RESEARCH ARTICLE

Matrix Metalloproteinase-13 - A Potential Biomarker for Detection and Prognostic Assessment of Patients with Esophageal Squamous Cell Carcinoma

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Abstract

Background: Matric metalloproteinase (MMP) 13 gene expression is increased in esophageal squamous cell carcinomas (ESCCs) and associated with increasing tumor invasion, lymph node involvement and decreased survival rates. Levels of the circulating enzyme may be elevated and used as a marker of tumor progression. In this study, clinical application of MMP-13 serum levels was evaluated for early detection, prediction of prognosis and survival time of ESCC patients. Materials and Methods: Serum levels of MMP13 were determined by ELISA in 66 ESCC patients prior of any treatment and 54 healthy controls for comparison with clinicopathological data through statistical analysis with Man Whitney U and Log-Rank tests. In addition, clinical value of MMP13 levels for diagnosis was evaluated by receiver operating characteristic (ROC) test. Results: The serum level of MMP-13 in patients (>250 pg/ml) was significantly higher than in the control group (<100 pg/ml) (p value=0.004). Also the results showed a significant correlation between MMP-13 serum levels with tumor stage (p value = 0.003), depth of tumor invasion (p value=0.008), involvement of lymph nodes (p value = 0.011), tumor size (p value = 0.018) and survival time. While there were no significant correlation with grade and location of tumors. ROC analysis showed that MMP-13 level is an accurate diagnostic marker especially to differentiate pre-invasive/ invasive lesions from normal controls (sensitivity and specificity: 100%). Conclusions: These findings indicate a potential clinical significance of serum MMP13 measurement for early detection and prognostic assessment in ESCC patients.

Keywords: Esophageal SCC - MMP13 - serum marker -ELISA - diagnosis - prognostic assessment

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Introduction

Esophageal cancer is the eighth most common cancer and the sixth leading cause of cancer deaths worldwide. It is also one of the ten most common malignant tumors with rapid progression and poor prognosis in patients. Because of the late occurrence of clinical symptom, the 5-year survival rate in patients is low, at approximately 13% (Ghadimi et al., 2011). The "Asian esophageal cancer belt," an area with a high prevalence of esophageal cancer, includes central Asia, Russia, Mongolia, and northern Iran. Esophageal squamous cell carcinoma (ESCC) is the dominant type of cancer in these areas, whereas adenocarcinoma is more common in areas with lower incidence of ESCC (Taziki et al., 2011).

Matrix metalloproteinases (MMPs) comprise a large group of zinc-dependent endopeptidases with the ability to cleave peptide bonds in most extracellular matrix proteins. Degradation of extracellular matrix occurs in many physiologic and pathologic processes (Yoon et al., 2003). To date, more than 26 members of this enzyme family have been identified and classified into eight groups, which include collagenase, gelatinase, metalloelastase, enamelysin, epilysin, stromolysin, matrilysin, and transmembrane metalloproteinase (Ghadimi et al., 2011; Sbardella et al., 2012).

MMPs are involved in many diseases including cancer, cardiovascular, central nervous system, fibrotic lung, and gum diseases, arthritis, and corneal and gastric ulcers. Moreover, MMPs break down biological molecules in various ways, inhibiting their abilities to participate in cellular signaling pathways (Mannello et al., 2006;

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Amalinei et al., 2007). Several studies have demonstrated that MMPs participate in cancerous changes that occur in tumorigenic cells. Cancerogenesis involves multiple processes that include the lack of dependence on growth factors, lack of response to growth factors, avoidance of programmed cell death (apoptosis), unlimited growth, proliferation, angiogenesis, the creation of new lymphatic vessels, and eventually, epithelial-mesenchymal cell transition (EMT) (Hanahan and Weinberg, 2000; Colotta et al., 2009). MMP13, also known as collagenase-3, has the ability to disrupt collagen types I, II, III, IV, VI, and X. In addition, MMP13 can degrade many non-collagenous compounds, including gelatin, tenascin, entactin, fibronectin, and fibrilin-1 (Leeman et al., 2002). Expression and activity of MMP13 is regulated by protein-induced transcription factors, post-transcriptional regulation by micro-RNA, activation of the pro-enzyme, and tissue inhibitors (Clark et al., 2008; Cruz-Munoz and Khokha, 2008; Akhtar et al., 2010). Increased MMP13 gene expression has been reported in various cancers including squamous cell carcinoma (SCC) of the cervix and vulva (Johansson et al., 1999), and breast (Zhang et al., 2008), lung (Hsu et al., 2006), esophageal (Etoh et al., 2000), gastric (Elnemr et al., 2003) and colorectal (Leeman et al., 2002) cancers. The increase in MMP13 expression in these cancers has been associated with patients' survival rates. In addition, in gastric cancer patients increased MMP13 expression was also associated with tumor invasion and progression. A recent study of Chinese ESCC patients found that increased MMP13 in serum was associated with tumor progression and survival rates (Jiao et al., 2014). To date, increased MMP13 expression has been reported in several esophageal carcinoma studies and correlated with tumor behavior and patient prognosis; however, MMP13 levels in esophageal cancer patients have not been thoroughly investigated. The aim of this study was to measure MMP13 levels in ESCC patients' sera and correlate MMP13 levels with patients' clinicopathological characteristics. The selection of patients at various disease stages was an important characteristic of this study.

