

Prognostic Roles of Endostatin, Matrix Metalloproteinase-2 & 9 and Tissue Inhibitors of Metalloproteinase-1 in Advanced Non-Small Cell Lung Cancer

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ABSTRACT

Background and aim of the work: Previous studies verified that Endostatin, matrix metalloproteinase (MMP) -2 and -9, in addition to tissue inhibitors of metalloproteinase (TIMP) -1 may play a crucial role in prognosis of non-small cell lung cancer (NSCLC). In this study we will investigate the changes in the pretreatment serum levels of these factors and to evaluate their clinical implication in patients with advanced non-small cell lung cancer (NSCLC).

Patients and methods: Pretreatment serum samples were collected from 25 patients and 10 control healthy individuals. The levels of Endostatin, MMP-2, MMP-9, and TIMP-1 were measured using a sandwich enzyme immunoassay kit.

Results: The pretreatment serum levels of Endostatin and TIMP1 were significantly elevated and correlated with their stages and survival ($P < 0.05$), where, the serum level of Endostatin in healthy subjects was 81.20 ± 23.99 ng/ml and in patients with NSCLC was 354.40 ± 164.01 ng/ml. The serum level of TIMP1 in healthy subjects was 1.49 ± 0.29 ng/ml and in patients with NSCLC was 2.96 ± 0.58 ng/ml. The serum level of MMP2 and 9 were non-significantly decreased in serum of NSCLC patients ($P > 0.05$), where the serum activity of MMP2 in healthy subjects was 0.14 ± 0.03 ng/ml and in patients with NSCLC was 0.09 ± 0.03 % and the serum activity of MMP9 in healthy subjects was 0.13 ± 0.019 ng/ml and in patients with NSCLC was 0.10 ± 0.03 %.

Conclusions: Our results indicated that the circulating levels of Endostatin, and TIMP-1 in patients with NSCLC may be valuable future tools for treatment planning and monitoring of treatment, however, these blood tests need to be standardized and validated in large-scale prospective clinical trials.

Keywords: Endostatin, matrix metalloproteinase, tissue inhibitors of metalloproteinase, non small cell lung cancer.

INTRODUCTION

The incidence of lung cancer in Saudi Arabia is dramatically increasing and it is expected to become the leading cause of cancer deaths in the near future.⁽¹⁾ Tumor angiogenesis is essential for primary tumor growth, spread, and growth of tumor cell metastasis.⁽²⁾ Endostatin is an angiogenesis inhibitor that is an endogenously produced proteolytic fragment of type XVIII collagen. It is a specific inhibitor of endothelial cell proliferation, migration, and angiogenesis.⁽³⁾ Administration of recombinant mouse Endostatin to tumor-bearing animals increases the apoptotic index of the tumor cells without affecting their proliferation index.⁽⁴⁾ Biologically active Endostatin was able to inhibit systemic angiogenesis, inhibit primary tumor growth, and prevent the development of primary metastatic lesions.⁽⁴⁾

Matrix metalloproteinases (MMPs) are proteolytic enzymes which are involved in

normal physiological processes such as embryogenesis and tissue remodeling and may be implicated in creating an environment that supports the initiation and maintenance of tumor growth.⁽⁵⁾ They are believed to play a crucial role in tumor invasion and metastasis, and they correlate with advanced clinical stage and considered by some investigators as a negative prognostic factor for survival.⁽⁶⁾

The Tissue Inhibitors of Metalloproteinase (TIMP) family is a natural inhibitor of several matrix metalloproteinase (MMPS) enzymes and are known to inhibit invasion and metastasis in animal models.⁽⁷⁾ Binding of the TIMPS to their specific MMPS result in an efficient inhibition of enzymatic activity of MMPS and it was suggested by some researchers that a broad spectrum MMP inhibitor might be considered as therapeutic method of reducing tumor invasion and metastasis in NSCLC.⁽⁸⁾

This study was done to investigate the changes in the pretreatment circulating levels of Endostatin, matrix metalloproteinase (MMP) -2 and -9, in addition of tissue inhibitors of metalloproteinase (TIMP) -1 and to evaluate their clinical implication in patients with advanced non-small cell lung cancer (NSCLC).

