



Brief report

Serum levels of carbonylated and nitrosylated proteins in mobbing victims with workplace adjustment disorders

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ABSTRACT

Aim: Today the most important problem in the work place is psychological abuse, which may affect the health because of high levels of stress and anxiety.

There is evidence that most psychiatric disorders are associated with increased oxidative stress but nothing is reported about the presence of oxidative stress in mobbing victims.

Methods: This study has been carried out in a group of 19 patients affected by workplace mobbing-due adjustment disorders, in comparison with 38 healthy subjects, to evaluate whether oxidative stress may be induced by mobbing.

Results: Serum levels of protein carbonyl groups and of nitrosylated proteins, biological markers of oxidative stress conditions, were higher than those measured in healthy subjects.

Conclusions: These findings may contribute to a better understanding of the redox homeostasis dysregulation occurring in victims of workplace mobbing.

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1. Introduction

The phenomenon of the physical, moral or psychical violence in the working environment, variously indicated as “mobbing”, “workplace bullying” or “workplace harassment”, is to date object of numerous studies, mostly of epidemiological type, which has yet to clear, in a sufficient way, the aspects of that phenomenon, the possible causes, risk factors, constituent characteristics and consequences. The concept of ‘mobbing’ includes workplace terrorizing, pressure, frightening, belittling and psycho-terror, or abstract violence. In fact, the most important problem in the work place today is psychological abuse, which has become more widespread because of legal regulations, particularly in public facilities, against physical violence.

Individuals who are exposed to psychological abuse experience various economical, psychological and social problems, and, most importantly, health problems (Meseguer de Pedro et al., 2008). In fact, occupational stress can have a direct impact on the immune system, exacerbating various medical conditions, including low bowel syndrome, headaches, and musculoskeletal symptoms. For example, events-induced stresses can very likely influence the

onset of an unbalance of pro-inflammatory and anti-inflammatory cytokines (Maes, 2008), both at systemic and local level, since the immune system and the neuroendocrine system machinery modulate each other and modulate the synthesis of pro-inflammatory cytokines, overproduction of which influence behaviour.

Furthermore several physiological and pathological processes including emotional or psychological stress increase the body concentration of reactive oxygen and nitrogen species (ROS and RNS), so compromising the delicate homeostatic mechanisms which involve neurotransmitters, hormones, oxidizing substances and numerous other mediators and triggering a condition of oxidative stress. Oxidative stress is one of the factors which contributes to an increase in the speed of the cell cycle and consequent premature cell death, leading to various disturbances at the level of the central nervous system (such as degenerative diseases and psychiatric pathologies), as well as at the level of peripheral systems (such as atherogenesis and cardiovascular pathologies). Over the last decade, mobbing has become a frequently reported problem in modern societies with serious effects on employees’ psychological and physical health. For example, Kudielka and Kern (2004) and Rocco et al. (2007) have evidenced stress-related changes of hypothalamus–pituitary–adrenal (HPA) axis in mobbing. In fact, flattened cortisol cycles and a significant inverse correlation between morning plasma

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cortisol levels and psychometric parameters in victims of mobbing have been reported. To the best of our knowledge, there are just few data on psychobiological consequences and concomitants of job stress, especially concerning oxidative stress. Tsuboi et al. (2006), Ishihara et al. (2008) and Fukuda et al. (2008) have evaluated inflammation and oxidative stress-related indices in female nurses, and have demonstrated an association between changes in such indices and job stress levels; furthermore also Casado et al. (2006) have found a positive correlation between blood malondialdehyde and occupational stress in workers of a prehospital emergency service. Maes (2009) has hypothesized that 'functional' symptoms, as occurring in chronic fatigue syndrome and somatization, have a genuine organic cause, that is activation of peripheral and central inflammatory and oxidative and nitrosative stress (IO and NS) pathways and gut-derived inflammation.

Today there is evidence that most psychiatric disorders, including amnestic, nicotine-related, psychotic, mood, anxiety, eating and sleep disorders, and sexual dysfunctions, are associated with increased oxidative stress. However nothing is reported in the scientific literature about the presence of a condition of oxidative stress in subjects undergone mobbing (Maes, 2008; Ng et al., 2008; Selek et al., 2008; Tsaluchidu et al., 2008; Zafir et al., 2009).

Since ROS and RNS may damage all types of biological macromolecules including proteins, that are key molecules playing an ultimate role in various structural and functional aspects of living organisms, we measured the serum concentrations of protein carbonyl groups and of nitrosylated proteins, in patients with workplace mobbing-due adjustment disorders. The most common protein modification provoked by oxygen free-radicals and ROS are carbonyl groups which can arise following oxidative attack on arginine, lysine, threonine and proline residues; RNS provides another source of protein damage because peroxynitrite, formed following the reaction of nitric oxide with the superoxide radical, can modify aromatic amino acids producing nitro-tyrosine, nitro-phenylalanine and nitro-tryptophan (Chevion et al., 2000; Gaston et al., 2003; Tuteja et al., 2004).

2. Materials and methods

2.1. Subjects

The study was performed on 19 patients (9 male and 10 female; mean age 53.2 ± 6.6 years). They had symptoms of somatization, anxiety and mood depression included on adjustment disorders due to repetitive episodes of mobbing in their workplaces.

A group of 38 healthy gender and age-matched subjects was included as control.

Tobacco smokers, alcohol abusers, patients affected by severe acute or chronic pathological conditions, including dyslipidemia, obesity and diabetes, or with history of significant psychiatric illness were excluded from the study; all participants were free of any drug in the last month before the analyses.

