MMP-2, TIMP-2, and MMP-2/TIMP-2 complex levels in epidermoid lung cancer

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Aim: To investigate serum levels of MMP-2, TIMP-2, and MMP-2/TIMP-2 complex in epidermoid lung cancer and determine whether one of these markers or any combination of them can be used as a prognostic factor and in the management of this disease.

Materials and methods: Twenty-eight patients with non-small cell lung cancer and 21 healthy, age-matched individuals participated in this study. MMP-2, TIMP-2, and MMP-2/TIMP-2 complex levels were measured in the sera of the patients and controls with the ELISA technique.

Results: MMP-2, TIMP-2, and MMP-2/TIMP-2 complex levels were significantly elevated in patients with non-small cell lung cancer compared to the controls (P = 0.018, 0.041, and 0.034, respectively).

Conclusion: MMP-2, TIMP-2, and MMP-2/TIMP-2 complex levels increased not only in lung tissues, but in the sera of the patients with non-small cell lung cancer. However, further studies are needed to fully evaluate the expression rates and diagnostic efficiency of these parameters in different histopathological subtypes of this cancer and in prospective research with a large number of participants.

Key words: Epidermoid lung cancer, MMP-2, TIMP-2, MMP-2/TIMP-2 complex

Introduction

The Ministry of Health declared in 1997 that lung cancer is the most frequent type of cancer in Turkey, accounting for 17.6% of all cancer cases. Each year, lung cancer cases comprise 15% of newly diagnosed cancer cases and cover 18% of cancer mortalities (1). The major cause of death in lung cancer is metastasis as in other malignancies (2).

The extracellular matrix acts as a barrier against tumor growth and invasion. Malignant tumors use matrix metalloproteinases to skip over this barrier (3–6) by degrading the extracellular matrix. MMPs are 28 extracellular proteases that play a role in physiological and pathological tissue degradation. They are synthesized from various epithelial and mesenchymal derived cells, including leukocytes, keratinocytes, fibroblasts, macrophages, chondrocytes, and smooth muscle cells. Arthritis, inflammation, multiple sclerosis, chronic wounds, chronic lung damage, bronchial asthma, and pulmonary hypertension are the major pathologies in which MMPs are involved apart than cancer (7). MMP-2 (gelatinase A) and MMP-9 (gelatinase B) are responsible for degradation of Type IV collagen and were found to be correlated with tumor aggressiveness and metastatic potential (8–10).

Furthermore, tissue inhibitors of metalloproteinases (TIMPs) play a pivotal role against the activity of MMPs and distant site metastasis (11,12). To date, 4 types of these inhibitors have been isolated in humans: TIMP-1, TIMP-2, TIMP-3, and TIMP-4 (13,14). Both MMPs and TIMPs have been investigated in lung cancers for diagnostic, prognostic, and therapeutic purposes.

Non-small cell lung cancer (NSCLC) is the general name of a broad cancer family with subgroups such as epidermoid carcinoma (squamous cell carcinoma), adenocarcinoma, and large cell carcinoma (15). Epidermoid carcinoma accounts for about 30%–40% of primary lung tumors. In this study, we aimed to investigate the serum levels of MMP-2, TIMP-2, and MMP-2/TIMP-2 complex in epidermoid lung cancer and to determine whether one of these markers or any combination of them can be used in the prognosis and management of this disease.

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2. Materials and methods

2.1. Study groups

This study was performed in 28 patients (27 males, 1 female) who were admitted to the pulmonary medicine and thoracic surgery departments with the diagnosis of NSCLC based on their physical examination, radiological findings, and corresponding bronchoscopic and pathological evaluations. A total of 21 healthy individuals (18 males, 3 females) participated as the control group. Patients who were newly diagnosed with NSCLC, lack of any other cancer history, acute or chronic disease history, or permanent drug use were included in the study. Blood samples were collected before any radiotherapy or chemotherapy treatment. Healthy individuals without an acute or chronic disease history, permanent drug use, or smoking habit participated in the study as the control group. The protocol of the study was approved by the local ethics committee and informed consent was obtained from all of the participants. The demographical characteristics of the study groups are shown in Table 1.