PATIENTS AND METHODS

This study included twenty five patients and 10 healthy individuals with matched age and gender, served as control group. They were selected from Al Hada Armed Force Hospital and King Abdul Aziz specialist Hospital, Taif Saudi Arabia from January 2011 to October 2015. The patient group had advanced NSCLC (stages IIIB and IV) and all patients were subjected to the following; history taking and examination, imaging which included chest x-rays and chest CT. Sputum cytology was performed and biopsies were obtained through fiberoptic bronchoscope, CT guided needle, or video-assisted thoroscopy. Metastatic work up included; abdominal and brain CT in addition to bone scan. Pretreatment venous blood samples were obtained from each subject participating in the study. Blood samples were subjected to the following investigations; serum Endostatin was measured by Enzyme-linked immunosorbent assay (ELISA) with monoclonal antibodies specific for Endostatin (Oncogen Research Products) according to the manufacturer's instructions. Serum TIMP-1 was measured by Enzyme-linked immunosorbent assay (ELISA) with monoclonal antibodies specific for TIMP-1 (Oncogen Research Products) according to the manufacturer's instructions. Serum MMP-2 and MMP-9 was measured by gelatin zymography technique. The study was conducted after approval of the ethical boards of the hospitals and written informed consents were taken from all participants. We could follow up 22 patients clinically, radiological and by body scan after a full course of chemotherapy and radiotherapy for a mean period of 9.4 ± 2.3 months.

Statistical analysis: SPSS 18.0 (SPSS, Chicago Illinois) was used for carrying out statistical analysis. Group differences were further analyzed by χ^2 and difference between means of continuous variables was tested by Student's t test. Correlation between parameters was

assayed by multiple regression analysis. Level of significance was determined at $P < 0.05$.

RESULT

Table 1 shows the clinicopathological criteria of NSCLC patients and control groups. Table 2 shows that pretreatment serum levels of Endostatin and TIMP1 in NSCLC patients were significantly elevated ($P < 0.05$) and the patient serum level of MMP2 and 9 were non-significantly decreased ($P > 0.05$). The mean period of survival of stage IIIA patients was 5.4 ± 1.2 months and that of stage IV patients was 4.9 ± 1.1 months (the difference was statistically insignificant). Multiple regression analysis verified a significant correlation between pretreatment levels of Endostatin and TIMP-1 and patients' stages and survival ($P < 0.5$).

DISCUSSION

Angiogenesis is the formation of new blood vessels from the existing vasculature, and neo-vascularization is a prerequisite for the growth of solid tumors beyond 1–2 mm in diameter.⁽²⁾ Pro-angiogenic stimuli may be released by tumors, stromal cells or inflammatory cells, and may trigger an angiogenic switch to allow the tumor to induce the formation of micro-vessels from the surrounding host vasculature.⁽³⁾ Angiogenic growth factors are produced not only by tumor cells, but also by normal bronchiolar and differentiated columnar epithelial cells and alveolar macrophages, however, the activity of these chemokines is balanced by antiangiogenic factors as Endostatin.^(3,4)

In the present study, the pretreatment serum level of Endostatin was higher in NSCLC patients than in healthy controls ($p < 0.001$). Dudek and Mahaseth⁽⁹⁾ reported similar results and concluded that the Pretreatment serum and plasma levels of Endostatin, VEGF and serum basic fibroblast growth factor (bFGF) levels were higher in NSCLC patients than in healthy controls ($P < 0.05, 0.001$ and 0.01 , respectively). Moses *et al.*⁽¹⁰⁾ found that Endostatin levels in the urine of patients with NSCLC were significantly higher than those in patients with the non-malignant diseases ($P = 0.0131$). Walker *et al.*⁽¹¹⁾ also found that the preoperative serum levels of vascular endothelial growth

factor (VEGF) and Endostatin were significantly higher in NSCLC patients with involvement greater than stage IB, compared to other patients.

