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Review Article

MMP-9 expression levels and the pathological differentiation of oral squamous cell carcinoma (OSCC): a meta-analysis

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Abstract: The purpose of this study is to evaluate the association of MMP-9 expression levels with the pathological parameters in the differentiation of oral squamous cell carcinoma (OSCC). Literature retrieval, selection and assessment, data extraction, and meta-analyses were performed according to the RevMan 5.3 guidelines. We used odds ratios (ORs) and 95% confidence intervals (CIs) to assess the association of MMP-9 expression levels with the differentiation degree of OSCC based on the results of a heterogeneity test. Nine eligible studies including 468 OSCC patients were analyzed. Statistically significant positive associations were found between high MMP-9 expression levels and poorer OSCC differentiation degrees (OR=2.15, 95% CI: 1.40-3.29; *P*=0.0005). Our meta-analysis shows that a high level of MMP-9 expression is an important sensitive coincident indicator for the poorer differentiation degree of OSCC.

Keywords: MMP-9, differentiation, oral squamous cell carcinoma, meta-analysis

Introduction

Oral cancer is a common cancer worldwide, with an incidence rate of about 5% of all malignant tumors, and the global annual incidence rate is 8.2 per 100,000 for males and 2.8 per 100,000 for females [1, 2]. More than 90% of all oral cancers are oral squamous cell carcinomas (OSCC) involving malignancy of the stratified squamous epithelium [3]. Many diseases, including cancer, result from abnormal changes in the epithelial cells as well as an imbalance in the tumor microenvironment (TME) [4]. The immune cells, capillaries, basement membrane, activated fibroblasts, and extracellular matrix (ECM) surrounding the cancer cells constitute the tumor stroma, which is a vital constituent of TME [5]. ECM remodeling facilitates the invasion of adjacent tissue by cancer cells in the TME, thereby making it an essential determinant of cancer cell behavior and progression. Hence, ECM remodeling is a current research hotspot [6, 7].

Matrix metalloproteinases (MMPs) belong to a family of zinc-dependent endopeptidases, which can degrade several types of collagen in the ECM. Hence, they play an important role in tissue repair and ECM remodeling and in turn promote cancer invasion [8, 9]. MMP-9, a member of the MMP family with collagenase and gelatinase activities, is widely known to be highly expressed at the invasive front of OSCC and associated with the progression and aggression of OSCC. However, some studies have shown conflicting results [10-12]. de Vicente et al. showed no association of MMP-9 expression levels with clinical variables, such as tumor stage and recurrence rate [13]. Similarly, Guttman et al. also showed no correlation between MMP-9 expression levels and the size of the primary tumor and neck metastasis in tongue SCC patients [14]. In contrast, increased levels of MMP-9 expression were associated with regional lymph nodes, distant metastases and a poor prognosis of OSCC patients in another study [15]. Ikebe et al. showed that increased levels of MMP-9 expression were related to invasion but not to the metastatic potential in OSCC [16]. Likewise, Tao and Gao conducted a meta-analysis on MMP-9 levels in OSCC patients with and without lymph node