2.2. Biochemical analysis

Fasting venous blood samples were obtained from all participants in the early morning and placed into anticoagulant-free serum separator tubes and were subsequently centrifuged at $2100 \times g$ for 10 min at 4 °C. The serum portions were separated and transferred into microtubes and stored at –60 °C until analysis. Serum levels of MMP-2, TIMP-2, and MMP-2/TIMP-2 were measured using the ELISA method. Quantikine Human MMP-2 (R&D Systems, USA & Canada, Cat. no: DMP200), Human TIMP-2 (RayBio, USA, Cat. no: ELH-TIMP-001), and Human MMP-2/TIMP-2 (R&D Systems, USA & Canada, Cat. no: DY 1497) kits were used for circulating serum levels of MMP-2, TIMP-2, and MMP-2/TIMP-2, respectively. The sensitivity levels of these assays for MMP-2, TIMP-2, and MMP-2/TIMP-2 were 0.16 ng/mL, 10 pg/mL, and 25 pg/mL, respectively. Briefly, aliquots of samples, standards, and controls were added to antibody-coated microplate wells and incubated. After aspiration and 4 washes, horseradish peroxidase-conjugated MMP-2, TIMP-2, or MMP-2/TIMP-2 complex antibodies were added. This step was followed by sequential incubation with substrate solution and stop solution. The absorbances of each well were determined at 450 nm (wavelength correction at 540 nm) using a microplate reader. Quantitations were achieved by the construction of standard curves using known concentrations of MMP-2, TIMP-2, and MMP-2/TIMP-2 complex.

2.3. Statistical evaluation

SPSS 13 for Windows was used for statistical evaluation of the data. The Shapiro–Wilk test was used to test the distribution pattern of the numerical data and the Mann–Whitney test was used to compare the medians between the groups. The results were presented as median (min–max). ROC curves were used to evaluate the diagnostic efficiency of these parameters. The ability of these parameters to distinguish cancer patients from healthy individuals was tested with logistic regression analysis. $P < 0.05$ was considered statistically significant.

Table 1. Demographic characteristics of the non-small cell lung cancer and control groups.

Parameter	Cancer	Control
N (female/male)		
1/27		
3/18		
Age (years)	53.75 ± 7.3	51.19 ± 7.34
Cigarettes (packs per year)	35 ± 13.54	
Histopathological type		
Adenocancer	3 (10%)	
Epidermoid cancer	25 (90%)	
Stage		
IB	2	
IIB	4	
IIIA	8	
IIIB	7	
IV	7	

3. Results

In our study, MMP-2, TIMP-2, and MMP-2/TIMP-2 complex levels were significantly higher in the cancer group compared to the control group (Table 2). Subgroup comparisons for different histopathological types of this cancer could not be performed because almost all of the cases were epidermoid carcinoma (n = 25). Because almost of the cases were epidermoid cancer and since it is the most common type of NSCLC we evaluated the data and formulated our commentary and conclusions about the levels of MMP-2, TIMP-2, and MMP-2/TIMP-2 in epidermoid lung cancer.

There was no statistically significant difference between the serum levels of these parameters for different stages of the cancer. MMP-2 levels were 17.56 ± 3.73 and 18.07 ± 3.4 ng/mL, TIMP-2 levels were 6.51 ± 0.49 and 6.99 ± 0.54 ng/mL, and MMP-2/TIMP-2 complex levels were 1.02 ± 0.30 and 0.91 ± 0.31 ng/mL in stage I+II and stage III+IV, respectively.

We performed ROC analysis and tested the diagnostic efficiency of these parameters However, none of these parameters ensured the required efficiency (P = 0.07, 0.08, and 0.06 for MMP-2, TIMP-2, and MMP-2/TIMP-2, respectively).

In logistic regression analysis, only MMP-2 has limited ability to distinguish epidermoid lung cancer cases from the healthy ones (P = 0.055).

4. Discussion

In this study we measured serum levels of MMP-2, TIMP-2, and MMP-2/TIMP-2 complex in epidermoid lung cancer. MMP-2, TIMP-2, and MMP-2/TIMP-2 complex levels were significantly higher in NSCLC patients compared to the controls.

MMPs, the proteolytic enzymes of the extracellular matrix, induce tumor invasion and the metastatic process based on the facilitation of the angiogenesis and degradation of extracellular matrix. However, MMPs' tissue inhibitors have been reported to inhibit tumor growth and invasion in NSCLC (16).

The results of the previous reports about MMPs in NSCLCs are heterogeneous and vary from weak positivity rates to significantly increased expression (16–20). Some researchers reported no significant difference in MMP-2 levels among different histopathological subtypes of NSCLCs (17,20,21). On the other hand, some others showed significantly higher levels of MMP-2 in squamous cell carcinoma and adenocarcinoma (18,19,22). Our results are consistent with the findings of these reports. Because nearly almost of the cases were epidermoid cancer we could not compare these parameters for different histopathological subtypes.

