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Assessment of glutathione peroxidase-1 polymorphisms, oxidative stress and DNA damage in sensitivity-related illnesses

Agnese Gugliandolo [a,1], Chiara Gangemi [a,1], Carlo Calabrò [a], Mercurio Vecchio [a], Debora Di Mauro [a], Marcella Renis [b], Riccardo Ientile [a], Monica Currò [a], Daniela Caccamo [a,*]

[a] Department of Biomedical and Dental Sciences and Morpho-functional Imaging, Polyclinic University of Messina, Via C. Valeria 1, 98125 Messina, Italy
[b] Department of Drug Sciences, University of Catania, Viale Andrea Doria, 95100 Catania, Italy

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ABSTRACT

Aims: Oxidative stress increase is a key event for development of sensitivity-related illnesses (SRIs). The aim of this work was to evaluate the influence of a genetic variant in the antioxidant enzyme glutathione peroxidase (GPx1) on oxidative stress development in SRIs.
Main methods: GPx1 rs1800668 genotype, as well as glutathione, ubiquinone, and DNA damage were assessed in 34 SRI patients and 36 healthy subjects.
Key findings: Total glutathione, reduced/oxidized glutathione, and ubiquinone were significantly decreased in cases compared with controls, while DNA fragmentation was significantly increased in patients. However, these differences were not associated to GPx1 genetic background.
Significance: GPx1 rs1800668 polymorphism does not play a major role in SRI-related oxidative stress development.

1. Introduction

Sensitivity-related illnesses (SRIs) are heterogeneous diseases caused by the exposure to low doses of environmental pollutants or xenobiotics, such as chemicals, drugs, heavy metals or radiations [1,2]. The most representative diseases of this group are multiple chemical sensitivity (MCS), fibromyalgia (FM) and chronic fatigue syndrome (CFS).

SRIs occur mainly in adults with higher prevalence in women, and usually arise after a single precipitating event caused either by a severe intoxication or by a chronic low dose exposure to environmental pollutants; then, the intolerance becomes chronic [3,4].

SRI symptoms include multi-organ systemic and skin events, chronic muscular fatigue, asthma, as well as neurological, gastrointestinal, cardiac, and autoimmune disorders [5,6].

SRIs diagnosis is based on the Cullen's inclusion anamnestic criteria [7], and results from the modified QEESI (Quick Environmental Exposure and Sensitivity Inventory), a multistep questionnaire that determines the levels of sensitization to chemical triggers as well as the life impact, and scores the type, localization and severity of symptoms after exposure [8]. Indeed, a modified QEESI score of 10 common environmental exposures and 10 major symptoms enables the differential diagnosis of MCS and SMCS (suspected MCS): full diagnosis ($20 \leq \text{Score} \leq 30$) or strongly suspected diagnosis, that is subjects fulfilling diagnostic criteria only partially ($10 \leq \text{Score} \leq 20$), or subjects not fulfilling diagnostic criteria ($0 \leq \text{Score} \leq 10$) [6,8].

Recent advances have highlighted the role of inherited or acquired impairment of xenobiotic metabolism in the individual hypersensitivity to xenobiotics and toxic endogenous metabolites. Alterations of this system may lead to incomplete removal of toxins or/and to excessive generation of toxic by-products, leading to redox state impairment, inflammation and cell damage [6,9,10]. Notably, erythrocyte catalase and glutathione-S-transferase (GST) activities, antioxidant potential as well as reduced glutathione (GSH) content, have been found to be lower in SRI patients than in healthy subjects [6]. On the contrary, in SRI patients glutathione peroxidase activity, as well as plasma levels of nitrites/nitrates, cytokines and chemokines have been reported to be higher than in healthy subjects; moreover, the erythrocyte membrane fatty acid profile is shifted to saturated compartment in SRI patients [6].

Redox and cytokine pattern impairment suggest that the expression/activity of detoxifying and antioxidant enzymes are inhibited in SRIs. Therefore, SRIs biological definition and diagnosis should include the assessment of oxidative stress and inflammatory markers.

Moreover, screening for the presence of GST, cytochrome P450 monooxygenase (CYP), and nitric oxide synthase (NOS)

* Corresponding author at: Polyclinic Hospital University, Via C. Valeria, Torre Biologica, 98125 Messina, Italy.
E-mail address: dcaccamo@unime.it (D. Caccamo).
[1] These two authors equally contributed to this work.

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polymorphisms should be considered; indeed, gene polymorphisms in some detoxification or pro-inflammatory enzymes have been associated with SRIs, and may be useful biomarkers to discriminate between different diseases [11,12].

Decreased antioxidant activity by glutathione peroxidase (GPx) can promote susceptibility to oxidative stress, allowing the accumulation of harmful oxidants; notably, it has been shown that the rs1800668 (C/T) variant within the promoter of GPx1 gene is able to affect enzyme activity [13,14]. The aim of this work was to assess oxidative and genotoxic stress in SRI patients by focusing on the possible association between stress markers and the genetic background at GPx1 locus.

