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Total Antioxidant Capacity and C-Reactive Protein Levels in Patients with Community-Acquired Pneumonia

Aims: The aim of this study was to evaluate the oxidative stress measured by serum total antioxidant capacity (TAC) and malondialdehyde (MDA) in patients with community-acquired pneumonia (CAP), and to evaluate their possible correlation with the serum C-reactive protein (CRP) and pneumonia severity index (PSI).

Materials and Methods: The PSI, chest X-ray (CXR) scores, and the serum TAC, MDA, and CRP levels were determined in 67 CAP patients on admission and compared to 45 healthy controls.

Results: In the whole study population, the TAC level was inversely correlated with CRP levels and WBC counts (r=-0.648, P=0.0001; r=-0.626, P=0.0001, respectively). Lower TAC and higher MDA levels were found in CAP patients compared with those of controls (P = 0.0001, P = 0.029). Although the mean serum MDA and TAC levels were similar between the groups of PSI class I-III (n=45) and PSI class IV-V (n=22), the radiological scores (2.36±1.23 vs 3.19±1.17) and CRP levels (138.67 ± 63.86 vs 177.14 ± 56.43) were significantly higher in the latter group (P = 0.010 and P=0.005, respectively).

Conclusions: Single measurement of serum MDA or TAC levels in CAP patients, in contrast to CRP level measurement, does not seem to predict the severity of disease.

Key Words: Community-acquired pneumonia, C-reactive protein, malondialdehyde, oxidative stress, pneumonia severity index, total antioxidant capacity

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Introduction

The imbalance between oxidants and antioxidants is referred to as oxidative stress and has been associated with various respiratory disorders. Increased oxidative stress participates in the pathogenesis of both airways and parenchymal lung diseases. Asthma, chronic obstructive pulmonary disease (COPD) and bronchiectasis have been associated with inflammation and increased levels of oxidative stress (1-4). Oxidative stress markers including hydrogen peroxide (H₂O₂), 8-isoprostanate or malondialdehyde (MDA) have been determined in various biological samples, as in blood, sputum, bronchoalveolar lavage (BAL) fluid, and exhaled breath condensate (EBC) collected from...
patients with lung diseases (1,5-8). During bacterial pneumonia, rapid and massive influx of activated phagocytes into the distal airways is observed (9). These phagocytic cells release excess reactive oxygen species (ROS) when they encounter bacteria, as part of the host defense against infection. ROS are also produced by bacteria during aerobic respiration. Enhanced ROS production may induce peroxidative lipid damage in which MDA is one of the most important products of the so-called thiobarbituric acid reactive substances (TBARS). This damage may further increase during the phagocytosis if the ROS are not adequately scavenged by blood antioxidants and antioxidant enzymes.

Community-acquired pneumonia (CAP) is the most common infection-related cause of death (10,11). Prognostic scores for CAP have been developed to assess pneumonia severity to make a clinical judgement and to guide decisions about treatment settings (12-14). Severity-based approach for the management of diagnosis and treatment of adult patients with CAP is recommended by most of the guidelines, including the American Thoracic Society (ATS), British Thoracic Society (BTS), and Turkish Thoracic Society (TTS) pneumonia guidelines (15-17). Several inflammatory biomarkers, including C-reactive protein (CRP), can be used for diagnosing or predicting the disease severity in patients with pneumonia (18).

There are a few clinical studies in which oxidative stress was evaluated in pneumonia patients (9,19-24). However, the relationships between oxidative stress and serum CRP or disease severity have not been extensively studied. Therefore, the aim of this study was to determine the level of oxidative stress assessed by serum total antioxidant capacity (TAC) and MDA, and to evaluate the relationship between oxidative stress and serum CRP as well as disease severity in patients with CAP.

