Increased plasma peroxides as a marker of oxidative stress in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS)

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Summary

Background: There is evidence that myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is characterized by activation of immune, inflammatory, oxidative and nitrosative stress (IO&NS) pathways. The present study was carried out in order to examine whether ME/CFS is accompanied by increased levels of plasma peroxides and serum oxidized LDL (oxLDL) antibodies, two biomarkers of oxidative stress.

Material/Methods: Blood was collected from 56 patients with ME/CFS and 37 normal volunteers. Severity of ME/CFS was measured using the Fibromyalgia and Chronic Fatigue Syndrome (FF) Rating Scale.

Results: Plasma peroxide concentrations were significantly higher in patients with ME/CFS than in normal controls. There was a trend towards significantly higher serum oxLDL antibodies in ME/CFS than in controls. Both biomarkers contributed significantly in discriminating between patients with ME/CFS and normal controls. Plasma peroxide and serum oxLDL antibody levels were both significantly related to one of the FF symptoms.

Conclusions: The results show that ME/CFS is characterized by increased oxidative stress.

key words: myalgic encephalomyelitis • chronic fatigue syndrome • CFS • inflammation • oxidative stress • antioxidants as a marker of oxidative stress

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**Background**

There is evidence that myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is accompanied by disorders in inflammatory, oxidative and nitrosative stress (O&NS) pathways. We have discussed elsewhere that an increased production of intracellular inflammatory mediators, like nuclear factor κB (NFκB), and consequently of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) are key factors in ME/CFS [1–4]. The inflammatory response in ME/CFS may explain the various findings on increased levels of pro-inflammatory cytokines ([5]); immune activation, with Th-1-like or Th-2-like responses and an increased expression of activation markers, e.g. CD38 ([6]); ex vivo immunosuppression as exemplified by lowered natural killer cell activity and decreased expression of activation markers, e.g. CD69 ([7,8]); dysregulation of the 2′-5′′ oligoadenylate synthetase / RNase L pathway ([9]); mitochondrial dysfunctions ([10,11]) and even apoptosis pathways ([12,13]).

Inflammatory reactions, like the increased production of NFκB, iNOS and cytokines may instigate the O&NS pathways [3,4]. Increased isoprostane, thiobarbituric acid reactive substances (TBARS), protein carbonyls, and urinary excretion of 8-OH-deoxyguanosine suggest that ME/CFS is accompanied by increased O&NS and that fatty acids, lipids and DNA are damaged by oxidation [14–18]. An increased production of nitric oxide and peroxynitrite may cause damage to proteins by nitration and nitrosylation, as reactive oxygen and nitrogen species (ROS/RNS) attack fatty acids, proteins, and mitochondria and mitochondrial DNA (mtDNA). The latter may cause mutagenic mtDNA lesions and accumulations of these lesions [19]. ME/CFS has been shown to be accompanied by mitochondrial damage [10,11,20,21] and mitochondrial dysfunctions and structural changes [22,23], which is important in that these mitochondrial and mtDNA lesions may cause lowered activity of the mitochondrial respiratory chain, which produces ATP and accounts for 98% of cellular energy [19].

In addition to damaging cells and tissues, increased O&NS can cause an autoimmune response [4,24–27]. During oxidation and nitration, the chemical structures of self-epitopes may be changed to generate new epitopes, or neoepitopes, which are highly immunogenic ([4,24–27]). Thus, oxidation of fatty acid autoantigens or membrane lipids may generate neoepitopes that are no longer hidden from the immune system. Similarly, during nitration of proteins, neoepitopes may be formed which are strongly immunogenic, such as nitrotyrosine (NO-tyrosine) [24]. Following the initial damage, the immune system may mount an IgG or IgM-mediated autoimmune response against these epitopes. There is evidence that ME/CFS is accompanied by an IgM-mediated autoimmune response against membrane fatty acids, like oleic, palmitic and myristic acid; by-products of lipid peroxidation, such as malondialdehyde (MDA) and azelaic acid; and functional lipid structures, such as phosphatidyl inositol (Pi) [25,26]. ME/CFS is also accompanied by a mounted IgM-mediated autoimmune responses against NO-derivates, like nitro-tyrosine, nitro-phenylalanine, nitro-arginine, nitro-tryptophan, nitro-cysteiny1 and NO-albumin [25,27].

