RESEARCH ARTICLE

# Serum Level of MMP-3 in Patients with Oral Squamous Cell Carcinoma - Lack of Association with Clinico-pathological Features

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## Abstract

**Background: MMP-3 is a proteolytic enzyme of the matrix metalloproteinase family. Protein degradation which is their fundamental action regulates different activities of tumor cell such as their growth, differentiation, apoptosis, migration, invasion, angiogenesis as well as their resistance to the immune system. Aim: The aim of this study was to determine MMP-3 serum levels in patients with OSCC and investigate if they correlate with clinicopathological features. Method and materials: Using an ELISA kit, we assessed and compared the circulating levels of MMP-3 in blood serum of 45 oral squamous cell carcinoma patients with 45 healthy control samples. Results: The serum MMP-3 level in OSCC patients was significantly higher (9.45±4.6 ng/ml) than healthy controls (5.9±3.6 ng/ml, p<0.001), especially in females and in older patients. However, there was no apparent correlation in serum MMP-3 concentration with the clinico-pathological features such as tumor location, stage, tumor size, nodal status, distant metastasis, histological grade and smoking. Discussion: This result suggests that the measurement of serum MMP-3 concentration might be helpful to diagnose OSCC but not to predict prognosis.**

**Keywords:** MMP-3 - serum - oral squamous cell carcinoma - diagnosis - prognosis

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## Introduction

The matrix metalloproteinase (MMP) is a member of zinc dependent endopeptidase group (Egeblad, 2002). Human MMP system consists of at least 23 MMPs which are divided into five groups based on their substrate and molecular structure: gelatinases, collagenases, stromelysins, membrane type MMPs and other less well characterized MMPs (Baker et al., 2006). MMPs are proteolytic enzymes and in cancer they regulate various cell behaviors by degradation of proteins. These include cancer cell growth, differentiation, apoptosis, migration, invasion and regulation of tumor angiogenesis and immune surveillance (Egeblad, 2002).

Matrix metalloproteinase-3 (MMP-3, stromelysin-1) is a member of MMP family which is capable to degrade a broad range of substrates including collagen type II, IV, IX, X and XI, fibronectin, gelatins, elastin, proteoglycanase, E-cadherin and osteopontin (Agnihotri et al., 2001; Sternlicht, 2001; Amălinei et al., 2007). MMP-3 is produced by different types of cells such as synovial cells, monocytes, macrophage, fibroblasts and chondrocytes (Tolboom et al., 2002; Constantin et al., 2002; Bar-Or et al, 2003). MMP-3 is produced as an inactive substrate that needs cleavage by proteolytic enzymes (like trypsin-2) for its activation (Moilanen, 2003). The role of MMP-3 in apoptosis induction, angiogenesis regulation, invasion and metastasis in cancer has been established. MMP-3 reveals pathological expression in many tumors; such as melanoma, skin squamous cell carcinoma, colorectal neoplasia, gastric cancer, head and neck squamous cell carcinoma (HNSCC) and breast cancer (Tsukifuji et al., 1999; Linkow et al., 2007; Gershtein et al., 2008; Tas et al., 2008; Jeffery et al, 2009; Yeh et al., 2012). MMP-3 expression has been used as an impending diagnostic and/or prognostic marker of some cancers (Fang et al., 2005; Liu et al., 2011). A few studies have found a correlation between the expression of MMP-3 and histopathological characteristic of tumor[(Kurahara SI,et al,1999),( Erdem NF,et al, 2007)]. The relation between tissue expression of MMP-3 and ivasion ,recurrence and metastasis of oral squamous cell carcinoma (OSCC) has demonstrated in different studies (Kurahara et al.,1999; Wiegand et al., 2005; Erdem et al., 2007). Tissue expression of MMP-3

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in oral squamous cell carcinoma has been investigated through different methods such as Immunohistochemistry, ELISA and polymerase chain reaction (PCR) (Kusukawa et al.,1995; Baker et al., 2006; Liu et al., 2007). However, MMP-3 serum level in patients with OSCC and its correlation with clinicopathological features of tumor is not utterly identified. Moreover, measurements of MMP-3 in blood is easier and faster than in tissue; hence we examined MMP-3 serum levels in patients with OSCC and assessed if it is correlated with clinicopathological characteristics of tumor.