Written informed consent according to the declaration of Helsinki was obtained from all subjects enrolled in the study.

The 58-item Profile of Mood States (POMS) was used to evaluate the effect of work-related psychological stress on immune system immediately before the analyses (McNair et al., 1992). The POMS answers are graded with 5-point scale ranging from "not at all" to "extremely". Following standard procedure, mood fluctuations were calculated by summing the POMS raw scores on the tension-anxiety, depression-dejection, anger-hostility, fatigue-inertia, and confusion-bewilderment subscales and subtracting score on the vigor-activity subscale.

A sample of blood was withdrawn from the antecubital vein. Sera were allowed to clot at room temperature for 2 h and separated by centrifugation at $1200 \times g$ for 15 min and stored at -80°C until used.

2.2. Determination of protein carbonyl groups

The serum content of protein carbonyl groups was evaluated according to the Levine's method as previously described (Morabito et al., 2004). Briefly, 100 μl of serum was incubated with 100 μl of a 20-mM 2,4-dinitro-phenylhydrazin solution for 60 min. Then, the proteins were precipitated from the solution with the use of 20% trichloroacetate; the protein pellet was washed three times with ethanol and ethyl acetate and then resuspended in 1 ml of 6 M guanidine at 37°C for 15 min. The carbonyl content was determined from the absorbance at 366 nm (molar

absorption coefficient: $22,000 \text{ M}^{-1} \text{ cm}^{-1}$). The serum concentration of protein carbonyl groups was normalized to the total protein amount determined by the Bradford assay, and results were expressed as nanograms of carbonyl groups for protein mgs. Each sample was analyzed in triplicate.

2.3. Determination of S-nitrosylated proteins

S-nitrosylated proteins in human sera were quantified by the Saville's method (Saville, 1958). In brief, 40 μl of serum was acidified by addition of 200 μl of 0.2N H_2SO_4 . After 2 min, 200 μl of a 0.5% solution of ammonium sulfamate was added and the mixture was allowed to react for 3 min. The mixture was then supplemented with 160 μl of a mixture of 0.25% HgCl_2 and 2.55% sulphaniilamide in 0.4N HCl and then with 160 μl of a solution of 0.38% N-1-naphthylethylenediamine in 0.4N HCl. After 5 min, absorbance at 540 nm was measured. S-nitrosylated proteins were quantified by extrapolation from a standard curve that had been generated with S-nitrosoglutathione. The serum concentrations of S-nitrosylated proteins were normalized to the total protein amount determined by the Bradford assay and results were expressed as nanomoles of S-nitrosylated proteins for protein mgs. Each sample was analyzed in triplicate.

2.4. Statistical analysis

Data were analyzed with a statistical package (SPSS 13.0 for Windows) by using a Mann-Whitney *U* test, and by calculations of Spearman's correlation. A *P* value of 0.05 (two-tailed) was chosen as the significant threshold.

3. Results

The patients exposed to mobbing and included in this study presented serum levels of nitrosylated and carbonylated proteins higher than those measured in healthy subjects (Figs. 1 and 2). In particular, as shown in Fig. 1, there was a significant difference ($P = 0.0025$) between the serum levels of carbonylated proteins in patients affected by mobbing (mean: 1.04, SD: 0.45, range: 0.55–2.27 ng/mg protein) and those measured in healthy subjects (mean: 0.74, SD: 0.35, range: 0.35–1.70 ng/mg protein). Furthermore, serum levels of nitrosylated proteins in patients suffering for mobbing were higher (mean: 30.80, SD: 48.64, range: 5.10–202.20 nmol/mg

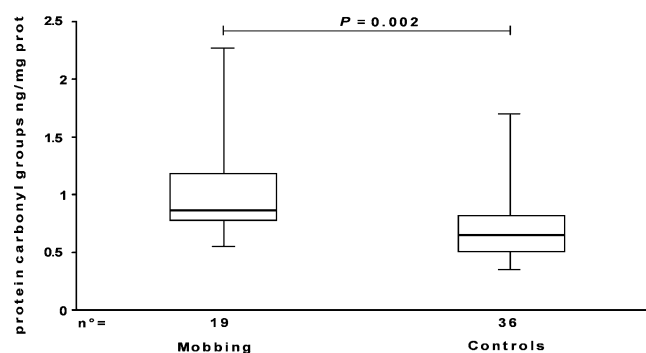


Fig. 1. Box plot illustrates median of carbonylated proteins in patients affected by mobbing and in healthy subjects.

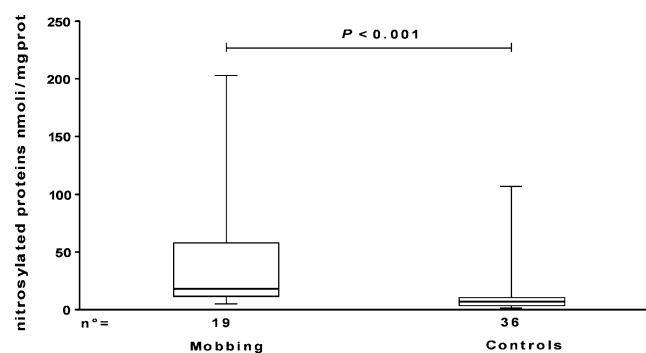


Fig. 2. Box plot illustrates median of nitrosylated proteins in patients affected by mobbing and in healthy subjects.

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