Leukocyte myeloperoxidase activity and serum CRP levels as predictive markers of inflammatory disorders amongst passive smokers

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Abstract

Aims and objectives: To analyse the leukocyte myeloperoxidase activity and serum C-reactive protein levels in smokers, passive smokers and non-smokers and to evaluate the risk of developing inflammation that may lead to lung cancer in smokers in general and passive smokers in particular, by comparing the levels with nonsmokers

Methodology: Study design comprised of three groups; active smokers (Smokers who have smoked more than 5 cigarettes/day for a minimum period of 2 years.) passive smokers (Smokers exposed to smoke for a duration of ≥30 minutes/day, ≥5 days a week for one year or more (waiters in a pub/restaurant) and controls (non-smokers). All patients with pneumococcal infections were eliminated from the study. Leukocyte MPO & serum CRP (semiquantitative assay) were performed with patient’s samples. Data was analysed by ANOVA followed by post hoc tests. Odds ratio was also calculated for the study groups.

Results: There was no significance in the study groups with respect to both MPO as well as CRP.

Conclusion: CRP or MPO may not be the ideal markers to predict the probability of developing an inflammatory disorder in persons exposed to passive smoke.

Keywords: myeloperoxidase, C reactive protein, passive smokers

1. Introduction

Smoking is one of the major lifestyle factors influencing the health of human beings. Lifelong cigarette smokers have a higher prevalence of common diseases like atherosclerosis and COPD with significant systemic impact. Smokers are also at a high risk of developing lung cancers. Passive smoking happens when people breathe in the smoke that drifts off the end of the burning cigarette or is blown out by a smoker. Passive smoking is also reported to be leading to lung cancer¹ and heart disease². Smoking enhances the binding of S. pneumoniae to pharyngeal cells³ and damage local defenses by impairing mucociliary flow, increasing the permeability of the respiratory epithelium, reducing the humoral responses to inhaled antigens and increasing susceptibility of the host to the respiratory viruses⁴. A causative role of smoking in the
pathogenesis of pneumococcal infection seems highly plausible. Indeed, previous hospital based case-controlled study\(^5\) found an increased risk of pneumococcal infection among current and former smokers\(^6\). Therefore the subjects with pneumococcal infection are not considered for the study as they are likely to interfere with our study reports.

Myeloperoxidase (MPO) has been shown to convert metabolites of the tobacco smoke procarcinogen benzo(a)pyrene to the highly reactive carcinogenic and mutagenic benzo(a) pyrenediolepooxide (BPDE)\(^7,8\). London \(et\ al\)^\(^9\) were the first to report an association between the MPO and lung cancer risk. Significant increase in MPO activity in smokers compared to non-smokers has been reported.

C-reactive protein (CRP) is an acute phase protein produced primarily by the liver in response to inflammatory cytokines such as interleukin-6. Recent data have shown that patients with non-small cell lung cancer have significantly higher levels of CRP and interleukin-6 which is correlated with prognosis\(^10\). Patients with increased CRP levels were nearly 10 times more likely to develop progressive lung cancer than those with lower levels\(^11,12\). According to certain reports, increased levels of CRP are more strongly associated with the risk of cancer death than cancer incidence\(^13\). CRP levels are elevated in smokers and significantly lower in long term smoking cessation\(^14\).

2. Methodology

2.1. Study design: Community based, prospective, case-control study.

2.2. Sample size: Blood samples from actives smokers (n=20), passive smokers (n=20) and non-smokers (n=20).

2.3. Exclusion Criteria

   Subjects with pneumococcal infection.

2.4. Inclusion Criteria

2.4.1. Active Smokers

   Smokers who have smoked more than 5 cigarettes/day for a minimum period of 2 years.

2.4.2. Passive Smokers

   Smokers exposed to smoke for a duration of >30 minutes/day, ≥5 days a week for one year or more (waiters in a pub/restaurant)

2.5. Controls: Persons who are in normal health and are not reported to have any exposure to cigarette smoke.

2.6. Questionnaires on Personal information (gender, age, diet, family history, alcohol consumption, etc.) in case of all the 3 groups.

   Number of cigarettes smoked and duration of smoking (active smokers) and duration of exposure to cigarette smoke (passive smokers), were collected.