## Materials and Methods

This study was enrolled by 45 OSCC patients (22 males and 23 females; mean age 57±16 years) and 45 healthy control subjects (22 males and 23 females; mean age 56.6±15 years). All the study patients were admitted to the ENT Department of Shiraz University of Medical Sciences and they had histopathological diagnoses of OSCC. Patients with other malignancies, inflammatory diseases, or infections were excluded. Control cases were healthy nonsmoker blood donors with no evidence of systemic or inflammatory diseases, or infections. They were also matched for age and gender. All participants were thoroughly informed about the research study and agreed to participate then signed a consent form. Serum samples were driven from centrifuged clotted blood at 4°C which were stored at -80°C since analysis time. Galectin-3 concentrations were measured by ELISA in accordance with the manufacturer's instructions (BM S 2014/2 INST; Bender Med Systems GmbH, Germany). Independent t-test was performed to compare the results of serum MMP-3 concentrations between controls and study participants. ANOVA and Fisher's exact test were used as appropriate to validate the MMP-3 level relation with clinicopathological features of OSCC.

## Results

Table 1 shows the clinical data of patients assayed for serum MMP-3 level. At the time of study, most of patients were in stage IV (49%). All of the patients had localized tumor (M0). A total of 21 (46%) tumors were well-differentiated, 13 (38%) moderately-differentiated and 7 (15%) were poorly-differentiated.Data for tumor grading of five patients were not accessible

The serum MMP-3 level in OSCC patients was significantly higher (9.45±4.6ng/ml, n=45) compared with the healthy controls (5.9±3.6ng/ml, n=45, p<0.001). The serum level of MMP-3 was higher (11.44±4.8ng/ml (in females compared to males (7.35±3.6ng/ml, p=0.002). Also, serum MMP-3 level was increased with increasing age (p=0.041). There was no apparent correlation in serum MMP-3 concentration with the clinico-pathological features such as tumor location, stage, tumor size, nodal status and histological grade. The serum MMP-3 level was not statically different between smoker and non smoker patients. (p=0.42)

**Table 1. Clinicopathological Profile of 45 Oral SCC Patients* and Correlation of it with MMP-3 Serum Level**

| Feature | | No. (%) | Pvalue |
|---|---|---|---|
| Gender | Male | 22 (48) | 0 |
| | Female | 23 (52) | |
| Tumor size | T1 | 6 (13) | 0.13 |
| | T2 | 26 (57) | |
| | T3 | 6 (13) | |
| | T4 | 6 (13) | |
| Regional lymph node involvement | N0 | 17 (37) | 0.28 |
| | N1 | 7 (15) | |
| | N2 | 5 (11) | |
| | N3 | 15 (33) | |
| Distant metastases | M0 | 45 (100) | |
| | M1 | 0 (0) | |
| TNM stage | I | 2 (4) | 0.10 |
| | II | 12 (26) | |
| | III | 8 (17) | |
| | IV | 22 (49) | |