Materials and Methods

Patients

From 2008 to 2013, 30 female and 36 male patients with histologic diagnoses of ESCC, and 23 male and 31 female controls were enrolled in the study. Patients ranged in age from 28 to 83 years and were divided into three subgroups based on the method of sample acquisition; these were in situ and invasive endoscopy, and esophagectomy (Table 1).

Inclusion criteria for admission of patients were access to their blood samples before receiving any treatment, including surgery, chemotherapy, or radiotherapy. The study was approved by the Ethical Committee of Mashhad University of Medical Sciences (MUMS) and consent forms were obtained from all patients before all procedures. After blood samples were drawn, all patients were treated with surgery or chemotherapy and follow-up information was collected from 45 patients. Of those 45 patients, 35 died during the study. Blood samples were also collected from 54 healthy control subjects. All samples were tested for hepatitis B and C viruses (HBV and HCV), and human immunodeficiency virus (HIV). Six samples from the original control group of 60 subjects were excluded due to hemolysis. From both groups, 5 ml blood samples were collected and centrifuged at 2000 x g. Serum was collected and stored at -80 °C. Before testing, all serum samples were diluted two-fold and serum MMP13 was measured by ELISA according to the manufacturer's protocol (Sigma, Germany).

Statistical Analysis

All statistical evaluations were performed using SPSS software (v. 11.5). Since MMP13 level was not normally distributed, its associations were assessed using non-parametric tests that included the Mann-Whitney test, Kruskal-Wallis test, and Spearman's Rho correlation coefficient. Receiver operating characteristic (ROC) analysis was carried out to find the diagnostic value of MMP13 levels in different histopathological situations. To analyze survival, we selected the median MMP13 concentration of 395 pg/mL as a criterion to stratify the patients based on MMP13 concentration, and then univariate survival analyses of time to death were performed using the Kaplan-Meier method. The log-rank test was used to analyze survival differences between groups. Statistical results with $p < 0.05$ were considered significant.

Results

Serum MMP13 concentrations

Preoperative MMP13 serum levels were higher in patients than in the control group (> 200 vs. <100 pg/ml, $p < 0.0001$). MMP13 levels in patients' sera correlated with tumor severity. Sera from patients with in situ carcinoma (endo-SCC-in situ), invasive carcinoma (endo-SCC-invasive), and surgical resections contained 250-350, 350-450, and > 450 pg/mL of MMP13, respectively (Table 2).

Serum levels of MMP13 and Cilinicopathological parameters

Statistical analysis serum levels of MMP-13 in ESCC patients before surgery showed that there were closely related to the depth of tumor invasion (T classification), lymph node involvement (N status), tumor stage, tumor size and survival rate.

Assess the relationship between MMP-13 with a depth of tumor invasion, lymph node involvement, tumor stage and location of the tumor showed that patients with greater the penetration into the lower layers of the esophagus

Table 1. Categorization of Patients Groups Based on Clinicopathological Character

Sex	Groups			
	Esophagectomy	Endo-SCC-invasive	Endo-SCC-in situ	Control
Male	16	15	5	23
Female	16	13	1	31
Total	32	28	6	54

Table 2. Relationship between Serum Levels of MMP-13 and Clinical Status of Patients

	In situ carcinoma (Endo-SCC-in situ)	Invasive carcinoma (Endo-SCC-invasive)	Tumor resection (Esophagectomy)	Control
Endo-SCC-in situ	-	NS*	-	P < 0.0001
Endo-SCC-invasive	NS(non-significant)	-	P = 0.036	-
Esophagectomy	-	P = 0.036	-	-
Control	P < 0.0001	-	-	-
Malignancies (Endo-SCC-in situ+ Endo-SCC-invasive+ Esophagectomy)	P = 0.017	-	-	-

