Journal of Clinical Neuroscience 22 (2015) 1282–1287

Contents lists available at ScienceDirect

Journal of Clinical Neuroscience

journal homepage: www.elsevier.com/locate/jocn

Clinical Study

Matrix metalloproteinase-9 and tissue inhibitor of matrix metalloproteinase-2: Prognostic biological markers in invasive prolactinomas

Güliz Demirelli Gültekin [a,*], Burak Çabuk [a], Çiğdem Vural [b], Savaş Ceylan [a]

[a] Department of Neurosurgery, Kocaeli University School of Medicine, Umuttepe, İzmit, Kocaeli, Turkey
[b] Department of Pathology, Kocaeli University School of Medicine, Umuttepe, Izmit, Kocaeli, Turkey

ARTICLE INFO

Article history:
Received 29 October 2014
Accepted 4 February 2015

Keywords:
Invasion
Matrix metalloproteinase-9
Pituitary adenoma
Prolactinoma
Tissue inhibitor of matrix metalloproteinase-2

ABSTRACT

In this study, the predictive roles of matrix metalloproteinase (MMP)-9 and tissue inhibitor of matrix metalloproteinase (TIMP)-1 and 2 in invasive and noninvasive prolactinomas were examined. Prognostic biomarkers to distinguish between invasive and noninvasive pituitary adenomas are required for the effective treatment of pituitary adenoma patients. We analyzed 57 prolactinoma patients classified as having invasive and noninvasive adenomas for MMP-9, TIMP-1 and TIMP-2 expression using immunohistochemical methods. Significantly higher MMP-9 expression was detected in invasive prolactinomas ($p = 0.004$). There was also a significant relationship between TIMP-2 expression and invasive behavior ($p = 0.004$) and TIMP-2 expression and recurrence ($p = 0.005$). Because MMP-9 expression is significantly increased in invasive prolactinomas, MMP-9 has potential as a marker for invasion. TIMP-2 may be a marker for both invasion and recurrence. These findings require further examination in large scale prospective studies.

1. Introduction

Pituitary adenomas are common neoplasms. Although they are generally benign in nature, they may invade adjacent tissues. Recurrence and resistance to treatment are serious problems in invasive pituitary adenoma patients, therefore, when treating pituitary adenomas, biological markers are needed to indicate the invasiveness of the tumor to effectively guide surgical, medical, and radiotherapy treatment modalities and to prevent recurrence. Pituitary adenomas are epithelial tumors that arise from the adenohypophysis. The most frequently detected pituitary adenomas are prolactinomas [1]. Prolactinoma treatment is primarily with dopamine agonist drugs (DA; cabegoline, bromocriptine) [2,3].

In this study, prolactinomas were investigated with a focus on the roles of matrix metalloproteinase (MMP)-9 and tissue inhibitor of matrix metalloproteinase (TIMP)-2 in pituitary adenoma invasion. Only prolactinomas were investigated in order to concentrate the research on the roles of MMP-9 and TIMP-2 in pituitary adenoma invasion.

The MMP family consists of zinc and calcium-dependent endopeptidases. These enzymes are associated with proteolytic degradation of the extracellular matrix (ECM) [4]. The role of MMP in tumor invasion and metastatic processes has been confirmed. ECM degradation by MMP is a critical process in the progression of malignant tumors, angiogenesis, and tumor invasion and metastasis. Gelatinases (MMP-9 and MMP-2) are thought to be key enzymes in ECM degradation as they degrade some of the main components of the ECM [5,6]. TIMP are low molecular weight proteins that are secreted and bind to active MMP, inhibiting their enzymatic activity [7].

Moreover, MMP-2 and MMP-9 are unique among the MMP in that the latent forms of these proteinases can form complexes with TIMP-2 and TIMP-1, respectively. In addition to their inhibitory role, TIMP can also take part in the activation of MMP [8]. TIMP-2 is a powerful inhibitor of MMP activity and high levels of TIMP-2 are positively correlated with an unfavorable prognosis in cancer patients [9,10].

Previous studies have investigated MMP-9, TIMP-1 and TIMP-2 as prognostic biological markers in breast, lung, colon and many other cancer types, and the correlations of these enzymes with invasion and metastasis have been determined [11–14]. However, MMP-9 studies in pituitary adenomas have yielded

* Corresponding author. Tel.: +90 050 57891621; fax: +90 380 5497060.
E-mail address: op.drgulizgultekin@yahoo.com (G.D. Gültekin).

http://dx.doi.org/10.1016/j.jocn.2015.02.021
0967-5868/

conflicting results and TIMP-1 and TIMP-2 have not been studied as potential prognostic biological markers in pituitary adenomas.

In this study, we evaluated the presence of MMP-9, TIMP-1 and TIMP-2 in invasive and noninvasive prolactinomas and investigated their potential as prognostic predictors using immunohistochemical methods.

2. Methods and materials

In the present study, we retrospectively reviewed the records of patients with prolactinoma who had undergone endoscopic transsphenoidal surgery in the Department of Neurosurgery, Kocaeli University School of Medicine between 1999 and 2011. Fifty-seven patients who were diagnosed with prolactinoma based on clinical, radiological and pathological data were included in this study. The mean ages of the patients in the invasive prolactinoma and noninvasive prolactinoma groups were 43.2 years and 35 years, respectively (range: 16–69). There were 11 females and 24 males in the invasive group (n = 35), and 18 females and four males in the noninvasive group (n = 22). The indications for surgery in the invasive group were as follows: resistance to DA treatment (n = 5), intolerance to DA (n = 6), and patient preference (n = 24). The indications for surgery in the noninvasive group were as follows: resistance to DA (n = 9), intolerance to DA (n = 6), and patient preference (n = 7). In the noninvasive group, all patients (n = 22) were first time surgeries, and 31/35 patients in the invasive group were first time surgeries. Four patients in the invasive group were second surgeries. Radiotherapy was not performed in any patient.