*There is missing clinico-pathologic data for one case

## Discussion

MMP-3 is an element of MMP system which performs a substantial role in apoptosis induction, angiogenesis, regulation of tumor growth, invasion and metastasis in cancer. These functions are known to be the fundamental changes in cancer physiology(Egeblad M, Werb Z,2002) .Pathologic appearance of MMP-3 has been found in many cancers (Linkow et al., 2007; Gershtein et al., 2008; Tas et al., 2008; Jeffery et al., 2009; Yeh et al., 2010). Most of investigators on SCC had construed high expression of MMP-3 in patients compared to control groups (Kusukawa et al., 1995; Kurahara et al., 1999; Baker et al., 2006; Vairaktaris et al., 2007). Baker et al. study revealed that expression of MMP-3 in OSCC was predominantly higher than normal tissue (Baker et al., 2006). We used an ELISA kit to measure serum MMP-3 concentration in patients with OSCC. To the best of our knowledge, this is the first report to do so. In our study ,the serum MMP-3 concentration in OSCC patients was significantly higher compared to healthy controls. Therefore the evaluation of MMP-3 serum level can be useful as OSCC marker and may help in diagnosis of OSCC. Linkow et al. demonstrated that serum MMP-3 level in patients with active untreated HNSCC was lower than healthy control groups and also treated patients (Linkow et al., 2007). They used LabMAP technique and evaluated HNSCC which contains different anatomic locatin, while we used ELISA kit and emphatically evaluated oral SCC and these may justify the different results from these two studies. In Choie et al. study, MMP-3 expression wasn't different in patients and control groups (Choi et al., 2008). They used quantitative real polymerase chain reaction (qRT-PCR) to evaluate MMP-3 expression. As PCR does not provide the information about the level of gene product, their different results compared with our study might be related to the different methods used. We didn't find any correlation between serum MMP-3 concentration and clinico-pathological features of tumor such as tumor

size, stage, nodal status and histopathological grade. Several previous studies have found correlations between MMP-3 expression and clinico-pathological features of OSCC (Kusukawa et al., 1995; Kurahara et al.,1999). For example Kusukawa et al. confirmed that tissue MMP-3 expression is positively correlated with tumor size, depth of tumor, disseminate invasive mode and the elevated incidence of lymph node metastasis(Kusukawa et al., 1995). In Kurahara et al. study, high expression of MMP-3 was directly related to high incidence of metastasis to lymph node (Kurahara et al., 1999). These diverse results may be allied to different methods of evaluating MMP-3 expression. In these two study researchers conveyed immunohistochemistry (IHC) for evaluating tissue expression MMP-3, but we employed ELISA kit to contemplate serum MMP-3 level in subjects. It is possible that all tissue MMP-3 is not released into to the serum,therefore serum level of MMP-3 cannot show the tissue level of MMP-3 .This will show that evaluation of both serum and tissue MMP-3 simultaneously will be useful. Moreover, MMP-3 releases inactively and ELISA can also asses the inactive form of MMP-3, hence using other methods for assessing the active form of MMP-3 is useful to evaluate correlation between serum MMP-3 level and clinico-pathological features of OSCC patients. However, some studies have not found any correlation between expression of MMP-3 and tumor pathology (Baker et al., 2006; Liu et al., 2007). Lue et al. didn't find any correlation between OSCC tissue MMP-3 expression and clinicopathological features of tumor (Liu et al., 2007). In their study, they used RT-PCR method ,a semi-quantitative method which does not convey any information about the products of the gene. Although ELISA test is a fully quantitative method, we didn't find any correlation between serum MMP-3 concentration and clinico-pathological features of tumor, yet.

MMP-3 serum level was positively related with age in our study. This positive correlation had been asserted in some previous studies, too (Thrailkill, 2005; Komosinska-Vassev et al., 2011). It is proposed that increase in MMP-3 serum level with increasing age is the result of hormonal change and physiological process (Thrailkill, 2005). In this study serum MMP-3 concentration was higher in females compared to males. Komosinska-vassevk et al. found different MMP-3 serum level in males and females (Komosinska-Vassev et al., 2011). These findings show the necessity of matching age and sex for analysis of MMP-3 in the pathological conditions.

In our study, the serum MMP-3 level was not statically different between smoker and non smoker patients. Yin et al. studied the changes of matrix metalloproteinase and tissue inhibitors of metalloproteinase (TIMPs) in human fibroblasts treated with tobacco smoke extract. They stated that the expression of MMP-1 and MMP-3 mRNA was considerably increased in a dose-dependent style (Yin et al., 2000). In our study none of the patients had distant metastasis. Some studies showed the relationship between MMP-3 expression and distant metastasis (Kusukawa et al., 1995; Kurahara et al., 1999). Future studies are suggested on patients with far metastasis. Moreover, Patients in this study have not been followed up, hence future studies on the recurrence of tumor and surveillance of patients is recommended.

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