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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[1-3]. 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[4]. MMP-2 is also capable in

laminin-5 cleavage which increases endothelial cell migration[4] 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[5]. CRP has a wide variety of biological properties and functions in routine clinical practice[6]. High levels of serum CRP have been observed in many pulmonary disorders, including pneumonia, malignancies, and pulmonary thromboembolism[7]. Increased CRP is associated with an increased risk of all the cancer, lung cancer, breast, prostate and colorectal cancer[8]. And high CRP level is positively correlated with weight loss, anorexia-cachexia syndrome, extent of disease, and recurrence in advanced cancer[9]. Tumor cells cause tissue inflammation and thus CRP level is increased, and malignant pleural effusion indicates the severity of disease and a poor survival[10]. 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[11].

MMPs are regulated by α2-macroglobulin (α2M) or tissue inhibitors of metalloproteinases (TIMPs) which are produced by macrophages, fibroblasts and other types of cells[12-14]. Thus, an imbalance between MMPs and their inhibitors is thought to be a causative factor in invasion and metastasis of cancers[15,16].

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[17,18]. 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 [19].

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[20]. 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 -80ºC 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

	Cases		Control		Total	
Gender	**No**	**%**	**No**	**%**	**No**	**%**
Female	16	13.33	15	25.00	34	18.89
Male	104	86.67	45	75.00	146	81.11
Total	120	100.00	60	100.00	180	100.00

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

Variable	NSCLC Cases (N=120) Mean ± SD Range	Healthy controls (N=60) Mean ± SD Range	P Value
MMP-2(ng / ml)	106.86 ±100.05 (3.6-320.0)	7.17 ±2.92 (2.6-16.3)	<0.001 S

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

Variable	NSCLC Cases (N=120) Mean ± SD Range	Healthy controls (N=60) Mean ± SD Range	P Value
CRP (mg/L)	39.1± 38.7 (1.2-108)	2.29 ± 2.03 (0.60-16.0)	<0.001 S

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

Correlations		
		MMP-2(ng/ml)
CRP(mg/L)	Pearson Correlation (r)	.884**
	Sig. (2-tailed) P Value	<0.001S
	N	120

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[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[35-37]. Present study findings are supported by the study of KANOH et al[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

1. Nagase H, Woessner Jr JF. Matrix metalloproteinases. J Biol Chem 1999;274 (31):21491 4.
2. Bonnans C, Chou J, Werb Z. Remodelling the extracellular matrix in development and disease. Nat Rev Mol Cell Biol 2014;15(12):786–801.
3. Vu TH, Werb Z. Matrix metalloproteinases: effectors of development and normal physiology. Genes Dev 2000; 14(17):2123–33.
4. Risau W (1997) Mechanisms of angiogenesis. Nature 386:671–674.
5. M.B. Pepys, G.M. Hirschfield, C-reactive protein: a critical update, J. Clin. Invest. 111 (2003) 1805–1812.
6. Hans C. Ablij, Arend E. Meinders, C-reactive protein: history and revival, Eur. J. Intern. Med. 13 (7) (2002) 412–422.
7. R.P. Smith, B.J. Lipworth, C-reactive protein in simple community-acquired pneumonia, Chest 107 (1995) 1028–1031.
8. Guo et al, Association between C - reactive protein and risk of cancer: a meta-analysis of prospective cohort studies, Asian Pacific J. Cancer Prev. 14 (1) (2013) 243–248.
9. F.A. Mahmoud, N.I. Rivera, The role of C-reactive protein as a prognostic indicator in advanced cancer, Curr. Oncol. Rep. 4 (2002) 250–255.
10. P.E. Postmus, E. Brambilla, K. Chansky, J. Crowley, P.Goldstraw, E.F. Patz Jr., et al, The IASLC lung cancer staging project: proposals for revision of the M descriptors in the forthcoming (seventh) edition of the TNM classification of lung cancer, J. Thorac. Oncol. 2 (2007) 686–693.
11. V. Søyseth, J.S. Benth, K. Stavem, The association between hospitalization for pneumonia and the diagnosis of lung cancer, Lung cancer 57 (2007) 152–158.
12. Arbeláez LF, Bergmann U, Tuuttila A, Shanbhag VP and Stigbrand T: Interaction of matrix metalloproteinases-2 and -9 with pregnancy zone protein and α2-macroglobulin. Arch Biochem Biophys 347: 62-68, 1997.
13. Beekman B, Drijfhout JW, Ronday HK and Tekoppele JM: Fluorogenic MMP activity assay for plasma including MMPs complexed to α2-macroglobulin. Ann NY Acad Sci 878: 150-156, 1999.