None of the prolactinoma patients had multiple endocrine neoplasia syndrome. Three patients with atypical meningioma and two with chordoma were included as a positive control group.

Grade 3 and 4 tumors, according to the Knosp classification [15], and grade 4D and 4E tumors, according to the modified Hardy classification [16], were considered to be invasive prolactinomas and radiological invasion was intraoperatively confirmed. Thirty-five patients were classified as having invasive prolactinomas and 22 with noninvasive prolactinomas. Treatment outcomes were evaluated according to prolactin (PRL) level in the blood and sella MRI on the first day after operation. Normal PRL values were considered to indicate remission and were monitored for recurrence or cure. A PRL level higher than 21 ng/dl was considered to indicate persistent recurrence. In the noninvasive group, 14/22 patients (64%) were cured and in the invasive group none were cured (0/35; 0%). Persistent recurrence was detected in 31/35 patients (88%) in the invasive group and 7/22 (32%) in the noninvasive group. In the invasive group, late recurrence was detected in 4/35 patients (12%) and the average time to late recurrence was 10 months after surgery. In the noninvasive group, late recurrence was detected in 1/22 patients (4%) and the time to recurrence for that patient was 4 years. Patients were observed for cure and recurrence from 3 to 14 years after surgery.

2.1. Histopathological examination

MMP-9 (Thermo Fisher Scientific, Waltham, MA, USA), TIMP-1 (Clone: 102D1; Thermo Fisher Scientific), and TIMP-2 (Clone: 3A4; Thermo Fisher Scientific) antibodies were applied to sections obtained from formalin fixed and paraffin embedded blocks. Sections (4 μm) were mounted on poly-L-lysine coated glass and de-paraffinized after being kept in a 56°C incubator overnight. They were placed in xylol for 30 minutes, absolute alcohol for 15 minutes, 96% alcohol for 15 minutes and then washed with water. Antigens were retrieved by boiling the sections in citrate buffer (pH 6) in a microwave oven for 15 minutes. Endogen peroxidase activity was blocked by quenching in a 3% hydrogen peroxide solution for 15 minutes. Tissue sections were incubated at room temperature for 30 minutes with primary antibodies against MMP-9, TIMP-1 and TIMP-2. A secondary antibody was applied and the sections were treated with streptavidin-biotin-peroxidase complex for 30 minutes. 3,3-diaminobenzidine was used as a chromogen and Mayer's hematoxylin was used for AEC ground staining. The sections were covered with a water-based cover material. Meningioma and chordoma tissues were used as the positive controls for MMP-9, TIMP-1 and TIMP-2 expression.

The stained tissues were examined by a single pathologist who was blinded to the clinical data. The extent and intensity of MMP-9 staining in the adenomas were evaluated. The extent of MMP-9 staining was scored as negative (0; from 0–30%), 1 (minimal; from 31–60%), 2 (focal; from 61–100%) or 3 (diffuse). The intensity of MMP-9 staining was scored as negative (0), weak (1), moderate (2) or strong staining (3). TIMP-1 and TIMP-2 were used to distinguish the adjacent pituitary tissue from the adenoma and were evaluated as positive or negative depending on whether staining was detected.

2.2. Statistical analyses

Research data were analyzed using the Statistical Package for the Social Sciences (SPSS statistics; version 13.0; IBM Corporation, Armonk, NY, USA). Descriptive statistics and the chi-squared test were used and the significance level was set at $p < 0.05$.

3. Results

3.1. MMP-9 and invasion

MMP-9 expression was detected in all patients (57; 100%). The relationship between MMP-9 expression and invasive behavior was evaluated by the extent of MMP-9 staining measured as the number of adenoma cells showing MMP-9 immunoreactivity. Figure 1 shows MMP-9 staining in one invasive and one noninvasive patient. In invasive prolactinomas, diffuse staining was detected in 85.7% of patients (30/35). A significant relationship was determined between MMP-9 expression and invasive behavior ($p = 0.004$; Table 1).

There was no correlation between MMP-9 staining intensity and invasive prolactinomas. This result conflicted with previous studies, therefore, the possible causative factors of this result were examined. In the invasive group, significant differences in MMP-9 staining intensity were found between patients who took and did not take DA drugs in the preoperative period. Figure 2 shows MMP-9 expression in two patients with invasive giant adenomas, one with DA and one without DA during the preoperative period. In the invasive group, the MMP-9 staining intensities for the DA treated group were strong staining in one (8%), moderate staining in nine (75%) and weak staining in two patients (16%). The MMP-9 staining intensities for the no DA treatment group were strong staining in 10 (75%), moderate staining in 10 (43%) and weak staining in three patients (12%).

3.2. TIMP-1 and invasion

TIMP-1 staining was detected in 31/57 patients (54%) and staining in the adenoma was observed in 16. There was no correlation between TIMP-1 expression and invasion.

3.3. TIMP-2 and invasion

TIMP-2 staining was detected in the adenoma and adjacent tissue sample in 32/57 patients (56%), while staining in the adenoma