SOD1	0.655	0.811	0.105	0.611	0.062	0.030	0.695	-	0.309	0.321	0.162	0.791
GPx Plasma	0.189	0.157	0.249	0.014	0.725	0.775	0.100	0.309	-	0.183	0.673	0.770
GPx RBC	0.352	0.581	0.603	0.371	0.870	0.543	0.112	0.321	0.183	-	0.854	0.401
GR Plasma	0.736	0.432	0.145	0.625	0.102	0.322	0.802	0.162	0.673	0.854	-	0.012
GR RBC	0.838	0.542	0.388	0.339	0.180	0.294	0.284	0.791	0.770	0.401	0.012	-

MDA, malondialdehyde; TBARs, thiobarbituric acid reactive substances; NTT, nitrotyrosine; GSSG, oxidized glutathione; GSH, reduced glutathione; GluT, total glutathione; SOD, superoxide dismutase; GR, glutathione reductase; GPx, glutathione peroxidase; RBC, red blood cell.

reduce peroxynitrite/peroxynitrous acid ($ONOO^-$/ONOOH) into nitrite (NO), thereby protecting cells against oxidative and nitrative reactions (60). The present data was not completely concordant with that obtained in the De Luca *et al* (57) study, which exhibited an overall statistically significant increase in GPx activity in RBCs in comparison with normal values. In addition, since GR activity was not measured in this latter study, it is not possible to confirm the accuracy of the present data demonstrating a normal level of GR activity in RBCs and plasma. In the present study, the evidence of oxidative stress in EHS self-reporting patients was, however, considerably reinforced by the evidence that, relative to normal values, the SOD1 mean value in RBCs was observed to be statistically significantly increased when considering the overall patient sample and the near 60% of the patients with mean values above the upper normal limit.

Such results were confirmed in patients with MCS in the De Luca *et al* (57) study, although not in patients with EHS; however, in these patients there was a strong tendency towards an increase in the SOD1 mean value in RBCs. The reason for such differences in comparison with the present data are not clear, and may be due to different inclusion criteria, since these criteria were not clearly detailed in the De Luca *et al* study, and/or to the use of a different dosage techniques.

SOD1 catalyzes the detoxification of the superoxide anion by dismuting this anion into hydrogen peroxide and molecular oxygen. $O_2^{\circ-} + O_2^{\circ-} + 2H^+ \rightarrow H_2O_2 + O_2$ [F].

A further consideration to be made concerning the increased SOD1 activity in EMFIS-bearing patients is that according to [B] and [C], excessive production of H_2O_2 may provide excessive OH° and $O_2H°$ free radical and OH^- ion production, and thus may amplify oxidative stress-induced detrimental health effects. Such a hypothesis is plausible since, in the present study, GPx activity was observed to be increased in only 10-18% of cases, thereby limiting its H_2O_2 detoxifying capacity. Another possibility for detoxifying H_2O_2 is catalase. However, catalase activity was not measured in the present study, although it was reported that in comparison with normal controls catalase activity tends to be decreased in EHS self-reporting patients (57), meaning that the H_2O_2 detoxifying capacity of catalase may be not sufficient in these patients.

It is possible, according to [D] and [F] by providing H_2O_2 in excess, that SOD1 may also indirectly contribute to the increased formation of GSSG, since as reported above it was demonstrated that GPx activity was normal or even increased in EHS self-reporting patients. In fact, whatever the resulting effects of the SOD1 increase in EHS self-reporting patients, it is notable that a similar increased level of SOD1 activity was reported in patients with Alzheimer's disease, with this increased level having been considered for the early diagnosis and therapeutic monitoring of this disease (61). This may also be the case for EMFIS-bearing patients.

During the oxidative process, peroxynitrite ($ONOO^-$) may also be generated from the reaction of the superoxide anion with nitric oxide in the framework of an oxidative/nitrosative stress process, according to the following formula: $O_2^{-\circ} + NO \rightarrow ONOO^-$ [G].