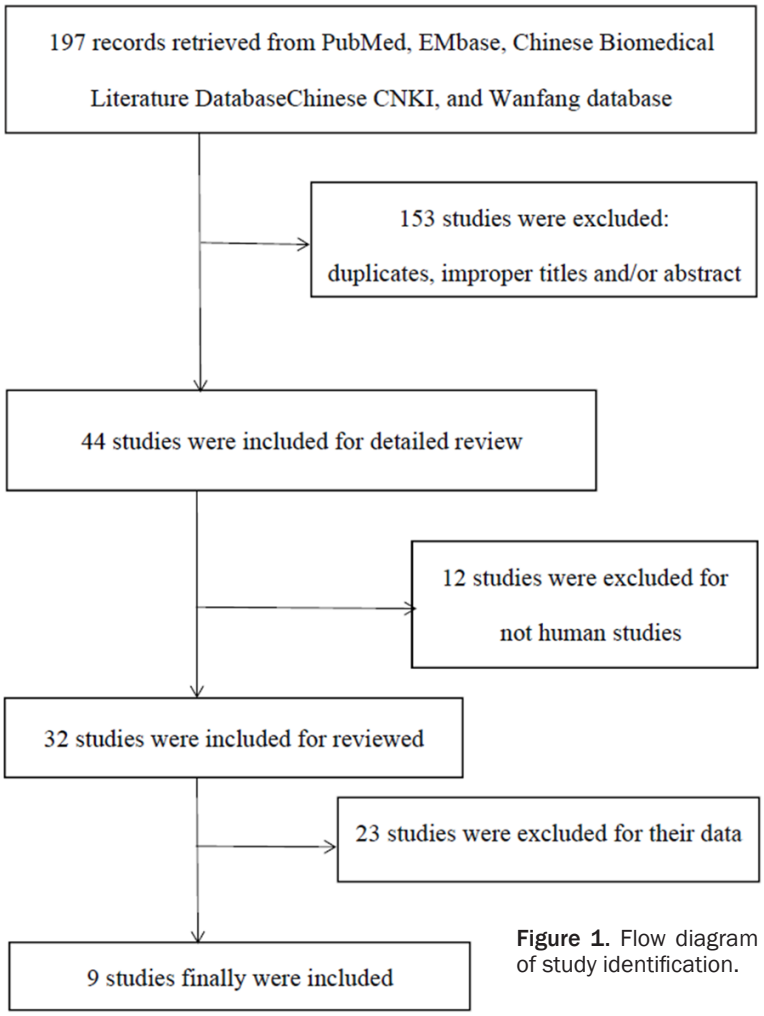

Figure 1. Flow diagram of study identification.

metastasis and concluded that patients with higher MMP-9 expression have fewer tumor metastases [17]. These conflicting results suggested that MMP-9 was not associated with all the clinical variables and pathological parameters of OSCC, so it is unlikely to be a universal promotor of OSCC progression. Cell differentiation disorders are the basic index in pathological parameters and play a critical role in the progression of squamous cancer. Hence, we conducted a retrospective analysis of relevant published studies to evaluate the association between MMP-9 expression levels and the differentiation degree of OSCC.

Materials and methods

Literature search and screening

We searched and identified studies in PubMed, Embase, the Chinese Biomedical Literature Database, Chinese CNKI, and the Wanfang database using the terms "MMP-9" or "Matrix metalloproteinase 9" and "oral cancer" or "oral squamous cell carcinoma" or "OSCC".

Inclusion criteria

We included any study that evaluated the association of MMP-9 expression with the differentiation degree of OSCC, and that was a cross-sectional type study, without restrictions on language or publication year, and that included information on histological grade and/or cell differentiation.

Exclusion criteria

We excluded any study that was case-only, that did not provide valid data required for the differential evaluation of patients with OSCC, that was a duplicate publication or that contained overlapping data or only provided the status of MMP-9 expression without the expression levels.

Data extraction

Information was independently extracted from all eligible publications by two investigators, based on the inclusion criteria. Any disagreements were resolved through discussion. The Cochrane Handbook 5.0 Quality evaluation criteria was used to evaluate the methodological quality of the included studies. The following data were extracted from each study: the first author's last name, year of publication, country of origin, ethnicity of the study population, characteristics of the cases and controls, disease states, detection methods and sample size.