Yurdakul et al. reported that 14.3% of their patients were MMP-2 positive and 85.7% were negative among NSCLC patients. Our results conflict with these findings. It can be suggested that enzyme expression in tumor tissue may not reflect the serum levels exactly.

There are conflicting reports about the relation between MMPs and stage of NSCLCs (23). Some reports suggested that no relationship exists between stage of cancer and MMP-2 levels in NSCLCs (19,22,24,25). Other researchers detected a significant association between stage and MMP-2 expression (17,18,21,25). We did not find a significant difference for serum levels of MMP-2 among different stages of the cancer.

The results of studies on TIMP-2 expression in NSCLCs are heterogeneous. The expression rates vary from 45% to 82% (16,22,26). Although we and many other authors demonstrated that TIMP-2 levels were increased in NSCLCs, Suemitsu et al. reported that TIMP-1 levels were elevated in NSCLC patients while TIMP-2 levels were decreased (27). They interpreted these results as showing that the reaction of immunohistochemical staining for TIMP-2 was mild in areas of in situ and invasive carcinoma, whereas the reaction was stronger in the basal cells at the interfaces between areas of basal cell hyperplasia or squamous metaplasia and dysplasia or carcinoma in situ. They concluded that TIMP-2 levels in

Table 2. Serum levels of MMP-2, TIMP-2, and MMP-2/TIMP-2 in the cancer and control groups.

Parameter	Cancer (median, min–max)	Control (median, min–max)	P
MMP-2 (ng/mL)	17.75 (13.43–28.46)	16.23 (8.06–18.97)	0.018
TIMP-2 (ng/mL)	6.97 (5.31–8)	6.569 (4.31–7.98)	0.041
MMP-2/TIMP-2 complex (ng/mL)	0.88 (0.53–1.8)	0.73 (0.46–1.14)	0.034

their patients with NSCLC were low because of the low-grade histological type.

The authors of previous reports are not in agreement about the impact of the histopathological subtype or the stage. Some researchers declared that there was no statistically significant association between histological subtype and TIMP-2 levels in NSCLCs (22,25,28). However, Albelda et al. reported that the difference in TIMP-2 levels was significant during the transformation of the metaplastic epithelium to SCC (29). In a recent article, it was demonstrated that the expression of TIMP-1 and TIMP-2 was higher in adenocarcinoma than in squamous cell carcinoma (30). Similarly, the results interpreting the effect of the tumor stage on TIMP-2 levels are conflicting. There are reports suggesting that the stage has no significant effect on TIMP levels (22,25). However, some authors demonstrated that the expression of TIMP-1 levels was correlated with tumor stage and patient survival data (28,31). We did not find any significant difference for serum levels of TIMP-2 among different stages of the cancer. We measured MMP-2, TIMP-2, and MMP-2/TIMP-2 levels in order to evaluate which one of these parameters would be more beneficial in distinguishing epidermoid lung cancer cases from the healthy ones and to test the diagnostic efficiency of these parameters. However, none of these parameters ensured the required efficiency. In logistic regression analysis, only MMP-2 presented limited ability to distinguish epidermoid lung cancer cases from the healthy ones.

Our study demonstrated that serum MMP-2, TIMP-2, and MMP-2/TIMP-2 complex levels were significantly elevated in patients with epidermoid subtype of NSCLC compared to the control group. Our findings indicating increased levels of both free and complex forms of MMP-2 with its tissue inhibitor TIMP-2 are in agreement with previous reports suggesting that epidermoid cancer invades and metastasizes by extracellular matrix degradation (3,8,9).

Because of the pattern of our study group (almost of the cases were epidermoid cancer) we could not make an evaluation and comment about MMPs, TIPMs, and MMP/TIPM complexes among different histopathological subtype of NSCLC.

Similarly, our results are in agreement with previous reports suggesting that the stage of the tumor also had no effect on either MMPs or TIMPs in NSCLCs.

There are few studies in the available literature evaluating both MMPs and TIMPs in lung cancer and most of them were performed in small cell lung cancer. Michael et al. investigated MMP-1, MMP-2, MMP-3, MMP-9, MMP-11, MMP-13, and MMP-14 and TIMP-1, TIMP-2, TIMP-3, and TIMP-4 expressions in small cell lung cancer using the immunohistochemical technique