Materials and Methods

Study Subjects

The study was conducted at Suleyman Demirel University Research and Practice Hospital, a tertiary-care teaching hospital, between May 2005 and December 2006. Consecutive adult CAP patients were enrolled in the study. Diagnosis of CAP was based on the presence of a new infiltrate on the chest radiographs and clinical findings. Clinical diagnosis of CAP was supported by at least two of the following signs and symptoms: cough, pleuritic chest pain, dyspnea, rales and/or pulmonary consolidation on physical examination, fever (axillary temperature >38°C), or leukocytosis (white blood cell [WBC] count >10 × 10^9/L), and no alternative diagnosis during follow-up (19). Sputum gram stain, sputum culture, blood and/or pleural fluid culture were performed to achieve an etiological diagnosis. Exclusion criteria from the study were: 1) immunosuppression or immunosuppressive therapy (defined as daily doses of ≥20 mg prednisolone equivalent for >2 weeks); 2) neutropenia (less than 1,000 neutrophils/mm^3); 3) prior antimicrobial treatment before the hospital admission; or 4) prior usage of medications with known antioxidative properties during the last month prior to admission. Diabetic patients with blood glucose levels >150 mg/dl were also not included in the study, in order to avoid falsely increased TAC values due to autoxidation of glucose.

Evaluation of Disease Severity

Patients underwent a posteroanterior and lateral chest X-ray (CXR) on the day of admission. Unilateral or bilateral consolidation, lobar consolidation and/or the presence of pleural effusion were recorded. The CXRs were evaluated by one of the authors (AB) and pneumonic changes were graded on a scale from 0 to 10 points as suggested by Majewska et al. (21). Briefly, the number of pulmonary fields involved by inflammatory infiltrate (from 0 to 6 points); the type of infiltration [nonhomogeneous and mostly hazy (1 point) or dense and coalesce (2 points)]; and the presence of pleural effusion [unilateral (1 point) or bilateral (2 points)] were evaluated.

Data about age, sex, smoking habits and comorbid diseases (COPD, cardiac, liver, renal diseases, central nervous system or digestive disorders and neoplasm) were collected. Severity indicators of pneumonia such as mental confusion, systolic and diastolic blood pressure, respiratory rate, creatinine, blood urea nitrogen, sodium, blood glucose, and arterial oxygen tension (PaO_2) were recorded. A total severity score was calculated for each patient according to pneumonia severity index (PSI) proposed by Fine et al. (12) within 24 hours of admission. Diagnostic work-up was performed according to the declaration of Helsinki ethical principles for medical research involving human subjects, and written informed consent was obtained from all study subjects.
Measurements

Blood samples were obtained from all patients for WBC counts, CRP level and other routine biochemical tests on the day of admission. The WBC levels were measured by COULTER® STKS™ Hematology Flow Cytometer and CRP concentration was measured using nephelometric method with a commercially available kit (Dade Behring, Marburg, Germany). For arterial blood gas analyses, blood was drawn from the radial artery while the patients were breathing room air. Arterial oxygen tension was analyzed with a blood gas analyzer (Roche OMNIFÜC C, Roche Diagnostics, Germany). Blood samples for TAC and MDA were obtained under fasting condition 12 h before sample collection. These samples were centrifuged at 1500 × g for 10 min and stored at -70°C until analyses. TAC was determined with an automated measurement method described by Erel (24). MDA was estimated by the measurement of TBARS. TBARS were measured in plasma with the method described by Draper and Hadley (25). Blood samples were also collected from 45 nonsmoker healthy subjects to establish the reference intervals of serum TAC and MDA.

Statistical Analysis

Values are presented as mean ± SD. Statistical analysis was performed with the SPSS 11.0 for Windows programs. Correlation analyses were performed by using Spearman rank correlation. Frequency comparison was done by chi-square test. For multigroup comparisons, one-way analysis of variance (ANOVA) with least square difference for posthoc comparison was applied. For data not normally distributed, the Mann-Whitney U test was used if only two groups were compared and the Kruskal-Wallis one-way ANOVA was used if more than two groups were being compared. A P value of less than 0.05 was considered to be significant for all tests.

