

Short communication

Elevated levels of protein carbonyls in sera of chronic fatigue syndrome patients

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Abstract

Protein carbonyl levels, a measure of protein oxidation, were found to be significantly elevated ($p < 0.0005$) in the sera of chronic fatigue syndrome (CFS) patients vs. controls. In contrast, the total protein levels in sera CFS patients were unchanged from those of controls. The elevated protein carbonyl levels confirm earlier reports suggesting that oxidative stress is associated with chronic fatigue syndrome and are consistent with a prediction of the elevated nitric oxide/peroxynitrite theory of chronic fatigue syndrome and related conditions. (*Mol Cell Biochem* **248**: 93–95, 2003)

Key words: reactive nitrogen species, reactive oxygen species, post-translational modification, serum proteins, oxidative etiology

Introduction

Chronic fatigue syndrome (CFS) is a medical condition characterized by profound, unexplained fatigue of at least 6 months duration accompanied by several other symptoms, such as multiorgan pain, cognitive dysfunction, post-exertional malaise and tender lymph nodes [1]. Among the objectively measurable correlates common in CFS are immune dysfunction, especially low NK cell activity and elevated inflammatory cytokine levels [2–5], orthostatic intolerance [6, 7] and elevated levels of a 37 kD RNase L protein [8]. The elevated nitric oxide/peroxynitrite theory of CFS etiology is a wide ranging theory of CFS etiology which ascribes CFS and certain related conditions to a biochemical vicious cycle mechanism generating chronically elevated levels of these two compounds in certain organs of the body [9–11]. It proposes that CFS etiology is centered on elevated levels of nitric oxide and its oxidant product, peroxynitrite, and that peroxynitrite may act by at least six known mechanisms, to increase the levels of its two precursors, nitric oxide and superoxide, thus producing more peroxynitrite [9]. In this way, a biochemical vicious cycle may be established,

causing the chronic nature of CFS. Infectious episodes, which often precede cases of CFS, initiate this cycle by increasing nitric oxide levels [9]. This theory predicts that measures of oxidative damage will be elevated in CFS but when first proposed, there was little evidence supporting this prediction. Since that time, there have been four studies providing support for elevated oxidative stress in CFS patients [12–15]. Antioxidants have been used in CFS treatment, suggesting that oxidative stress has a role in the generation of CFS symptoms [16, 17]. The current study measures protein carbonyl levels in the sera of CFS patients versus controls, because such protein carbonyl levels have been established by studies of Stadtman, Levine *et al.* to be a measure of protein oxidation [18, 19]. Protein carbonyls are reported to be the ‘most frequently studied marker of oxidative damage to proteins’ [20] and elevated levels of protein carbonyls are reported to occur in aging, rheumatoid arthritis, oxygen-treated premature infants, pre-eclampsia, Alzheimer’s disease, Parkinson’s disease and amyotrophic lateral sclerosis [20, 21]. We report below that protein carbonyl levels are elevated in the sera of CFS patients.

Materials and methods

Sera from CFS patients and controls were obtained from Scott Rigden M.D. (Scottsdale, AZ, USA), Albert G. Corrado M.D. (Richland, WA, USA) and Nancy Gregory ARNP, CDE (Palouse Medical P.S., Pullman, WA, USA). The serum donors also provided information on age, gender and medical status by filling out a questionnaire and were certified by the medical personnel listed above that they met the 1994 CDC criteria for CFS diagnosis [1] (CFS sera). Both CFS and control donors were in all cases, certified by the same medical personnel to have no known autoimmune diseases. All donors of the sera had previously signed informed consent forms, that had been previously approved by the Washington State University IRB which had also approved of the experimental protocol, so that these experiments were in accordance with the relevant ethical standards for human experimentation. Serum samples were stored at -20°C for up to 1 week before being shipped on dry ice to the principal investigator and were subsequently stored at -80°C until they were assayed. The CFS serum donors were 41.9 ± 8.3 years old (mean \pm S.D.), 78% female, whereas the control donors were 45.1 ± 11.1 years old (mean \pm S.D.), 100% female.

Protein carbonyl levels were assayed using the 2,4-dinitrophenylhydrazine protocol of Levine *et al.* [19]. Protein concentrations were measured using the Bradford assay [22], using bovine serum albumin as a standard. All compounds used were of reagent grade.

Results and discussion

Data comparing several properties of the CFS patients versus controls are listed in Table 1. The data show increased levels of proteins carbonyls in the sera of CFS patients. When either the protein carbonyl level per ml of serum or the protein carbonyl level per mg of protein were analyzed using Student's one tailed *t*-test, both of the differences between

Table 1. Protein carbonyl levels in the sera of chronic fatigue syndrome patients and controls

	CFS patients	Controls
Number of serum samples	36	16
Protein carbonyl levels (nmoles/ml of serum)	16.79 ± 2.69	13.88 ± 2.18
Serum protein (mg/ml of serum)	34.11 ± 1.66	34.34 ± 2.98
Protein carbonyl levels (nmoles/mg protein)	0.492 ± 0.0747	0.409 ± 0.800

The data for protein carbonyl levels and serum protein levels are all expressed as the mean \pm S.D. for the 36 or 16 samples.

CFS patients and controls were highly significant ($p < 0.0005$ for both comparisons). These results support the view that an etiologic mechanism characterized by increased levels of one or more oxidants, is involved in the generation of CFS. Specifically it is consistent with prediction of the elevated nitric oxide/peroxynitrite theory of CFS because peroxynitrite reacts to produce protein carbonyls [23], but cannot distinguish that proposed mechanism with other possible etiologic mechanisms that may also involve elevated levels of protein oxidation. Using the same set of sera as in the current study, it was shown that the levels of citrulline, the coproduct of nitric oxide synthases, were elevated in CFS sera versus controls [24], providing support for the view that nitric oxide synthesis is elevated in CFS. The current studies provide further confirmation for earlier results showing that markers of oxidative stress are elevated in CFS [12–15].

It has been reported that the blood volume of CFS patients may be less than that of controls, suggesting an inference that CFS sufferers experience changes similar to those produced by dehydration [25]. Dehydration would be expected to produce an increase in serum protein levels. Accordingly, we used the data on serum protein levels to determine whether this inference is supported by our data. From these samples (Table 1), the serum protein levels of CFS patients was 34.11 ± 1.66 mg/ml compared with 34.34 ± 2.98 , where both are expressed as the mean \pm S.D. There is no significant difference between these values by either a one or two tailed *t*-test. We infer that there is no evidence for dehydration from studies of our CFS and control serum samples.

Antioxidants have been commonly used to treat CFS [16, 17], suggesting that oxidative stress contributes to the generation of symptoms of that condition and hydroxocobalamin, a potent scavenger of nitric oxide, is also often used in such treatment [11]. The current study, then, may be part of a pattern of evidence suggesting a role of oxidants and possibly reactive nitrogen species in CFS etiology.

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