Table 3. Relation to Serum Level of MMP-13 with Pathological Parameters

Pathological parameters	Pathological parameters features	N	Serum MMP13 concentration Mean (95% CI)	P value
Tumor depth	T1+T2	12	379.1 (330.8-427.4)	0.008
	T3	19	494.5 (429.8-559.1)	(Mann-Whitney U test)
Metastasis to regional lymph nodes	N0	21	413.1 (359.2-466.9)	0.011
	N1	8	546.8 (445.7-647.9)	Mann Whitney U test (Nx omitted)
	NX	2	447.9 (381.7-514.1)	
Tumor stage	Stage II	22	411.7 (360.5-462.9)	0.003
	Stage III	7	570.1 (468.9-671.3)	(Mann Whitney U test)
Tumor grade	G1	5	435.6 (270.4-600.9)	NS
	G2	23	435.5 (381.2-489.8)	(Kruskal-Wallis Test)
	G3	3	583.6 (422.4-744.4)	
Tumor size	Size < 5cm	21	411.2 (365.3-457.1)	0.02
	Size > 5cm	10	530.8 (428.2-633.5)	(Mann Whitney U test)
Tumor location	Mid-third	19	436.5 (375.4-497.6)	NS
	Low-third	11	454.6 (371.7-537.6)	(Mann Whitney U test)

Table 4. Summary of ROC Analysis and Diagnostic Indices

	AUC	P value	Cut-off	Sensitivity	Specificity	PPV	NPV	PLR	NLR
Preinvasive/invasive vs. normal	1	0	191	100%	100%	100%	100%	-	0
insitu vs. normal	1	0	213	100%	100%	100%	100%	-	0
Invasive vs. insitu	0.791	0.02	371	64.40%	100%	22.20%	100%	2.8	0
low stage vs. high stage carcinomas	0.864	0.004	479	86.40%	85.70%	95.00%	66.70%	6	0.2

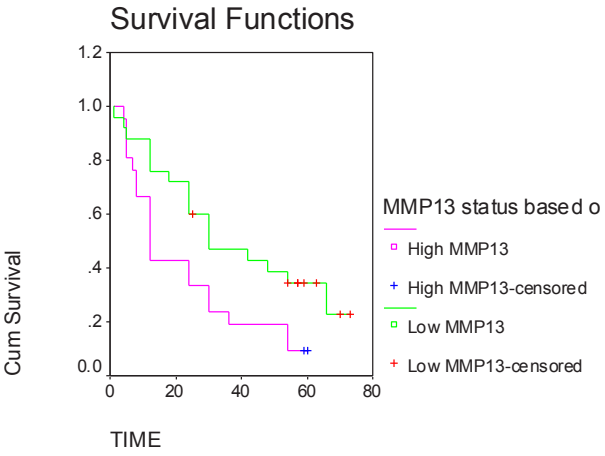

Figure 1. Overall Survival Curves. Analysis was according to MMP13 serum level by using Kaplan-Meier estimate with log rank test. (p value = 0.048)

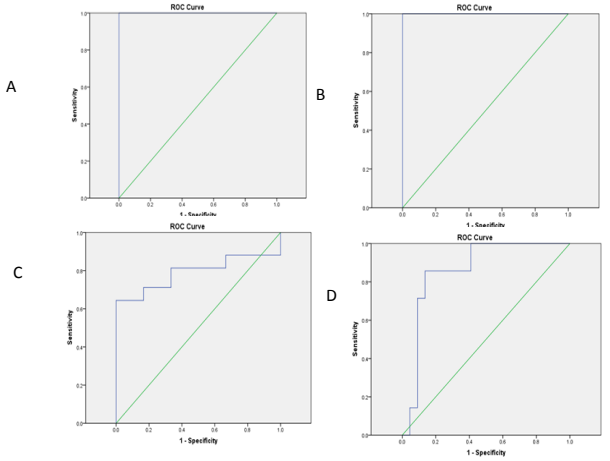

Figure 2. ROC Curve Representing Diagnostic Performance of Serum MMP13 levels in Segregating Different Hitopolthologic Condition. Areas under the ROC curve, sensitivities, and specificities were calculated and compared. A: control vs. malignant, B: in situ vs. normal, C: Invasive vs. in situ, D: low stage vs. high stage carcinomas

tissue had higher levels of MMP-13 in circulation (Mann Whitney U-test, p value = 0.008). Also, patients had metastasis to lymph nodes (p value = 0.011), and higher stage have higher levels of MMP-13 in the serum (p value = 0.003). In addition, there is no correlation between tumor location and the blood levels of MMP-13 (p value = NS). Relationship between tumor grade and levels of MMP-13 were determined by using the Kruskal-Wallis Test and results showed that the degree of differentiation