Results

Sixty-seven adult CAP patients (84% male, age range: 19-82 years) and 45 nonsmoker healthy control subjects (87% male, age range: 20-72 years) were included in the study. The age and gender distribution were similar in these two groups. Baseline clinical features of the study population are presented in Table 1. In the whole study population, serum TAC concentration was inversely correlated with CRP levels and WBC counts (r=-0.648, P=0.0001; r=-0.626, P=0.0001, respectively); however, no such correlation was observed for the serum MDA concentrations. The mean TAC level was significantly lower and the mean MDA level significantly higher in CAP patients compared with levels in control subjects (P=0.0001 and P=0.029, respectively), (Table 1).

In CAP patients, serum TAC levels were correlated with smoking history (pack-years), fever, and erythrocyte sedimentation rate (r=0.254, P=0.039; r=0.249, P=0.042; r=0.252, P=0.05, respectively). Thirty-six patients (54%) were current smokers and serum mean TAC level was significantly higher in this group than in nonsmokers (2.056 ± 0.686 vs 1.725 ± 0.621, P=0.043, respectively).

At least one comorbid disease was detected in 34% (n=23) of patients, as follows: diabetes mellitus: 11 (but not hyperglycemic more than 150 mg/dl), COPD: 5, congestive heart failure: 4, cerebrovascular disease: 3, and others: 3 (esophageal dysmotility: 1, mental retardation: 1, chronic liver disease: 1). The CAP patients with comorbid disease were older and had higher PSI scores than patients without comorbid disease (P=0.0001 and P=0.0001, respectively), but serum MDA and TAC concentrations were not significantly different in these two groups (Table 2).

Pleural effusion (PE) was detected in 18 (27%) CAP patients on admission. Although not statistically significant, these patients were older and had higher PSI

| Table 1. Some clinical and laboratory variables in CAP patients in comparison with control subjects. |
|-------------------------------------------------------|------------------|------------------|
| Healthy controls | n = 45 | CAP patients | n = 67 |
| Gender F/M | 6/39 | 11/56 |
| Mean age, years | 41 ± 22 | 40 ± 23 |
| Smokers | 0 | 36 |
| Nonsmokers | 45 | 31 |
| WBC, 10^9/L | 7.8 ± 1.0 | 17.3 ± 8.9 |
| CRP, mg/L | 2.3 ± 0.9 | 151.0 ± 63.3 |
| MDA, mmol/L | 2.875 ± 0.552 | 3.195 ± 0.861 |
| TAC, mmol Trolox equivalent/L | 3.139 ± 0.329 | 1.903 ± 0.673 |
| PSI | 66.4 ± 45.4 |

CAP: community-acquired pneumonia; WBC: white blood cell count; CRP: C-reactive protein; MDA: malondialdehyde; TAC: total antioxidant capacity; PSI: pneumonia severity index.
Table 2. The evaluation of CAP patients according to presence of predisposing factors or pleural effusion.

<table>
<thead>
<tr>
<th></th>
<th>Pleural Effusion</th>
<th>Comorbid Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(-)</td>
<td>(+)</td>
</tr>
<tr>
<td>n</td>
<td>49</td>
<td>18</td>
</tr>
<tr>
<td>Gender F/M</td>
<td>8/41</td>
<td>3/15</td>
</tr>
<tr>
<td>Mean age, years</td>
<td>39 ± 23</td>
<td>45 ± 24</td>
</tr>
<tr>
<td>Smokers</td>
<td>28</td>
<td>8</td>
</tr>
<tr>
<td>Nonsmokers</td>
<td>21</td>
<td>10</td>
</tr>
<tr>
<td>WBC, n x 10^9/L</td>
<td>17.9 ± 8.3</td>
<td>15.7 ± 10.7</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>151.7 ± 64.0</td>
<td>149.3 ± 63.1</td>
</tr>
<tr>
<td>MDA, nmoL</td>
<td>3.333 ± 0.874</td>
<td>2.821 ± 0.719</td>
</tr>
<tr>
<td>TAC, mmol Trolox equivalent/L</td>
<td>1.955 ± 0.697</td>
<td>1.761 ± 0.597</td>
</tr>
<tr>
<td>PSI</td>
<td>56.4 ± 38.3</td>
<td>93.1 ± 53.0</td>
</tr>
</tbody>
</table>

CAP: community-acquired pneumonia; WBC: white blood cell count; CRP: C-reactive protein; MDA: malondialdehyde; TAC: total antioxidant capacity; PSI: pneumonia severity index.

