Association between C - reactive protein and Matrix metalloproteinase-2 in Non-small cell lung carcinoma

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Abstract
Matrix metalloproteinases (MMPs) are enzymes which are involved in irreversible degradation of ECM (extra cellular matrix). MMP-2 is capable in laminin-5 cleavage which increases endothelial cell migration and in the secretion of VEGF that promote angiogenesis in physiological conditions as well as tumor development also. C-reactive protein (CRP) is an acute-phase protein which is used as marker of systemic inflammation. Increased CRP is associated with an increased risk of all the cancer. In the present study serum levels of MMP-2 and CRP is quantified in the NSCLC patients to establish the clinical significance of these biomarkers during NSCLC disease progression. This is a cross sectional study which includes 120 NSCLC patients and 60 age and sex matched healthy controls attending the medical oncology OPD of our institute from July, 2017 to March, 2019. Blood Samples were collected from patients and healthy controls. CRP was measured by turbidimetric technique and MMP-2 is measured by ELISA. The independent t-test was used to compare the level of MMP-2 and CRP in healthy controls and NSCLC patients. Pearson’s correlation (r) was used to correlate the MMP-2 level and CRP level in NSCLC patients. Serum MMP-2 level and serum CRP levels were found significantly high in NSCLC patients when compared with healthy controls (p<0.001). Furthermore a significant positive correlation was also observed between MMP-2 level and CRP level in NSCLC patients. Therefore measurement of MMP-2 level and CRP level could be a marker for disease progression of NSCLC and to see the association between inflammation and pathogenesis of NSCLC.

Keywords: Matrix metalloproteinases, C - reactive protein, NSCLC.

Introduction
Matrix metalloproteinases (MMPs) are the Zn dependent enzymes which are involved in irreversible degradation of ECM (extra cellular matrix), processing and cleavage of chemokines, and shedding of cell membrane proteins during homeostatic processes and in pathological states.¹ For the development of a tumor, new vascular system is required because tumor cannot grow without new blood vessels. MMP-2 & MMP-9 are involved in the process of enabling proteolytic degradation of the vascular basal membrane and in the migration of endothelial cells to form new blood vessels.² MMP-2 is also capable in
laminin-5 cleavage which increases endothelial cell migration and in the secretion of VEGF that promote angiogenesis in physiological conditions as well as tumor development also.

C-reactive protein (CRP) is an acute-phase protein which is used as a sensitive, but nonspecific, marker of systemic inflammation. CRP has a wide variety of biological properties and functions in routine clinical practice. High levels of serum CRP have been observed in many pulmonary disorders, including pneumonia, malignancies, and pulmonary thromboembolism. Increased CRP is associated with an increased risk of all the cancer, lung cancer, breast, prostate and colorectal cancer. And high CRP level is positively correlated with weight loss, anorexia-cachexia syndrome, extent of disease, and recurrence in advanced cancer. Tumor cells cause tissue inflammation and thus CRP level is increased, and malignant pleural effusion indicates the severity of disease and a poor survival. CRP may be increased due to cancer-related infection, specially a post stenotic pulmonary infection in the case of lung tumors. It is well known that pneumonia can be the first symptom that indicates lung cancer. MMPs are regulated by α2-macroglobulin (α2M) or tissue inhibitors of metalloproteinases (TIMPs) which are produced by macrophages, fibroblasts and other types of cells. Thus, an imbalance between MMPs and their inhibitors is thought to be a causative factor in invasion and metastasis of cancers.

The production of CRP in liver cells is regulated by interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) which are secreted from macrophages. α2M is the main proteinase inhibitor in blood, and also involved in the inflammatory reaction through its function as a carrier protein of IL-6. However, the correlation between the serum levels of MMP-2 and CRP in patients with NSCLC progression has not been established. Therefore, in the present study serum levels of MMP-2 and CRP is quantified in the different stages of NSCLC patients to establish the clinical significance and changes of these biomarkers during NSCLC disease progression.

Material & Method
The Present cross-sectional study was conducted in the Biochemistry department, in association with Medical oncology department of SMS Medical College and hospitals at Jaipur from July, 2017 to March, 2019.

Study Population:  Study subjects included total 180 subjects. 120 NSCLC patients and 60 age matched healthy controls who voluntarily participated in study. The NSCLC patients recruited were diagnosed on the basis of histological and cytological examinations. Patients of NSCLC who have received CT/RT or surgery, patients with cardiovascular diseases, renal diseases, hepatic diseases or uncontrolled infection, patients with chronic inflammatory conditions and pregnant patients were excluded from this study. All the 120 NSCLC patients were categorized into 2 groups on the basis of stage of NSCLC. Group 1 consist of patients of stage I and stage II NSCLC patients. Group 2 consist of patients of stage III and stage IV NSCLC patients. The lung cancer patients were staged according to the 7th edition of the International Staging of Lung Cancer, 2009. The study protocol was approved by the institutional CTSC (Clinical Trial and Screening Committee) and Ethics Committee with number: 2157, MC/EC/2016. Informed written consent was obtained from all the study subjects.

Sample Collection: Samples were collected from patients and healthy controls by venipucture. Serum was separated and stored at -80°C till analysis. CRP was measured by turbidimetric technique and MMP-2 is measured by ELISA.

Statistical Analysis: The presentation of the results is in the form of mean ± standard deviation. SPSS for windows (version 21, Chicago, IL, USA) was used for the analysis of data collected. The independent sample t-test was used to compare the means of different variables in the two groups and Pearson’s correlation (r)
was used to correlate the variables. For all statistical assessment a value of p<0.05 was accepted to be significant.