2.7. Methodology:

2.7.1. Screening test for pneumococcal infection

   The screening test for pneumococcal infection was done by Gram staining procedure. The staining technique consists of preparation of a thin smear of sputum on a clean glass slide and primarily staining with dye such as crystal violet; application of a dilute solution of iodine; decolourisation with an organic solvent such as ethanol or acetone; counterstain with dye such as carbon fuchsin or safranine. The presence of Gram positive cocci in chains is indicative of pneumococcal infection.

2.7.2. Estimation of leukocyte myeloperoxidase activity

   Blood samples were collected using anticoagulant. Leukocytes were separated from erythrocyte by collecting the buffy coat\(^15\). Leukocyte suspension was prepared in distilled water. This suspension was used for estimating myeloperoxidase activity (method of Mattheson \(et\ al\)^\(^16\)). Myeloperoxidase activity was expressed as units/mg protein.

2.7.3. Leukocyte protein estimation

   Leukocyte protein estimation was done by Lowry method\(^17\).

2.7.4. Estimation of serum CRP level by latex serology

   Serum CRP levels were estimated by latex serology test (method of Singer \(et\ al\)^\(^18\)). Positive CRP levels were
obtained at a CRP serum concentration of 6 gm/L and above. Negative results were obtained at a CRP concentration below 6gm/L.

2.8. Statistical analysis

ANOVA to compare the MPO values obtained in the 3 groups, active smokers, passive smokers and controls. Chi square test was applied to compare the percentage distribution of CRP levels between the 3 groups. Variation in CRP and MPO levels with the number and duration of smoking and alcohol consumption was analysed by chi square and student ‘t’ test respectively. Odds ratio was calculated for CRP levels in the 3 groups.

Informed consent was obtained from all subjects included in the study and approval from Institutional Ethical Committee was also obtained.

3. Results

**Table 1: Leukocyte MPO levels (values are Mean±S.D)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>0.046±0.096</td>
</tr>
<tr>
<td>Active smokers</td>
<td>0.0088±0.005</td>
</tr>
<tr>
<td>Passive smokers</td>
<td>0.03654±0.068</td>
</tr>
</tbody>
</table>

**Table 2: Plasma CRP levels (Percentage Distribution)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Percentage Distribution</th>
<th>Pearson chi square</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>100%&lt;0.6g/dl</td>
<td></td>
<td>p=0.05</td>
</tr>
<tr>
<td>Active smokers</td>
<td>90%&lt;0.6g/dl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Passive smokers</td>
<td>94.7%&lt;0.6g/dl</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Odds ratio for plasma CRP levels

<table>
<thead>
<tr>
<th>Group comparison</th>
<th>Odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control and active smokers</td>
<td>0</td>
</tr>
<tr>
<td>Control and passive smokers</td>
<td>0.5</td>
</tr>
</tbody>
</table>

**Table 4: Comparison of alcohol consumption with the parameters between the groups**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pearson chi square</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol consumption and plasma CRP levels</td>
<td>10.483</td>
<td>p=0.065</td>
</tr>
<tr>
<td>Alcohol consumption and leukocyte MPO levels</td>
<td>&gt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

**Table 5: Comparison of smoking status with the parameters within the active smokers**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pearson chi square</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocyte MPO</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Plasma CRP</td>
<td>2.222</td>
<td>p=0.136</td>
</tr>
</tbody>
</table>

**Table 6: Comparison of duration of exposure to smoking with the parameters within the passive smokers**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pearson chi square</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocyte MPO</td>
<td>1.287</td>
<td>p=0.45</td>
</tr>
<tr>
<td>Plasma CRP</td>
<td>1.287</td>
<td>p=0.45</td>
</tr>
</tbody>
</table>

No significance was observed in MPO or CRP levels when active and passive smokers were compared with controls. However the values of 2active smokers correlated well with the number of cigarettes smoked and duration of smoking, but did not contribute to statistical significance within the active smokers. No such correlation was observed amongst passive smokers with respect to duration of exposure to cigarette smoke. But percentage of passive smokers having a CRP value < 0.6g/dl (cut off value for positive results) was significantly less compared to controls. Alcohol consumption, dietary habits or family history, in addition to smoking did not show any correlation in the parameters studied. Odds ratio for CRP levels between active smokers and controls or passive smokers and controls was not equal to 1 which suggests that...
CRP could not be used as a marker for deciding the risk of developing the disease amongst active or particularly passive smokers, in the present study.