Table 3. The relation between pneumonic infiltration on chest X-ray with the laboratory variables.

<table>
<thead>
<tr>
<th></th>
<th>Unilobar involvement</th>
<th>Multilobar involvement</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=51</td>
<td>n=16</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>40 ± 23</td>
<td>41 ± 25</td>
<td>ns</td>
</tr>
<tr>
<td>WBC, n x 10^9/L</td>
<td>17.9 ± 9.2</td>
<td>15.6 ± 8.1</td>
<td>ns</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>153.1 ± 64.2</td>
<td>144.5 ± 61.8</td>
<td>ns</td>
</tr>
<tr>
<td>MDA, nmoL</td>
<td>3.212 ± 0.862</td>
<td>3.142 ± 0.881</td>
<td>ns</td>
</tr>
<tr>
<td>TAC, mmol Trolox equivalent/L</td>
<td>1.938 ± 0.688</td>
<td>1.791 ± 0.630</td>
<td>ns</td>
</tr>
</tbody>
</table>

WBC: white blood cell count; CRP: C-reactive protein; MDA: malondialdehyde; TAC: total antioxidant capacity; ns: not significant.

scores and had statistically significantly lower MDA levels than PE (-) CAP patients (P=0.006 and P=0.030, respectively). There were no statistical differences in serum TAC or CRP levels and WBC counts between the CAP patients with or without PE (Table 2). In PE (+) CAP patients, the CXR scores were found to be higher than in PE (-) patients (P=0.0001).

According to findings on CXR, CAP patients were grouped as having unilobar or multilobar involvement in 51 (86%) and 16 (24%) patients, respectively. However, there were no statistically significant differences between the lobar and multilobar involvement in terms of serum MDA, TAC and CRP levels or WBC counts (Table 3). We also evaluated the patient’s CXR according to Majewska et al. (21), and no association was found among the parameters studied with the radiological scores.

According to the calculated PSI scores, our patients were grouped as follows: group I: 31 patients, group II: 5 patients, group III: 9 patients, group IV: 16 patients, and group V: 6 patients. Only one of the patients in group V died due to complication of the insertion of a chest tube. We could not find any significant differences among these groups in terms of mean levels of MDA, TAC, CRP and WBC counts. Although the CXR scores were also not different among these groups (P=0.054), they showed good correlation with PSI scores (r=0.420, P=0.0001). The CAP patients were also grouped as low risk (PSI I-III) and high risk (PSI IV-V) and the results of the parameters studied in these groups are shown in Table 4.

The etiological agent was identified in 21 patients (30%) based on cultures from sputum, blood, and pleural fluid. The most frequently isolated microorganisms were
Table 4. Comparison of the two groups of CAP patients with low- or high-risk according to Fine’s scale.

<table>
<thead>
<tr>
<th></th>
<th>Low-risk group ≤ 90 points</th>
<th>High-risk group &gt; 90 points</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSI</td>
<td>41.4 ± 24.9</td>
<td>120.0 ± 30.5</td>
<td>0.0001</td>
</tr>
<tr>
<td>CXR score</td>
<td>2.36 ± 1.23</td>
<td>3.19 ± 1.17</td>
<td>0.010</td>
</tr>
<tr>
<td>WBC, n x 10⁹/L</td>
<td>17.7 ± 9.2</td>
<td>15.9 ± 8.2</td>
<td>ns</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>138.7 ± 63.9</td>
<td>177.1 ± 56.4</td>
<td>0.005</td>
</tr>
<tr>
<td>MDA, nmol/L</td>
<td>3.164 ± 0.842</td>
<td>3.243 ± 0.936</td>
<td>ns</td>
</tr>
<tr>
<td>TAC, mmol Trolox equiv/L</td>
<td>1.930 ± 0.682</td>
<td>1.858 ± 0.682</td>
<td>ns</td>
</tr>
</tbody>
</table>