The aim of the present study was to examine two biomarkers of O&NS: plasma peroxides and serum oxidized low-density lipoprotein (oxLDL) IgG antibodies. Peroxides are one type of ROS that can be found in peripheral blood that indicates the presence of oxidative stress. Increased oxLDL IgG autoantibodies are a footprint for lipid peroxidation and the consequent immune responses that take place in vivo.

**Material and Methods**

**Subjects**

Ninety-three subjects participated in the present study, 56 ME/CFS patients and 37 normal controls.

All subjects with ME/CFS were outpatients admitted to the Maes Clinics, Antwerp, Belgium. Subjects with a life-time diagnosis of psychiatric disorders, according to the DSM-IVR [28], including depression, bipolar disorder, anxiety disorders, psychotic and organic mental disorders were excluded from this study.

Additional exclusionary criteria for study participation included any subjects who: a) had been treated with anti-psychotic drugs, anticonvulsants or mood stabilizers; b) had medical illnesses, such as inflammatory bowel disorders, diabetes type 1 or type 2, hypertension, and arteriosclerosis; c) abnormal blood tests, e.g. thyroid stimulating hormone (TSH), total protein and positive IgM antibody titers for EBV or CMV; d) had acute infections within two months of the study; e) were treated with statins and beta-blockers; and f) had been taking dietary supplements with antioxidants. Patients and controls gave written informed consent after the study protocol was fully explained. The study was approved by the local ethical committee.

The diagnosis “ME/CFS” was made using the Centres for Disease Control and Prevention (CDC) criteria [29]. The severity of ME/CFS was measured by means of the Fibromyalgia and Chronic Fatigue Syndrome Rating Scale (FF scale) [30]. This scale measures pain, muscular tension, fatigue, concentration difficulties, failing memory, irritability, sadness, sleep disturbances, autonomic disturbances, irritable bowel, headache, and subjective experience of infection. The total sum on this scale was employed as a measure of the severity of illness. The total sum on the FF scale is computed as a measure for severity of illness. All diagnostic assessments in the patients were carried out by physicians. The normal controls were recruited from laboratory personnel or family members of the personnel.

**Methods**

Fasting blood was sampled between 8.30 a.m. and 11.30 a.m. for the assay of peroxides and oxLDL antibodies. Plasma peroxide levels were determined by means of the colorimetric assay Oxystat (Biomedica Medizinprodukte GmbH & Co KG, A-1210 Wien) for the quantitative determination of peroxides in EDTA plasma (Cat No BL-5007). This method is based on the reaction of the biological peroxides with the enzyme peroxidase and a subsequent color reaction using tetra-methyl benzidine (TMB) as substrate. After addition of a stop solution, the developed color is measured photometrically at 450 nm. A calibrator is measured in parallel and used to calculate the concentration of circulating biological peroxides in the sample, in a one point calibration protocol. The detection limit
Table 1. Measurements of age and gender ratio, and plasma peroxide and serum oxidized LDL (oxLDL) antibody levels in patients with myalgic encephalomyelitis / chronic fatigue syndrome (ME/CFS) versus controls.

| Variables          | Controls | ME/CFS | F or χ² | Df     | P     |
|--------------------|----------|--------|---------|--------|-------|
| Age                | 42.5 (11.4)* | 38.2 (14.0)* | 2.5     | 1/91   | 0.10  |
| Female/Male Ratio  | 25/12    | 50/6   | 5.4     | 1      | 0.02  |
| Peroxides (mol/L)  | 349.9 (246.3)* | 550.9 (398.9)* | –       | –      | –     |
| oxLDL (mU/mL)      | 270.3 (297.7)* | 424.9 (378.9)* | –       | –      | –     |

* Results shown as mean (±SD) values; ** Results shown as median values (q25 – q75 values).

Figure 1 shows that the residualized plasma peroxide levels were significantly higher in ME/CFS patients than in normal controls. A factorial ANCOVA with diagnosis and gender as factors and age as covariate showed significantly higher peroxide values in ME/CFS patients than in controls (F=5.48, df=1/83, p=0.02). There were significant gender differences (F=17.9, df=1/83, p=0.0001), and no significant diagnosis X sex interaction (F=0.91, df=1/83, p=0.70) and no significant effects of age (F=0.32, p=0.6). Least Significant Difference (LSD) analysis at p<0.05 showed that the plasma peroxide levels were significantly higher in ME/CFS males and females as compared to their healthy counterparts. The residualized plasma peroxide values showed no significant diagnostic performance for ME/CFS, where the area under the ROC curve was only 62.6%.