In this *in vivo* reaction, the radical coupling of NO with $O_2^{-\circ}$ to form the non-free radical anion $ONOO^-$ is fast enough to outcompete the protective endogenous effect of SOD1. Although NO is regarded as a physiological cellular regulating agent, due to its rapid intra-tissue diffusion, it is also considered to be a crucial mediator of cellular damage occurring in different inflammation-associated pathological conditions, more particularly in neurodegenerative diseases, including Alzheimer's disease (62). Since NO is produced in large quantities in the brain, it is thought that it may serve a major contributing role in amplifying the peroxynitrite-induced toxicity in the central nervous system, thereby accounting for the fact that Alzheimer's disease is associated with peroxynitrite-associated oxidative stress. In fact, contrary to what was believed in the past, that the majority of oxidative stress-associated toxic effects may be attributed to NO, it is known that *in vitro* NO may inhibit lipid peroxidation (63); and it is now clearly established that due to the almost instantaneous formation of peroxynitrite each time NO and superoxide collide, peroxynitrite is the true toxic tissue damaging agent; peroxynitrite is a powerful oxidant that has been proven to cause pathogenic damage by interacting at a relatively slow rate and diffusion-limiting capacity with intracellular lipids, proteins and DNA (62).

A good example of such a selective reaction is the nitration of tyrosine residues in proteins and the formation of NTT, which thereby serves as a marker of peroxynitrite formation (64) and is a marker of oxidative/nitrosative stress (65).

The present study therefore included NTT in the battery of oxidative stress biomarkers used. Considering the overall sample of 60 EHS self-reporting patients investigated, wit was possible to define three categories of patients according to their NTT values: One-third of the patients exhibited values within the normal-range values, and another one-third exhibited values above the upper normal limits, while a further one-third of the patients presented with values below the lower normal limits. Notably, these findings were corroborated by the fact that in the latter two last categories, patients with abnormal mean values, these values were statistically significantly increased or decreased relative to normal values.

These data strongly suggested that one-third of the patients studied, those with statistically significantly increased NTT mean values, presented with detectable oxidative/nitrosative stress in the peripheral blood; overall, these data called into question why two-thirds of the patients presented with normal or statistically significantly decreased NTT values. The increase in NTT values in one-third of the patients may be easily explained by the increased peroxynitrite formation, and also by the fact that a decrease in GSH, usually an efficient scavenger of peroxynitrite (60), may result in a decrease in peroxynitrite detoxification. Furthermore, since SOD1 may also catalyze peroxynitrite-mediated tyrosine nitration (24), it may be hypothesized that the increased SOD1 activity that was identified in a number of patients may also contribute to the increased NTT detection in these patients.

Explaining the normal or decreased NTT values in two-thirds of the patients, however, is more problematic. A plausible hypothesis may be that according to [F], the increased SOD1 activity may strongly detoxify the organism of superoxide anions, thus decreasing the formation of peroxynitrite to such a level that the NTT may have been normalized or even decreased. If such hypothesis were to be validated, it may further confirm the existence of oxidative

stress in these patients, since this detoxification process would involve increased SOD1 activity. A second hypothesis may be associated with the scavenging of peroxynitrite by GSH since, as reported above, peroxynitrite is able to directly oxidize low molecular weight thiols, including GSH; this hypothesis is in agreement with the decrease in GSH bioavailability that was observed in the patients. Finally, a third hypothesis may involve the inactivating effects of peroxynitrite on certain enzymes, by inducing the nitration of tyrosine and the oxidation of cysteine. In agreement with the present data, this may be the case for GR in a majority of patients, and for GPx and SOD1 in a number of them, and it may be the case for catalase, according to the data obtained by De Luca *et al* (57). Tyrosine nitration may indeed affect the structure and function of selective proteins (66), and consequently must be considered to be a central process of peroxynitrite-mediated toxicity. It is notable that tyrosine nitration and, more particularly, GSH depletion, in association with increased peroxynitrite toxicity, has been proposed to contribute to the occurrence and progression of a number of inflammation-associated diseases, particularly the neurodegenerative diseases Parkinson's disease (67), Alzheimer's disease (68) and amyotrophic lateral sclerosis (69). A key molecular mechanism that may account for the occurrence of these pathological disorders may involve the activation of the proinflammatory transcription factor nuclear factor (NF)-κB by hydrogen peroxide (70) and/or peroxynitrite, possibly through the classical inhibitor of NF-κB kinase-dependent cell-type specific pathway (71). Further research in this field of molecular biology is required in order to elucidate the molecular causal role of oxidative stress in the onset of inflammation and, more particularly, inflammation-associated disease.