PSI: pneumonia severity index; CXR: chest X-ray; WBC: white blood cell count; CRP: C-reactive protein; MDA: malondialdehyde; TAC: total antioxidant capacity; ns: not significant.

Streptococcus pneumoniae (13 cases); the others included gram-negative microorganisms (Klebsiella pneumoniae, 5 cases; Pseudomonas aeruginosa, 1 case) and Staphylococcus aureus (2 cases). Multiple pathogenic bacteria were isolated in only two patients. There was no statistically significant difference in MDA and TAC concentrations measured in patients with or without a positive microbiologic result.

Discussion

The respiratory tract has a great external surface area that directly interfaces with the external environment and exposes it to inhaled noxious antigens and particles including etiologic agents of pneumonia. This makes it a vulnerable target for oxidative injury. TBARS (mostly MDA) and free radicals have been reported to be increased in inflammatory lung diseases including pneumonia (20). In the present study, we found statistically significantly high MDA levels and low TAC levels in the serum of CAP patients compared to those in control subjects, indicating increased oxidative stress in the blood of patients with pneumonia. Our results are supported by the studies reported previously (9,18-23,26,27). The occurrence of oxidant/antioxidant imbalance through decreased serum total antioxidant status (TAS) in patients with pneumonia was concluded in the preliminary results of the study by Katsoulis et al. (9). Increased oxidative stress and decreased enzymic and non-enzymic antioxidant activities in children with acute pneumonia were also concluded in the study by Cemek et al. (18). Similarly, Duflo et al. (20) studied alveolar and serum oxidative stress in ventilator-associated pneumonia (VAP) and found that plasma and alveolar TBARS increased significantly in patients who developed VAP compared with those who did not, by 43% and 259%, respectively. In the study by Majewska et al. (21), the occurrence of oxidative stress in the respiratory tract related to lower airways infection was proven by the demonstration of elevated H₂O₂ and TBARS exhalation. Compatible with the aforementioned studies, Nowak et al. (22) stated that an enhanced process of lipid peroxidation occurs during bacterial pneumonia and increased levels of circulating markers of lipid peroxides return to normal values quicker than the concentration of MDA during recovery. In another study by Li et al. (23), the serum levels of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and MDA were all significantly higher, while TAC was significantly lower in 68 patients with pneumonia, and it was concluded that determination of serum SOD, GSH-Px, TAC and MDA might be helpful for the diagnosis and treatment of pneumonia. In the current study, in contrast to the previous ones, the serum TAC level was measured instead of specific components of the antioxidant defense system with an assay suitable for serum samples (24). Most of the antioxidants that take part in this colorimetric method are extracellularly located, although there is evidence that major intracellular antioxidant enzymes such as SOD and GSH-Px are also detectable extracellularly even in smaller amounts.

In contrast to Katsoulis et al. (9), the current study showed a mildly positive correlation between serum TAC level and smoking status, and an increased TAC level on admission if smoking coexisted. This finding is in accordance with the previous study by Abou-Seif (29), which stated that erythrocyte SOD and catalase as well as plasma SOD activities were elevated in smokers compared...
with the control group. Stolarek et al. (28) studied the differential effect of cigarette smoking on H$_2$O$_2$ and TBARS exhaled in patients with CAP, and demonstrated a decreased level of exhaled H$_2$O$_2$ and increased concentration of exhaled TBARS within up to five days after the diagnosis of CAP and initiation of antibiotic therapy. The attenuated exhalation of H$_2$O$_2$ level in EBC may indicate the enhanced decomposition of H$_2$O$_2$, impaired inflammatory response to bacterial infection or the attenuation of phagocyte oxidative response early in the course of infection, whereas increased TBARS seems to be associated with a delayed effect of increased oxidative stress. Pre-existing chronic bronchitis enhances lung antioxidant defense, thus additional oxidant burden related with pneumonia did not result in further rise in H$_2$O$_2$ exhalation (28).

