Oxidative Protein Damage Is Associated With Elevated Serum Interleukin-6 Levels Among Older Moderately to Severely Disabled Women Living in the Community

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Background. Elevated interleukin (IL)-6 is associated with adverse outcomes. Our objective was to determine whether serum protein carbonyls, an indicator of oxidative protein damage and oxidative stress, were associated with IL-6.

Methods. Serum protein carbonyls and IL-6 were measured in 739 women, age ≥65 years, in the Women’s Health and Aging Study I.

Results. Geometric mean of protein carbonyls was 0.082 nmol/mg. After adjusting for age and smoking status, loge serum protein carbonyls were associated with log e IL-6 (β = 0.143, standard error [SE] = 0.048, p = .003) in linear regression analyses and with elevated IL-6 (≥2.5 pg/mL) (odds ratio = 1.38, 95% confidence interval, 1.02–1.86, p = .037) in logistic regression analyses.

Conclusion. Oxidative damage to proteins is independently associated with serum IL-6 among older women living in the community. Increased oxidative stress may be a factor involved in the pathogenesis of the proinflammatory state that occurs in older adults.

Key Words: Inflammation—Interleukin-6—Oxidative stress—Protein carbonyls.

A low-grade proinflammatory state characterized by elevated inflammatory mediators is common among older adults (1), and is associated with pathological processes in multiple systems such as endothelial dysfunction (2), metabolic syndrome (3), atherosclerosis (4), and decline of muscle strength and mass (5–7) that are characteristic of declining health in older individuals. Among older adults, elevated interleukin (IL)-6 levels have been associated with disability (8), decline in physical function (5,9), cognitive decline (10), frailty (11), and increased mortality (12). The underlying triggers for the age-related proinflammatory state have not been well characterized. Reactive oxygen species are ubiquitous reactive derivatives of O2 metabolism that are found in all biological systems, and they are formed as intermediates in reduction-oxidation (redox) processes that lead from oxygen to water. Reactive oxygen species have often been considered as damaging molecules, but there is considerable evidence that they participate in cell signaling. Reactive oxygen species play a critical role in the activation of nuclear factor kappaB (NF-κB) (13,14) and activator protein-1 (AP-1) (15), redox-sensitive transcription factors that are involved in the upregulation of cytokines such as IL-6 (11). Thus, reactive oxygen species may affect health in old age by two parallel mechanisms: directly by damaging biomolecules and indirectly by upregulating the production of proinflammatory cytokines.

Protein oxidation is the covalent modification of a protein that is induced directly by reactive oxygen species (16), by products of lipid and free amino acid oxidation (17), or by reactive nitrogen species (18,19). Oxidation preferentially affects certain protein side chains, especially Pro, Arg, Lys, and Thr residues, and when oxidized, these produce carbonyl groups (aldehydes, ketones) (20). Protein carbonyls are the most studied marker of protein oxidation (18–20). In model systems, induced oxidative stress increases protein carbonyls in serum and tissues (16,21). Although protein carbonyls are an important general marker for oxidative protein damage and have been widely applied in epidemiologic studies, the relationship between protein carbonyls and the age-related proinflammatory state has not been well described in humans. We hypothesized that elevated serum protein carbonyls are associated with elevated serum IL-6 in older adults. To examine this hypothesis, we characterized serum protein carbonyls and serum IL-6 among older, moderately to severely disabled women living in the community.

METHODS

Participants in this study were women, aged 65 years or older, who also participated in the Women’s Health and Aging Study I (WHAS I), a population-based study designed to evaluate the causes and course of physical disability in
the one-third most disabled older women living in the community. WHAS I participants were recruited from an age-stratified random sample of women aged 65 years or older selected from Medicare enrollees residing in 12 contiguous ZIP code areas in Baltimore (22). Women were screened to identify self-reported physical disability that was categorized into four domains. The domains of disability were ascertained in a 20- to 30-minute home interview that included questions related to (i) mobility and exercise tolerance, that is, walking for a quarter of a mile, walking up 10 steps without resting, getting in and out of bed or chairs; (ii) upper extremity function, that is, raising the arms up over the head, using fingers to grasp or handle, lifting or carrying something as heavy as 10 pounds; (iii) higher functioning tasks (a subset of instrumental activities of daily living, not including heavy housework, that is, using the telephone, doing light housework, preparing meals for self, shopping for personal items); and (iv) basic self-care tasks (a subset of non-mobility-dependent activities of daily living, that is, bathing or showering, dressing, eating, using the toilet). WHAS I enrolled women with disability in two or more domains. Of the 1409 women who met study eligibility criteria, 1002 agreed to participate in the study in 1992. There were no major differences in socioeconomic or reported health characteristics between eligible participants and those who declined to participate (22).