Figure 2 shows that there is a trend towards higher oxLDL antibodies in ME/CFS patients than in normal controls. A factorial ANCOVA with diagnosis and gender as factors and age as covariate showed no significant differences in serum oxLDL antibodies between ME/CFS and controls (F=1.8, df=1/83, p=0.2), no significant gender (F=0.1, df=1/83, p=0.70) and age (F=2.5, df=1/83, p=0.09) effects, and no significant interaction between diagnosis and gender (F=1.8, p=1/83, p=0.20). The serum oxLDL antibodies showed no
significant diagnostic performance for ME/CFS, where the area under the ROC curve was only 62.7%.

Using LDA, we found that both the residualized plasma peroxide and oxLDL antibodies were significantly discriminating variables ($\chi^2=9.15, F=4.71, df=2/160, p=0.01$). There was only one FF symptom that correlated with the oxidative stress markers, i.e. headache. We found significant and positive correlations between headache and the residualized peroxide ($r=0.27, p=0.05$) and oxLDL antibody ($r=0.34, p=0.01$) values. There was a weak albeit significant correlation between the residualized plasma peroxide and serum oxLDL antibodies ($r=0.27, p=0.01$).

**DISCUSSION**

The major finding of this study is that patients with ME/CFS have significantly increased peroxide levels compared to normal controls. This indicates increased ROS and oxidative stress in ME/CFS. As such the results corroborate earlier findings that ME/CFS is accompanied by induction of O&NS pathways. These data also extend previous studies that show ME/CFS is accompanied by a lowered antioxidant status. A significantly lowered antioxidant capacity may impair the defenses against ROS/RNS and O&NS [31]. Recently, it was reported that ME/CFS is accompanied by induction of O&NS pathways, e.g. the forced swimming test and administration of LPS to mice. Thus, forced swimming (one 6-minute session a day during 7–15 days) induces ROS and RNS and decreases antioxidant defenses [35–37]. Administration of LPS to mice induces a behavior complex, characterized by an increased immobility period, post swim fatigue and thermal hyperalgesia, which is associated with increased O&NS and reduced antioxidant levels [38].

In the present study we were unable to find significant differences in serum oxLDL antibodies between ME/CFS patients and normal controls. At first sight, these findings do not corroborate previous findings on increased IgM-mediated autoimmune responses against neoepitopes formed by oxidative damage to fatty acids [25]. First, the oxLDL antibodies measured here are of the IgG subtype, whereas the reactions against neoepitopes of Pi, and oleic, palmitic and myristic acid are IgM mediated [25]. Secondly, the latter are typical membrane fatty acids/lipids which reside on the inner and outer membrane layers, whereas the oxLDL antibodies are directed against LDL particles in the cardiovascular system. Interestingly, we found a weak albeit significant correlation between plasma peroxide concentrations and serum oxLDL antibodies. This may be explained since lipid peroxidation (as indicated by increased oxLDL antibodies) is induced by increased ROS (as indicated by increased plasma peroxide levels).

We found that only one of the 12 FF symptoms was significantly correlated with the oxidative markers, i.e. headache. Previous research on the other hand observed significant correlations between different key symptoms of ME/CFS and oxidative markers or antioxidant levels. For example, aches and pain, muscular tension and fatigue were significantly correlated to decreased antioxidant defenses [14], and increased serum IgM levels directed against lipid peroxidation was reported to be associated with increased O&NS [25]. Jammes et al. [16] reported that O&NS is a causal factor in fatigue, pain and muscle tension. These authors found that the response to incremental exercise in patients with ME/CFS associates increased O&NS with marked alterations of muscle membrane excitability.

It is interesting to note that there is a strong co-occurrence of ME/CFS and depression and that shared disorders in O&NS pathways may underpin this co-occurrence ([39]). Thus, lower levels of both zinc and coenzyme Q10, and oxidative damage to fatty acids, proteins and DNA are hallmarks of both ME/CFS and depression ([39]). Recently we found significantly increased peroxide levels and oxLDL antibody levels in patients with ME/CFS.
levels in depression (140]). This indicates that while there are similarities in O&NS markers between ME/CFS and depression, oxLDL antibodies are more strongly associated with depression that with ME/CFS.

Conclusions

In conclusion, the results of the present study show that plasma peroxide concentrations are significantly increased in ME/CFS, whereas serum oxLDL antibodies showed a trend toward increased levels in ME/CFS. These findings further underscore that O&NS pathways are involved in the pathophysiology of ME/CFS.

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