Whatever the precise molecular mechanism to be considered, the present data strongly suggest that EHS self-reporting patients, more precisely EMFIS-bearing patients, present with oxidative/nitrosative stress. This has been evidenced by measuring TBARs, MDA, GSSG/GSH and NTT in the plasma, and the inducible enzymes SOD1 in RBCs and GPx in RBCs and plasma. The search for a correlation between these different parameters confirmed the coherence of the present molecular dissection analysis.

A major finding of the present study was that by using a limited number of oxidative stress biomarkers, 70-80% of EHS self-reporting patients were able to be characterized by the existence of oxidative stress. Consequently, as is the case for numerous chronic pathological disorders, including cancer (44-47), diabetes (48), cardiovascular diseases (49,50), neurodegenerative diseases (51), and similar pathological syndromes including CFS (51-55) and MCS (57), the present data strongly suggested that EMFIS may be characterized by some degree of chronic inflammation (3,4) in addition to oxidative stress. This means that EMFIS (as for MCS and CFS) is a novel pathological disorder which merits recognition by the international biomedical community and classification as such by the WHO.

There remains no clear explanation as to the causal origin of oxidative stress in EHS self-reporting patients. The hypothesis that a nocebo effect may have been initially causal in the onset of oxidative stress is unlikely, since this is unable to explain the molecular abnormalities that were evident in the present study (4). The hypothesis that certain environmental stressors may be causally implicated in the onset of this pathology requires further investigation. Since it was previously demonstrated that MCS is frequently associated with EHS in EHS self-reporting patients (3), man-made chemicals may theoretically be these environmental stressors. However, in the present study, all patients with MCS that may have been associated with EHS were excluded, thus EMF exposure, as reported by the patients, may be an environmental stressor. This hypothesis merits consideration since numerous *in vitro* and animal experimental studies have reported that extremely low frequencies (ELF) radiation exposure (72,73), and more importantly radio-frequencies (RF) EMF exposure (74-79), are associated with oxidative stress occurrence, with the resulting biological effects including alterations in differentiation (72,73), inflammatory responses and DNA damage (77,80); all these detrimental effects occur more frequently in the brain (74,76-79).

Finally, it may be concluded that regardless of its causal origin, EMFIS may be biologically characterized as a novel pathological disorder, and thus may be diagnosed in medical practice on the basis of clinical symptoms, and more objectively by measuring: Inflammation-associated biomarkers, including histamine, protein S100B and the cellular stress chaperone proteins Hsp70 and Hsp27 (3); oxidative stress biomarkers, including TBARs, MDA, GSS and NTT in plasma; and anti-oxidative defense biomarkers, including SOD in RBCs, and GSH and GPx in plasma.

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

PI and DB designed the study and developed data collection tools. DB, as principal investigator, led the overall study with respect to data collection, data management, data analysis and interpretation. PI and DC directly provided technical input and guidance for participant selection, data collection, and data analysis and interpretation. DB wrote the manuscript, and PI and DB directly provided critical input to frame and

finalize the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study, part of a therapeutic clinical trial using Fermented Papaya Preparation to treat EHS self-reporting patients (4), was agreed by the European Cancer and Environmental Research Institute (ECERI) scientific/ethical advisory committee, and was conducted according to currently accepted ethical guidelines, including informed written consent approval signed by all patients prior to inclusion. This investigation has been also registered in the European Clinical Trials Database ('EudraCT') under the registration no. 2017-003937-27.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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