Standardized questionnaires were administered in the participant’s home by trained interviewers. Mini-Mental Status Examination (MMSE) was recorded (22). Medication use was recorded (22). Race was assessed in a questionnaire as black, white, or other; current smoking status as yes or no; and education as 0–8, 9–11, 12 years, or >12 years as the highest level of formal education achieved. Two weeks later, a trained registered full-time study nurse conducted an examination of each study participant in her home, using a standardized protocol that included physical performance measures and a standardized physical examination. Approximately 75% of women also consented to phlebotomy performed during a separate visit by a trained phlebotomist who followed a standardized protocol. Further details on the methods and sampling design of the WHAS studies are published elsewhere (22).

There were 1002 women enrolled in the WHAS I, 739 of whom participated in the blood drawing and had serum protein carbonyl and IL-6 measurements at baseline. There were no significant differences in race or body mass index between those who did and did not participate in the blood drawing, but women who did and did not participate in the blood drawing were different by age (77.4 vs 80.7 years, respectively; \( p < .0001 \)). Nonfasting blood samples were obtained by venipuncture between 9 AM and 2 PM. Processing, aliquoting, and freezing were carried out at the Core Genetics Laboratory of The Johns Hopkins University School of Medicine following a standardized protocol. Blood samples were delivered to Quest Diagnostics Laboratories (Teterboro, NJ) and in part stored continuously at \(-70^\circ C\) until the time of analyses for serum protein carbonyls and IL-6. Serum protein carbonyls were measured using a commercial enzyme-linked immunosorbent assay (ELISA) (Zentech PC Test, Protein Carbonyl Enzyme Immuno-Assay Kit; Zenith Technologies, Dunedin, New Zealand). Protein carbonyls are stable under long-term storage at \(-70^\circ C\) (23). The assay has a minimum detectability of 0.02 nmol/mg protein, which is well below that range found in healthy human controls. Intra-assay and inter-assay coefficients of variation (CVs) for protein carbonyl measurements were 10.1% and 18.2%, respectively. Serum IL-6 was measured using a commercial ELISA (Quantikine Human IL-6; R & D Systems, Minneapolis, MN). The minimum detection limit for the IL-6 ELISA reported by the manufacturer is 0.039 pg/mL. Intra-assay and inter-assay CVs for IL-6 measurements were 4% and 6%, respectively.

Descriptive statistics were used to characterize the study population and to describe biochemical measurements of serum protein carbonyls and IL-6. Both serum protein carbonyls and IL-6 were transformed by \( \log_e \) to achieve a more normal distribution. Body mass index was categorized as underweight (<18.5 kg/m²), normal range (18.5–24.9 kg/m²), overweight (≥25–29.9 kg/m²), or obese (≥30 kg/m²), according to World Health Organization criteria (24). Age and serum protein carbonyls were compared between groups using the Wilcoxon two-sample test. Linear regression analysis was used to examine the relationship between serum protein carbonyls and other factors and serum IL-6 as a continuous outcome variable. Logistic regression analysis was used to examine the relationship between serum protein carbonyls and other factors and elevated serum IL-6. Elevated IL-6 was defined in two ways: using either (i) an absolute, data-independent cutoff of 2.5 pg/mL or (ii) a data-dependent cutoff of the highest quartile of IL-6. We used a cutoff for an elevated serum IL-6 of ≥2.5 pg/mL because this level has prognostic significance and has been associated with a greatly increased risk of mobility disability among older adults (5).

**RESULTS**

Overall, among 739 women, the geometric mean level of serum protein carbonyls was 0.082 nmol/mg. The relationships between demographic factors, morbidity, serum protein carbonyls, and elevated serum IL-6 are shown in Table 1. Serum protein carbonyls were significantly different between women with and without elevated serum IL-6. The proportion of women who were current smokers or had congestive heart failure, peripheral artery disease, or diabetes mellitus was significantly higher among those with elevated serum IL-6 than among those without elevated serum IL-6. There were no significant differences between groups by age, race, education, body mass index, MMSE score, use of nonsteroidal antiinflammatory drugs, hypertension, coronary heart disease, stroke, depression, or cancer. The data were also examined for interactions between race and protein carbonyls and body mass index and protein carbonyls, and no significant interactions were found.