Similar results were found in the study by Katsoulis et al. (9), with respect to oxidative stress levels in patients with comorbid disease. In this group, the MDA or TAC levels were not different than in the group without comorbid disease (Table 3). Pneumonic CXR score is a simple and inexpensive measure to evaluate the extent of pneumonic infiltration in the lung, but it should be noted that size and severity of inflammatory alveolar infiltrate have some limitations that may affect analyzed correlations. In the present study, there was no correlation between pneumonic CXR score and serum MDA or TAC levels. There are discordant results in the literature about the relationship between the radiological extension and oxidant/antioxidant balance in pneumonia patients. In the study by Katsoulis et al. (9), no significant difference was reported in TAS levels on the day of admission according to the CXR findings, complications or distribution of the pneumonic infiltration. However, the TAS difference (ΔTAS, between admission and the 7th day) was found to be different in the radiologic distribution groups, as lobar vs non-lobar. In another study by Majewska et al. (21), weak or moderate correlations between exhaled markers of systemic inflammatory response and pneumonic CXR score were detected. In this study, it was also suggested that EBC H$_2$O$_2$ and TBARS had a limited predictive value for monitoring with respect to size and severity of pneumonic infiltrate. Thus, we can speculate that some correlation between the radiological scores and laboratory variables might be detected if we had used thorax computed tomography (CT) images to evaluate the spatial distribution of the infiltration.

In the current study, a positive bacterial culture result was achieved in 30% of patients (21 cases), and Streptococcus pneumoniae was the most frequently isolated microorganism (in 13 cases). However, no statistically significant differences in MDA and TAC concentrations were obtained in the patients with or without a positive microbiologic result. These results were supported by the study of Laskaj et al. (19), who studied gamma-glutamyltransferase activity and TAS in serum and platelets of 60 CAP patients. They confirmed bacterial pneumonia in 53.3% (32/60), atypical pneumonia in 38.3% (23/60) and interstitial pneumonia in 8.3% (5/60) of the study patients, and reported no statistically significant difference in the study parameters according to the etiology of pneumonia. In this regard, Katsoulis et al. (9) reported an increased TAS change in patients with gram-negative pneumonia, although no significant difference had been found in TAS on admission between the groups of pneumonia due to gram-positive or gram-negative agents.

It is well known that inflammatory cells such as neutrophils, lymphocytes and macrophages can play a significant role in releasing ROS during the phagocytic process. Although TAC levels negatively correlated with WBC counts (r = -0.646, P = 0.0001) in the whole study population, there was no such correlation among the WBC and TAC or MDA levels when the population was restricted to CAP patients. This result was similar with the previous studies (9,22). A negative correlation was found between the TAS change and total WBC, and it was suggested that other sources of ROS may be involved in peripheral oxidant/antioxidant imbalance in CAP patients (9). In the present study, it is of interest to note that CRP concentration was not correlated with serum MDA or TAC levels in the CAP patients, although it was negatively correlated with TAC in the whole study population. CRP concentration is a very useful nonspecific biochemical marker of inflammation, and its measurement in CAP patients contributes to diagnosis and assessment of the severity of the disease. The lack of relationship between CRP levels and TAC may be attributed to the presence of a high serum CRP level in all CAP patients and to the fact that TAC represents a marker of global defense against free radicals. To our knowledge, there is no other study in the literature evaluating serum CRP levels and oxidant/antioxidant status assessed by MDA and TAC levels in patients with CAP. Nevertheless, a supportive finding of our results was reported in a recent study done.
References