Hong *et al.*⁽¹²⁾ concluded in his study that addition of angiogenesis inhibitors to the treatment protocol of advanced NSCLC patients were superior to non-angiogenesis inhibitors protocols in terms of objective response rates, disease control rates, progression-free survival, and overall survival.

Members of the matrix metalloproteinase (MMP) family, such as MMP-2 and MMP-9 were found to be implicated in the expansion and metastasis of cancers through degradation of type collagen, which is one of the main constituents of basement membranes and is considered to be the first barrier to tumor metastasis.^(5, 6) These metalloproteinase are inhibited by tissue inhibitors of TIMPs which are generally inhibitors of angiogenesis^(7, 8, 13) In our study, The serum level of TIMP1 in patients with NSCLC was significantly higher in patients with NSCLC compared to normal subjects, however, we found no significant difference between normal subjects and patients with NSCLC in the serum activity of MMP-2 and MMP-9.

Di Carlo *et al.*⁽⁵⁾ detected that MMP-1, MMP-9, and TIMP-1 expression levels were increased in tumor samples compared with matched normal tissues and were correlated significantly with the evolution of tumor, lymph node, and metastasis. The over-expression of TIMP-1 was verified by many authors to be an independent prognostic marker in patients with NSCLC, and evaluating TIMP-1 may be important for identifying patients who are at greater risk of disease recurrence.^(2, 13)

Previous studies showed that both MMP9 and TIMP-1 mRNAs were significantly correlated to lymph node invasion and cancer stage and survival analysis revealed that high levels of expression of MMP9 mRNA was significantly associated with an unfavorable outcome in NSCLC patients.⁽⁵⁻⁸⁾ Pesta *et al.*⁽¹³⁾ in their study reported that increased expression of the two mRNAs, even not necessarily equate their enzymatic activities, seems to parallel a major cancer aggressiveness. High TIMP-1 indicated a poor prognosis of NSCLC, especially in

squamous cell variant and it was recorded in previous studies that more than 50% of lung cancer patients with a plasma TIMP-1 level < 3.0 ng/ml survived for 12 months while less than 30% of the lung cancer patients with level above 3.0 ng/ml survived for more than one year.^(11, 14)

Similar results were obtained in our study where multiple regression analysis verified that the pretreatment levels of Endostatin and TIMP-1 were significantly correlated with patients' stages and survival ($P < 0.5$).

CONCLUSIONS

Our results indicated that the circulating levels of angiogenic growth factors (VEGF), Endostatin, and TIMP-1 in patients with NSCLC may be valuable future tools for treatment planning and monitoring of treatment, however, these blood tests need to be standardized and validated in large-scale prospective clinical trials.

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Table (1): Clinicopathological criteria of NSCLC patients and control groups

Parameters	Total (N)	Mean± SD Age (years)
Healthy control subjects.	10	63.1± 12.6
NSCLC patients.	25	63.7± 12.4
Histopathological types:		
Squamous cell carcinoma	10	64.7± 15.2
Adenocarcinoma	13	62.9± 14.8
Large cell carcinoma.	2	63.8± 15.2

Table 2: Serum levels of angiogenic growth factors in NSCLC patients

	NSCLC Group N=25	Control group N=10	Significance
Endostatin Mean ± SD	354.40 ± 64.01 Ng/ml	81.20 ± 13.99 Ng/ml	P< 0.001 S
TIMP1 Mean ± SD	2.96 ± 0.58	1.49 ± 0.29	P< 0.01 S
MMP1 Mean ± SD	0.09 ± 0.03 Ng/ml	0.14 ± 0.03 Ng/ml	P> 0.05 NS
MMP9 Mean ± SD	0.10 ± 0.03 Ng/ml	0.13 ± 0.019 Ng/ml	P> 0.05 NS

14. Significant; S Non Significant; NS