and demonstrated that their productions had increased at alternating rates in the tumor tissues (32). There are also a few reports that have evaluated both MMP-2 and TIMP-2 in NSCLC. Eren et al. also demonstrated through immunohistochemistry that tissue expressions of MMP-2 and TIMP-2 were increased in patients with NSCLC (25). In another study, it was demonstrated that MMP-2, MMP-9, and TIMP-1 levels were elevated, while TIMP-2 levels were decreased in tumor tissues compared to normal tissues in NSCLC (26). In our study, TIMP-2 levels were increased in epidermoid lung cancer. Our results are in agreement with the results reported by Eren et al. We suggest that the higher percentage of advanced stage cancer cases might be the cause of the higher TIMP-2 levels. In a recent article, expression of MMP-2, TIMP-1, and TIMP-2 was evaluated and it was suggested that all of these parameters were higher in adenocarcinoma than in squamous cell carcinoma (30). Lim et al. also measured the expression levels of both MMP-2 and TIMP-1 and reported that they were correlated with tumor stage and patient survival data (31).

Most authors who measured the levels of MMP-2 and TIMP-2 in lung cancer reported their concentrations at tissue levels (16–26). There are 2 reports in the literature evaluating the serum levels of MMP-2 and TIMP-2 in lung cancer patients. Garbisa et al. measured MMP-2 levels in the serum of lung cancer patients and observed that serum levels increased with advancing stages and were significantly increased in patients in stage IV compared to the control group (33). We did not find any significant difference between the serum levels of MMP-2 among different stages of epidermoid cancer.

Ylisirniö et al. detected serum MMP-2, MMP-9, TIMP-1, and TIMP-2 levels as prognostic markers and also MMP-2/TIMP-2 complex levels in order to demonstrate both free and complex forms of MMP-2. Conflicting with the previous reports and our data, they suggested that both serum TIMP-2 and MMP-2/TIMP-2 complex levels were decreased in cancer patients compared to healthy ones (28). In our study, MMP-2, TIMP-2, and MMP-2/TIMP-2 complex levels were increased in epidermoid lung cancer compared to the healthy controls. It can be suggested that MMP-2/TIMP-2 complex levels also increase as a tissue defense mechanism and this accompanies the overproduction of these enzymes involved in tissue degradation.

The underlying mechanisms of the increased expression of MMPs and TIMPs in NSCLC are still unclear. It had been suggested that TIMP-2 prevented the proliferation and invasion of cancer cells by inhibiting MMP-2. However, Bourboulia et al. observed that TIMP-2-mediated inhibition of tumor growth occurs independently of MMP inhibition and is caused by both the direct effects

of TIMP-2 on tumor cells and modulation of the tumor microenvironment (34); but it is known that MMP-2 can also be inhibited by TIMP-2, not only by TIMP-2.

We measured both free and complex forms of MMP-2 and TIMP-2 to determine whether one of these markers or any combination of them can be used in the prognosis and management of this disease. We observed that both of the free fractions, as well as the complex form, were significantly increased. In ROC analysis the diagnostic efficiency of these parameters was tested, but none of these parameters ensured the required efficiency. According to logistic regression analysis results, only MMP-2 has limited ability to distinguish epidermoid lung cancer cases from healthy ones. In epidermoid lung cancer overproduction of MMP-2/TIMP-2 complex levels accompanies the overproduction of MMP-2. This can be explained by the defense mechanism of the tissue. It can also be suggested that any of these parameters can be used for monitoring the response to therapy and prognostic evaluation of the patient with this type of cancer. However, none of these parameters achieved the sufficient diagnostic specificity and sensitivity.

The small number of patients in the study group was the major limitation of our study. We would have compared the levels of these parameters in different histopathological types and we would have probably demonstrated the diagnostic efficiency of one or more of these parameters if a higher number of participants had been included in the study.

Expression of MMPs and TIMPs in tumor tissues was determined with immunohistochemistry, in situ hybridization, RT-PCR, and zymography (16–27). We measured serum levels and used the ELISA technique in our study. Our results are almost parallel with the results obtained at the tissue level. Serum analysis of these data is non-invasive and the ELISA technique is simple, easy to perform, economical, and suitable for routine analysis.

We suggest that MMP-2, TIMP-2, and MMP-2/TIMP-2 complex levels are elevated not only in lung tissues but also in the sera of the patients with NSCLC. Measurement of MMPs and their tissue inhibitors in the sera of patients is easier and more practical than determining them in tissue samples, which can be obtained only by invasive procedures such as bronchoscopy or surgery. Measuring the serum levels of these parameters will also provide the clinician with information about the expression rates of MMPs and TIMPs and be sufficient to formulate an idea about the proteolytic characteristics of non-small cell tumors. However, further studies with a larger number of participants are needed to perform a full evaluation of the expression rates and ensure the diagnostic efficiency of these parameters in different histopathological subtypes of lung cancer.

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