Linear regression models were used to examine the relationship between serum protein carbonyls and serum IL-6 as a continuous outcome measure (Table 2). In a multivariate model adjusting for age (model 2), \( \log_e \) protein carbonyls were associated with \( \log_e \) IL-6 (\( \beta = 0.145, SE = 0.048, p = .0004 \)), and when adjusted for age and smoking status (model 3), \( \log_e \) protein carbonyls were associated with \( \log_e \)
Oxidative protein damage and IL-6

Table 1. Characteristics of Women in the Women’s Health and Aging Study I at Baseline With Serum IL-6 Levels Above and Below 2.5 pg/mL (N = 739)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>&lt;2.5 pg/mL (N = 394)</th>
<th>≥2.5 pg/mL (N = 345)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>76.0 (71.0–85.0)</td>
<td>76.0 (71.0–86.0)</td>
<td>.85</td>
</tr>
<tr>
<td>White race, %</td>
<td>74.6</td>
<td>69.4</td>
<td>.26</td>
</tr>
<tr>
<td>Education ≤12 y, %</td>
<td>80.2</td>
<td>85.1</td>
<td>.08</td>
</tr>
<tr>
<td>Current smoker, %</td>
<td>7.1</td>
<td>17.4</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>&lt;18.5</td>
<td>3.3</td>
<td>.87</td>
</tr>
<tr>
<td></td>
<td>18.5–24.9</td>
<td>25.2</td>
<td>.36</td>
</tr>
<tr>
<td></td>
<td>25.0–29.9</td>
<td>36.8</td>
<td>.46</td>
</tr>
<tr>
<td></td>
<td>≥30</td>
<td>34.6</td>
<td>.76</td>
</tr>
<tr>
<td>Mini-Mental Status</td>
<td>Examination score &lt; 24, %</td>
<td>15.5</td>
<td>.25</td>
</tr>
<tr>
<td>Use of nonsteroidal antiinflammatory drugs (NSAIDs), %</td>
<td>29.9</td>
<td>.45</td>
<td></td>
</tr>
<tr>
<td>Protein carbonyls, nmol/mg</td>
<td>0.081 (0.059–0.109)</td>
<td>0.087 (0.062–0.116)</td>
<td>.037</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>58.3</td>
<td>.93</td>
<td></td>
</tr>
<tr>
<td>Coronary heart disease, %</td>
<td>21.6</td>
<td>.12</td>
<td></td>
</tr>
<tr>
<td>Congestive heart failure, %</td>
<td>8.1</td>
<td>.015</td>
<td></td>
</tr>
<tr>
<td>Peripheral artery disease, %</td>
<td>16.5</td>
<td>.0005</td>
<td></td>
</tr>
<tr>
<td>Stroke, %</td>
<td>4.6</td>
<td>.12</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>13.7</td>
<td>.13</td>
<td></td>
</tr>
<tr>
<td>Chronic obstructive pulmonary disease, %</td>
<td>25.4</td>
<td>.11</td>
<td></td>
</tr>
<tr>
<td>Depression, %</td>
<td>16.0</td>
<td>.26</td>
<td></td>
</tr>
<tr>
<td>Cancer, %</td>
<td>10.9</td>
<td>.67</td>
<td></td>
</tr>
</tbody>
</table>

Note: *Median (25th, 75th percentile) for continuous variables or percentages as noted.

Table 2. Multiple Linear Regression Models of Protein Carbonyls and Other Risk Factors With loge IL-6 (pg/L) as the Outcome, Among Women Aged ≥65 Years in the Women’s Health and Aging Study I (N = 739)

<table>
<thead>
<tr>
<th>Variable</th>
<th>β</th>
<th>SE</th>
<th>Standardized β</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>Loge protein carbonyls</td>
<td>0.146</td>
<td>0.049</td>
<td>0.109</td>
</tr>
<tr>
<td>Model 2</td>
<td>Loge protein carbonyls</td>
<td>0.145</td>
<td>0.048</td>
<td>0.108</td>
</tr>
<tr>
<td></td>
<td>Age, y</td>
<td>0.004</td>
<td>0.003</td>
<td>0.050</td>
</tr>
<tr>
<td>Model 3</td>
<td>Loge protein carbonyls</td>
<td>0.143</td>
<td>0.048</td>
<td>0.107</td>
</tr>
<tr>
<td></td>
<td>Age, y</td>
<td>0.006</td>
<td>0.003</td>
<td>0.075</td>
</tr>
<tr>
<td></td>
<td>Current smoking</td>
<td>0.309</td>
<td>0.075</td>
<td>0.151</td>
</tr>
</tbody>
</table>

Note: IL = interleukin; SE = standard error.