2. Methods

2.1. Patients

Thirty-four Sicilian SRI patients (11 M/23 F; 27.4 ± 5.1 years) were recruited for this study at "Istituto di Ricerca Medica e Ambientale" (IRMA) in Acireale (Catania, Italy). Diagnosis was set based on the medically-assessed results of the modified QEESI score > 21, diagnosed sensitivity to chemicals or heavy metals, and genetic background positive for the presence of one or more CYP and GST variants (CYP2C9*2, CYP2C9*3, CYP2C19*2, CYP2D6*4, CYP2D6*41, GSTM1, GSTT1), that have been shown to be associated with SRI [6,11,15]. In particular, 17.6% were bearing one mutant allele CYP2C19*2, 5.9% were bearing one mutant allele CYP2C9*2. The 14.7% and 11.8% of our patients presented, respectively, one mutant allele *4 and *41 of CYP2D6, but the homozygous genotype *41/*41 was represented in only the 2.9% of the population. Some of the variants were presented in combination, in particular the haplotypes CYP2C9 *1/*3/CYP2D6 *4/*41, CYP2C19 *1/*2/CYP2D6 *1/*41, CYP2C19 *1/*2/CYP2D6 *4/*41, CYP2C9 *1/*3/CYP2D6 *1/*41, CYP2D6 *1/*2A/CYP2C19 *1/*17, CYP2C9*1/*3/CYP2D6 *2/*2A/CYP2C19 *1/*17 were represented, each one, with a frequency of about 3%. All participants were mutated for GSTT1 (44%) or GSTM1 (52%) or both (4%).

Symptoms manifested by our cohort were: muscular weakness and fatigue, confusion and memory loss, minor and major depression, general anxiety, panic disorders and post-traumatic distress, respiratory distress, chronic bronchitis and asthma, ear-nose-throat disturbances, autoimmune disorders, gastrointestinal and genitourinary tract malfunction, migratory joint pains, migraine, dermatitis, odor hypersensitivity. Symptoms appeared after exposure to trace concentrations of common odorous substances, including volatile organic compounds (VOCs), perfumes, fresh paint, cleaning chemicals, print and toners, carpeting, selected drug categories and water or food additives and contaminants.

Fifty Sicilian healthy subjects were initially recruited as controls among staff and students at University of Messina. However, fourteen were excluded due to genetic background positive for the presence of CYP or GST isoform variants. Thus, the control group for this study consisted of 36 subjects (11 M/25 F; 29.6 ± 5.8 years) selected according to the established inclusion criteria of: (i) an absence of any clinically diagnosed disease, (ii) no drug or nutraceutical supplement since at least six weeks, at the time of blood sampling, (iii) known genetic background negative for the presence of any CYP or GST isoform variants.

Among the enrolled participants, 81.4% of patients and 85.2% of controls were non-smokers. None of the subjects made use of drugs or alcohol.

All subjects provided written informed consent to blood sampling and anamnestic data collection. The study protocol was in accordance with the ethical standards of Polyclinic University of Messina, and the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

2.2. Measurement of redox markers

Total and reduced glutathione (GLT, GSH) and ubiquinone (CoQ10) plasma concentrations were assessed by HPLC using Glutathione HPLC kit and Ubiquinone/Coenzyme Q10 HPLC kit (ImmunDiagnostik, Bensheim, Germany), according to manufacturer instructions. Reference values for these methods were: GLT 763–1191 μmol/L, GSH 620–790 μmol/L; GSH/GSSG 81–93%; CoQ10 0.67–0.99 μg/ml. Intra-assay CV were 3.9% for GLT, 3.3% for GSH and 4.4% for CoQ10. Inter-assay CV were 4.2% for GLT, 3.3% for GSH and 5.6% for CoQ10. Low concentration and high concentration control samples, included in the kits, were used as reference material.

Oxidized glutathione (GSSG) content was calculated as the difference between total glutathione and GSH.

2.3. Analysis of DNA damage by Single Cell Electrophoresis (SCGE).

Lymphocyte DNA damage was evaluated by Alkaline Comet assay, after isolation of lymphocytes by HISTOPAQUE (Sigma Aldrich), according to Tomasello et al. [16]. Low melting agarose embedded nucleoids were examined 24 h after staining by DMIRB fluorescence microscope at a 400× magnification (Leica Microsystems Heidelberg, Mannheim, Germany), equipped with a digital camera (Canon Power Shot S50, Milan, Italy).

Samples were run in duplicate, and images of 50 cells per slide for a total of 100 cells per sample were randomly acquired and analyzed using CASP software (Comet assay software project, http://www.casp.sourceforge.net).

The parameters evaluated for each comet were: head length (HL), tail length (TL), comet length (CL), percentage of DNA in head (% H-DNA) and tail (% T-DNA), tail moment (TM), and olive tail moment (OTM).

2.4. Assessment of GPx1 genotype

Genomic DNA was isolated from peripheral blood lymphocytes by Gentra Pure Gene DNA Purification System (Qiagen, Milan, Italy), according to the manufacturer's protocol. Genotyping for GPx1 rs1800668 polymorphism (C>T, assay ID: C_7912052_40) was carried out by Real-time PCR allelic discrimination using a pre-designed TaqMan SNP Genotyping assay available from Applied Biosystems (Applera Italia, Monza, Italy).

Genotyping reactions were set up in a 96-well plate on a 7500HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA), and were carried out in a final volume of 10 μL containing 1× TaqMan Genotyping Master Mix, 1× TaqMan-specific assay, and 10 ng genomic DNA, using thermal cycling conditions suggested by manufacturer's protocols. Sample with GPx1 rs1800668 known genotype, previously assessed by DNA direct sequencing, were also run as controls for genotyping.

2.5. Statistical analysis

Continuous data are expressed as means ± standard deviation (S.D.), and the categorical variables as number and percentage. The unpaired t-test and the Fisher exact test were used to test the difference between cases and controls in terms of continuous and categorical variables, respectively. Compliance of genotype distribution to the Hardy–Weinberg equilibrium was estimated by Fisher's exact test, based on a Web program (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl).

The between-groups variability was analyzed by the factorial ANOVA followed by the Bonferroni post-hoc analysis. A p-value ≤ 0.05 was considered statistically significant for all the analyses. Statistical analyses were performed using GraphPad Prism 4 (San Diego, CA, USA). Post-hoc power of the study was estimated using GPower 3.1.