Results
Demographic Characteristics: A total of 180 subjects were included in this study. Of these 136 were males and 34 were females (Table-1).

Table-1: Distribution of the subjects according to gender

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th></th>
<th>Control</th>
<th></th>
<th>Total</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>Gender</td>
<td>16</td>
<td>13.33%</td>
<td>15</td>
<td>25.00%</td>
<td>34</td>
<td>18.89%</td>
</tr>
<tr>
<td>Male</td>
<td>104</td>
<td>86.67%</td>
<td>45</td>
<td>75.00%</td>
<td>146</td>
<td>81.11%</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>100.00%</td>
<td>60</td>
<td>100.00%</td>
<td>180</td>
<td>100.00%</td>
</tr>
</tbody>
</table>

Table-2: Comparison of Serum MMP-2 levels between NSCLC cases and Healthy controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>NSCLC Cases (N=120) Mean ± SD Range</th>
<th>Healthy controls (N=60) Mean ± SD Range</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-2(ng/ml)</td>
<td>106.86 ±100.05 (3.6-320.0)</td>
<td>7.17 ±2.92 (2.6-16.3)</td>
<td>&lt;0.001 S</td>
</tr>
</tbody>
</table>

Table-3: Comparison of Serum CRP levels between NSCLC cases and Healthy controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>NSCLC Cases (N=120) Mean ± SD Range</th>
<th>Healthy controls (N=60) Mean ± SD Range</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP (mg/L)</td>
<td>39.1± 38.7 (1.2-108)</td>
<td>2.29 ±2.03 (0.60-16.0)</td>
<td>&lt;0.001 S</td>
</tr>
</tbody>
</table>

Table-4: Correlation of serum CRP (mg/L) with serum MMP-2 (ng/ml) levels in NSCLC cases

<table>
<thead>
<tr>
<th>Correlations</th>
<th>MMP-2(ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP(mg/L)</td>
<td>Pearson Correlation (r) = 0.884**</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>P Value &lt;0.001S</td>
</tr>
</tbody>
</table>

Discussion
Present cross-sectional study showed that serum MMP-2 levels (Table-1) were significantly high in NSCLC patients when compared with healthy controls. These results are supported by Study of Suzuki et al. [21], they found that MMP-2 was detected in 5/5 NSCLC by zymography and immunohistochemistry, whereas MMP-9 was detected in only 1/5 NSCLC, which showed that MMP-2 play an important role in NSCLC progression. Interstitial collagenase (MMP-1) and type IV collagenases (MMP-2, MMP-9) are responsible for breakdown of collagen and basement membrane components during tumor development and angiogenesis [22-24]. Table-2 shows that serum CRP levels were also significantly high in NSCLC than Healthy controls. This finding is supported by a meta-analysis study by Yong-Guo et al. [25], in which it is observed that increased serum CRP is associated with risk of cancers especially lung cancer. In general, higher CRP concentration is observed in cancer patients than healthy controls and participants with benign diseases [26]. There may be several mechanisms for the relationship between CRP and cancer. CRP levels may be increased due to inflammation caused by tumor development [27, 28]. Immune system responds to tumor antigens, which can increase the CRP level [29-31]. Production of inflammatory proteins is increased by tumor cells, which could explain the high level of CRP in cancer patients. These mechanisms support that high levels of CRP is a response to the cancer progression and thus CRP could be a marker for cancer for early stage and disease progression also. It is observed in previous studies that the serum CRP levels are highly increased in lung cancer patients when compared with healthy control [32].

Chronic inflammation is involved in the development and progression of cancer. Cells and cytokines of immune system of lung have different functions under normal physiological conditions. The ratio of immune cells and cytokines remains within the normal range, so there is no harmful immune responses to the host. Biomarkers which are secreted in response to imbalance in immune system of lungs caused by lung cancer can serve as biomarker and predictive factors in relation to immunotherapy [33]. The inflammation which is caused by immune system imbalance is associated with carcinogenesis by
promoting angiogenesis and proliferation of tumor cells\cite{34}.

In present study we also found positive correlation between MMP-2 and CRP levels in NSCLC patients (Table-3), which shows that there is an association between inflammation and pathogenesis of NSCLC. Many biological processes such as cell proliferation, differentiation, migration, activation, and cell growth are regulated by cytokines as well as the tumor development\cite{35-37}. Present study findings are supported by the study of KANOH et al\cite{38}, they observed that there is a significant positive correlation between CRP and MMP-2 levels in metastatic NSCLC patients which is considered to reflect the tissue disturbance and inflammation that are associated with invasion and metastasis of NSCLC, and can also predict tumor progression and poor prognosis of NSCLC.

**Conclusion**

In conclusion, present study shows that serum MMP-2 levels and serum CRP levels are markedly increased in NSCLC patients than in Healthy controls. Furthermore a significant positive correlation was also observed between MMP-2 level and CRP level in NSCLC patients. Therefore measurement of MMP-2 level and CRP level could be a marker for disease progression of NSCLC and to see the association between inflammation and pathogenesis of NSCLC.

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**References**


17. Yap SH, Moshage HJ, Hazenberg BP, Roelofs MH, Bijlzet J, Limburg PC, Aarden LA and van Rijswijk MH: Tumor necrosis factor (TNF) inhibits interleukin (IL)-1 and/or IL-6 stimulated synthesis of C-reactive protein (CRP) and serum amyloid A (SAA) in primary cultures of human hepatocytes. Biochim Biophys Acta 1091: 405-408, 1991.