IL-6 (β = 0.143, SE = 0.048, p = .003). We did not adjust for morbidities that were significantly associated with elevated IL-6, that is, congestive heart failure, peripheral artery disease, or diabetes mellitus, because oxidative stress and elevation of IL-6 occur quickly and in close association. However, when an alternative model was adjusted for these three morbidities, age, and smoking, log protein carbonyls was still associated with elevated IL-6 (OR = 1.38, 95% CI, 0.96–1.77; p = .09). We also considered alternative models in which high IL-6 was defined as serum IL-6 in the highest quartile (quartile cutoff of >3.71 pg/mL), because nearly half the women in this study had IL-6 ≥2.5 pg/mL (Table 3). In a multivariate model adjusting for age (model 2), loge protein carbonyls were associated with elevated IL-6 (odds ratio [OR] = 1.38, 95% confidence interval [CI], 1.03–1.87), and when adjusted for age and smoking status (model 3), loge protein carbonyls were associated with elevated IL-6 (OR = 1.38, 95% CI, 1.02–1.86). We did not adjust for morbidities that were significantly associated with elevated IL-6, that is, congestive heart failure, peripheral artery disease, or diabetes mellitus, because of the same reasons noted above for the linear regression models. However, when an alternative model was adjusted for these three morbidities, age, and smoking, log protein carbonyls was still associated with elevated IL-6 (β = 0.114, SE = 0.047, p = .017).

Logistic regression models were used to examine the relationship between serum protein carbonyls and elevated serum IL-6 (≥2.5 pg/mL) (Table 3). In a multivariate model adjusting for age (model 2), loge protein carbonyls were associated with elevated IL-6 (OR = 1.38, 95% CI, 0.96–1.77; p = .09). We also considered alternative models in which high IL-6 was defined as serum IL-6 in the highest quartile (quartile cutoff of >3.71 pg/mL), because nearly half the women in this study had IL-6 ≥2.5 pg/mL (Table 3). In a multivariate model adjusting for age, log protein carbonyls were associated with the highest quartile of IL-6, and when adjusted...
for age and smoking status (model 3), loge protein carbonyls were associated with elevated IL-6. When congestive heart failure, peripheral artery disease, and diabetes mellitus were adjusted for in the model, loge protein carbonyls were associated with elevated IL-6 (OR = 1.78, 95% CI, 1.24–2.53; p = .0016).

Additional analyses were conducted in which protein carbonyls were divided into quartiles. In linear regression models, adjusting for age and current smoking, the β values increased with increasing quartile of protein carbonyls, and the highest quartile of protein carbonyls was associated with IL-6 (β = 0.168, SE = 0.067; p = .013).

**DISCUSSION**

This study suggests that elevated serum protein carbonyls, an indicator of oxidative protein damage and oxidative stress, are independently associated with elevated serum IL-6 among older, moderately to severely disabled women living in the community. To our knowledge, this is the first report of an association between serum protein carbonyls and serum IL-6 in older adults. The results are consistent with the presumed underlying biological mechanism in which the redox-sensitive transcription factor NF-kB is involved in the upregulation of IL-6 under conditions of increased oxidative stress. Oxidative stress, as indicated by oxidative damage to proteins, may be a key triggering factor in the low-grade proinflammatory state that is found in older adults. In addition to upregulating proinflammatory cytokines, the oxidative damage that reactive oxygen species impart on proteins can lead to loss of structural integrity and cell function (25,26) and to increased susceptibility to proteolysis (27,28). Although oxidative stress has been shown to upregulate IL-6 in various in vitro and animal models, it is not possible to draw definitive conclusions about the direction of the association in this cross-sectional study, and it may be possible that elevated IL-6 increases oxidative stress.

In humans, elevated serum protein carbonyls have been described in older compared with younger adults (19,29,30). Elevated protein carbonyls have been described in inflammation-related conditions such as Alzheimer’s disease (31), atherosclerosis (32), chronic renal disease (33), diabetes mellitus (34,35), and peripheral artery disease (36). We have recently shown that elevated protein carbonyls are associated with poor grip strength (37). Elevated serum IL-6 levels have also been described in Alzheimer’s disease (38), atherosclerosis (39), chronic renal disease (40), diabetes mellitus (41), and cardiovascular disease (42). However, previous studies have not characterized the relationship between serum protein carbonyls and serum IL-6 in these studies.

The present study was conducted among older, moderately to severely disabled women living in the community, and it is not clear whether serum protein carbonyls and serum IL-6 will be significantly associated in younger populations, among less disabled older women, and among men. Further studies are needed to expand these investigations to other populations. In addition, oxidative protein damage was used as the only indicator of oxidative stress in the present study. A single marker of oxidative damage and a single marker of oxidative damage cannot provide exhaustive conclusions about relationships existing between two very complex and strongly connected mechanisms. Indicators of oxidative damage to lipids and oxidative damage to DNA could complement these investigations, and currently, such laboratory studies are in progress.

**Summary**

Serum protein carbonyls were independently associated with serum IL-6 levels, a finding which is consistent with the presumed biological mechanism by which redox-sensitive transcription factors upregulate IL-6. This finding may represent an important first step toward characterizing the relationship between oxidative stress and the low-grade proinflammatory state that is common in older adults.

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