14. Chen WT and Wang JY: Specialized surface protrusions of invasive cells, invadopodia and lamellipodia, have differential MT1-MMP, MMP-2 and TIMP-2 localization. Ann NY Acad Sci 878: 361-370, 1999.
15. Ding S, Zhang M, Zhao Y, Chen W, Yao G, Zhang C, Zhang P and Zhang Y: The role of carotid plaque vulnerability and inflammation in the pathogenesis of acute ischemic stroke. Am J Med Sci 336: 27-31, 2008.
16. Alvarez B, Ruiz C, Chacon P, Alvarez-Sabin J and Matas M: Serum values of metalloproteinase-2 and metalloproteinase-9 as related to unstable plaque and inflammatory cells in patients with greater than 70% carotid artery stenosis. J Vasc Surg 40: 469-475, 2004.
17. Yap SH, Moshage HJ, Hazenberg BP, Roelofs MH, Bijizet J, Limburg PC, Aarden LA and van Rijiswijk 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.
18. Smith JW and McDonald TL: Production of serum amyloid A and C-reactive protein by HepG2 cells stimulated with combina-tions of cytokines or monocyte conditioned media: the effects of prednisolone. Clin Exp Immunol 90: 293-299, 1992.
19. Matsuda T, Hirano T, Nagasawa S and Kishimoto T: Identification of α2 macroglobulin as a carrier protein for IL-6. J Immunol 142: 148-152, 1989.
20. Edge S, Byrd DR, Compton CC, Fritz AG, Greene FL, Trotti A. AJCC Cancer Staging Manual [eds.] 7th edition. Springer, New York, 2010.
21. Suzuki M, Lizasa T, Fujisawa T, Baba M, Yamaguchi Y, Kimura H, Suzuki H: Expression of matrix metalloproteinases and tissue inhibitor of matrix metalloproteinases in non-small cell lung cancer. Invasion Metastasis 1999;18:134–141.
22. Schütz A, Schneidenbach D, Aust G, Tannapfel A,Steinert M, Wittekind C: Differential expression and activity status of MMP-1, MMP-2 and MMP-9 in tumor and stromal cells of squamous cell carcinomas of the lung. Tumour Biol 2002;23:179–184.
23. Thomas P, Khokha R, Shepherd FA, Feld R, Tsao MS: Differential expression of matrix metalloproteinases and their inhibitors in non-small cell lung cancer. J Pathol 2000;190:150–156.
24. Atkinson JM, Pennington CJ, Martin SW, Anikin VA, Mearns AJ, Loadman PM, Edwards DR, Gill JH: Membrane type matrix metalloproteinases (MMPs) show differential expression in non-small cell lung cancer (NSCLC) compared to normal lung: correlation of MMP-14 mRNA expression and proteolytic activity. Eur J Cancer 2007;43:1764–1771.
25. Y.Z. Guo, L. Pan, C.J. Du, D.Q. Ren, X.M. Xie, Association between C-reactive protein and risk of cancer: a meta-analysis of prospective cohort studies, Asian Pac. J. Cancer Prev. 14(1) (2013) 243–248.
26. C.S. Wang, C.F. Sun, C-reactive protein and malignancy: clinico-pathological association and therapeutic implication, Chang Gung Med. J. 32 (5) (2009) 471–482.
27. D. Basso, C. Fabris, A. Meani, et al, C reactive protein in pancreatic cancer and chronic pancreatitis, Ann. Clin. Res. 20 (1988) 414–416.
28. D.M. O'Hanlon, J. Lynch, M. Cormican, H.F. Given, The acute phase response in breast carcinoma, Anticancer Res. 22 (2002) 1289–1293.

29. M.G. Alexandrakis, F.H. Passam, I.A. Moschandrea, et al,Levels of serum cytokines and acute phase proteins in patients with essential and cancer-related thrombocytosis, Am. J. Clin. Oncol. 26 (2003) 135–140.
30. F. Balkwill, A. Mantovani, Inflammation and cancer: back toVirchow?, Lancet 357 (2001) 539–545.
31. A.D. Blann, G.J. Byrne, A.D. Baildam, Increased soluble intercellular adhesion molecule-1, breast cancer and the acute phase response, Blood Coagul. Fibrinolysis 13 (2002)165–168.
32. Chung HW, Kim JW, Lee JH, Song Sy, Chung JB, Kwon OH, et al. Comparison of the validity of three biomarkers for gastric cancer screening: carcinoembryonic antigen, pepsinogens, and high sensitive C-reactive protein. J Clin Gastroenterol. 2009;43(1):19-26.
33. Domagala-Kulawik J.The role of the immune system in non-small cell lung carcinoma and potential for therapeutic intervention. Transl Lung Cancer Res. 2015;4(2):177– 190.
34. Lu H, Ouyang W, Huang C. Inflammation, a key event in cancer development. Mol Cancer Res. 2006; 4(4):221–233. PubMed
35. Tarrant JM Blood cytokines as biomarkers of in vivo toxicity in preclinical safety assessment: considerations for their use. Toxicol Sci. 2010;117(1):4–16. PubMed
36. Cobos C, Figueroa JA, Mirandola L, Colombo M, Summers G, et al.The role of human papilloma virus (HPV) infection in non-anogenital cancer and the promise of immunotherapy: a review. Int Rev Immunol. 2014; 33 (5):383–401. PubMed.
37. Vacchelli E, Aranda F, Bloy N, Buque A, Cremer I, et al., Trial Watch-Immunostimulation with cytokines in cancer therapy. Oncoimmunology. 2016; 5(2): PubMed e1115942.
38. Yuhsaku Kanoh, Tadashi Abe, Noriyuki Masuda and Tohru Akahoshi. Progression of non-small cell lung cancer: Diagnostic and prognostic utility of matrix metalloproteinase-2, C-reactive protein and serum amyloid A: Oncology Reports 29: 469-473, 2013.