4. Discussion

It has long been accepted that cigarette smoking is a classical and major risk factor in the development of cardiovascular disease (CVD) and atherosclerosis\(^\text{19, 20}\). More recently, CVD has even been referred to as an inflammatory disease\(^\text{21,22}\). In addition, a link has been established between several other chronic inflammatory diseases and smoking, including, Chronic inflammatory diseases, including chronic obstructive pulmonary disease (COPD)\(^\text{23}\), rheumatoid arthritis, systemic lupus erythematosus\(^\text{24}\) and Crohn’s disease\(^\text{25}\) has been associated with smoking. Although the mechanisms linking smoking to these diseases are not well understood, inflammatory markers may be valuable in providing explanations for smoking-mediated morbidity and mortality.

Smoking induces inflammatory pathways. Smokers have increased numbers of white blood cells, mainly because of a particular increase in polymorphonuclear neutrophils, which are released from the bone marrow and recruited to inflamed tissue\(^\text{26}\). IL-\(\beta\) and IL-6, which are increased in response to lung inflammation and are implicated in the induction of CRP gene expression, may mediate the stimulation of bone marrow cells\(^\text{27}\). CRP was found to be significantly higher in male and female smokers compared with non-smokers\(^\text{28}\) in certain studies.

On the contrary, Helmersson \textit{et al} have reported that although smoking status correlated with a significant elevation in levels of IL-6 and serum amyloid protein A, another acute phase protein, the increase in CRP levels observed in smokers was not found to be statistically significant\(^\text{29}\). A dose dependent correlation between CRP and smoking habits was demonstrated in the ‘Speedwell’ survey of British men with respect to CRP levels which increased proportionately with the increase in number of cigarettes smoked\(^\text{30}\). However, another study conducted in people of Japanese ethnicity (the Iwate Kenpoku Cohort study) failed to identify any significant relationship between serum CRP concentration and the number of cigarettes smoked per day\(^\text{31}\). MONICA study from Germany, reported significantly high serum CRP concentrations in male smokers with no significant difference in women smokers\(^\text{32}\). In contrast, another study examining CRP levels in patients with moderate to severe COPD reported that, although there was a significant difference in CRP levels in the COPD patients, there was no difference in CRP status between smokers and non-smokers\(^\text{33}\).

CRP levels were significantly increased in children who were exposed to second hand smoke\(^\text{34}\). Contradictory results have been observed in never-smoking adults exposed to second hand smoke with one group reporting an increase in CRP levels\(^\text{35}\), while a second study did not observe a significant change in CRP status, even though other biomarkers of CVD risk (fibrinogen, homocysteine) were increased\(^\text{36}\).

MPO levels were significantly higher in patients who developed CAD in a 8 year follow up study, compared to controls, and correlated with CRP. Risk of future CAD increased in consecutive quartiles of MPO concentration with an odds ratio of 1.49 in a report published by the Epic-Norfolk prospective population study\(^\text{37}\). Also MPO was found to be an early marker of systemic inflammation in a 6 year follow up study in smokers without severe airway symptoms\(^\text{38}\).

However the results of present work suggests that neither CRP nor MPO seem to be effective or authentic markers for predicting the development of inflammatory disorders in smokers in general and passive smokers in particular. Significant correlation may be obtained if the study is performed with larger number of subjects and if a more reliable high sensitivity CRP method is adopted.

References
5. Lipsky B A, Boyko E J, Inui T S. Risk Factors for Acquiring pneumococcal infection. \textit{Arch intern Med}. 1986; 146:
Kumarchandra et al

2179-2185.


29. Lowe GDO, Yarnell JWG, Rumley A, *et al.* C-reactive protein, fibrin D-dimer, and incident ischemic heart disease in

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36. Marjn C Meuwese, Erik SG Strois, Stanley L Hazen, Joram N Van Miert et al. S.MPO levels are associated with the future risk of CAD in apparently healthy individuals: The epic – Norfolk prospective population study. *Journal of the American College of Cardiology* 2007;50(2);159-165.