of the tumor cannot be associated with levels of MMP-13. The results of Spearman Rho correlation test showed that there was a significant correlation between tumor size and levels of MMP-13 patients (p value = 0.018, r = 0.422). The median overall survival time was 24.0 months (95% CI: 16.1-31.9) for all patients. Censored rate was 22.2%. The patients with high MMP-13 levels (> 395 pg/mL) showed a significantly lower OS than patients with low MMP-13 levels (<395 pg/mL) (median OS; 12.0 (8.4-15.6) versus 30.0 (9.2-50.8), Log Rank test, p-value= 0.0263). Figure 1 shows the corresponding Kaplan Meier curve. Receiver operating characteristic (ROC) analysis showed that MMP-13 level is a useful diagnostic test to differentiate pre-invasive/invasive lesions from normal controls, in situ lesions from invasive ones, in situ lesions from controls, and low stage carcinomas form high stage carcinomas. Table

Discussion

Early cancer detection is critical to treatment strategies and outcomes. Previous studies have shown that even diagnostic methods based on direct sampling of esophageal cells cannot always identify malignancies due to the insufficient sensitivities and specificities of these methods(Gholamin et al., 2010). For this reason biomarkers that can easily and quickly aid in diagnoses are necessary. Several studies have shown increased MMP13 expression in cancer tissues and that this increased expression in some cases can predict tumor behavior and patients' survival times(Chang et al., 2009). For example, a study of Chinese gastric cancer patients found that serum MMP11 levels were higher and survival rates lower in patients with lymph node metastases than those of patients without metastases(Yan et al.). In patients with melanoma, serum MMP9 and MMP1 levels can be considered markers for fast metastasis(Nikkola et al., 2005). In our study serum MMP13 levels were significantly higher in Iranian ESCC patients than in normal subjects; therefore, MMP13 can be used to differentiate patients with malignant disease from healthy individuals. We also observed MMP13 levels in the three groups of patients differed; MMP13 in the endo-SCC-in situ, endo-SCC-invasive, and surgical resection were 250-350, 350-450, and > 450 pg/mL, respectively. Depth of tumor invasion into surrounding tissues and lymph node involvement define tumor stages, and in fact, the best predictors of tumor behavior in all types of cancer(Vazquez-Sequeiros et al., 2003; Rice et al., 2010). Patients with MMP13 concentrations greater than 450 pg/mL who underwent resections were found to be in stage II of cancer with progression to lymph nodes and adventitia layer. Tumor size is expected to increase as cancer progresses. Tumors were measurable in 31 patients who had undergone surgical resection. In patients with tumors < and > 5 cm in size, the mean serum MM13 concentrations were 411.2 and 530.8 pg/mL, respectively. Survival data was collected from 45 patients. At the end of the study, 22.2% of the patients remained alive (censored). Patients with serum MMP13 concentrations > 395 pg/mL had lower survival rates than patients with serum levels < 395 pg/mL. No association was found between serum MMP13 levels and tumor location or differentiation. MMP13 concentration as a serum marker significantly correlated with patients' clinical and pathological characteristics; therefore, it can be used to predict tumor behavior and for early diagnosis and treatment. The study also showed that serum MMP13 levels are accurate indicators of histological states, particularly for differentiating malignant from pre-malignant neoplastic lesions of free samples, with sensitivities and specificities of 100%. Serum MMP13 concentration may be useful as an indicator of as neoplastic lesions in patients with indigestion (dyspepsia), a common symptom in ESCC patients. A diagnosis of the invasive state can be rejected if serum the MMP13 concentration is < 371 pg/mL, with 100% specificity. This information could be especially useful to pathologies examining small endoscopic biopsies with suspicious areas. MMP13 concentrations can also be used to differentiate between advanced vs. low-level disease and thus can be effective in guiding future therapies. Currently, biochemical markers and tumor-related microenvironmental factors are utilized for tumor staging and prognosis. Our results indicate that the MMP13 level can be an important component of an ESCC prognosis system. Larger and prospective studies are recommended to verify these findings to determine more precisely the most critical MMP13 values.

In conclusion, based on this study, serum MMP13 levels in patients with ESCC could be utilized as a tumor marker. MMP13 levels in ESCC patients were significantly associated with the degree of tumor invasion, lymph node involvement, tumor size, and patient survival rates. In addition, measurement of serum MMP13 can be a valuable diagnostic test to differentiate histological conditions, especially in cases requiring endoscopy.

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