Data analysis

The pooled odds ratios (ORs) with 95% confidence intervals (95% CIs) were used to evaluate the strength of the association between the MMP-9 expression levels and differentiation

Table 1. Basic features and quality assessments of the included studies

First author-year (Refs)	Designs	Patient source	Outcomes measure	Sample size	Gender		Age (Mean age)
					Male	Female	
Andisheh-Tadbir-2016 [18]	Cross-sectional	Iran	Immunohistochemistry	42	28	14	35-81 (54.47)
Fan-2014 [19]	Cross-sectional	China	Immunohistochemistry	74	50	24	38-79 (57.10)
Jafarian-2015 [20]	Cross-sectional	Iran	Immunohistochemistry	100	58	42	20-84 (60.50)
Kim-2006 [21]	Cross-sectional	South Korea	Immunohistochemistry	38	23	15	33-74 (56.20)
Monteiro-2016 [11]	Cross-sectional	Britain	Immunohistochemistry	52	43	17	25-96 (62.00)
Ou-Yang-2008 [24]	Cross-sectional	China	Immunohistochemistry	50	33	17	40-90 (61.00)
Barros-2011 [22]	Cross-sectional	Brazil	Immunohistochemistry	32	NA		NA
Sun-2008 [23]	Cross-sectional	America	Immunohistochemistry	44	NA		NA
Chang-2008 [25]	Cross-sectional	China	Immunohistochemistry	50	27	23	NA

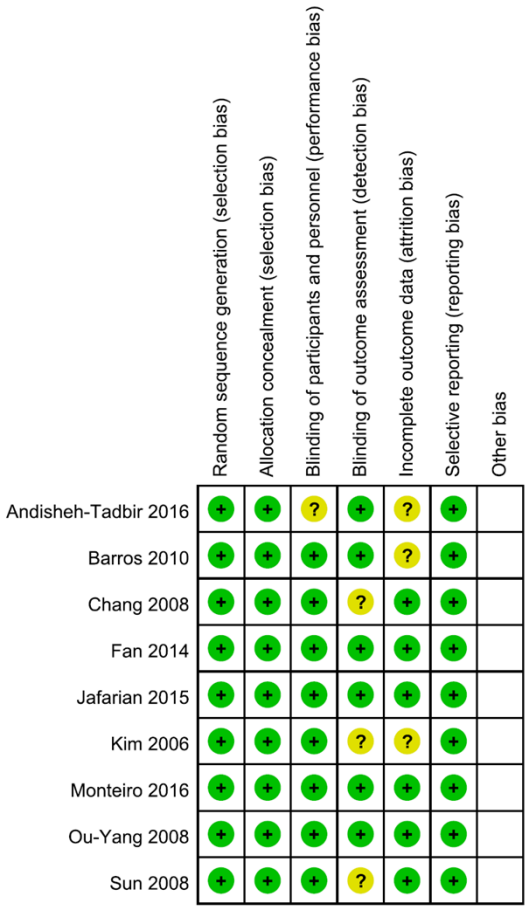

Figure 2. Risk of bias summary for each included study.

degree of OSCC. *Q* and I^2 tests were used to examine the heterogeneity of each study. For the heterogeneity test, the fixed-effects model was selected if $P>0.05$, and the random-effects model was selected if $P<0.05$, in order to calculate the combined OR. $P<0.05$ was considered a significant difference. The publication bias was tested by a funnel plot. All analyses were performed with RevMan 5.3 software provided by the Cochrane Collaboration.

Results

Literature screening

As shown in **Figure 1**, a total of 197 studies were preliminarily identified, of which 188 were excluded due to duplication, no OR and 95% CI data, not clinical, or not human studies. Finally, a total of nine studies reporting [11, 18-25] data on 468 patients with OSCC were analyzed.

Characteristics of the included studies

Of the nine studies, seven were published in English [11, 18-23] and two in Chinese [24, 25]. The sample sizes ranged from 32 to 100. As shown in **Table 1**, six studies were conducted in Asia [18-21, 24, 25], and three in America and Europe [11, 22, 23].

The methodological quality of the included studies is reported in **Figure 2**.

Methodology assessment of MMP-9 expression detection

All the studies adopted immunohistochemistry (IHC) staining for MMP-9 detection.

MMP-9 expression and differentiation of OSCC

The inter-study heterogeneity ($P=0.10$, $I^2=40\%$) was insignificant to confirm any association between high MMP-9 expression and poorer differentiation grade of OSCC. Hence, we used the fixed-effects model to calculate OR,

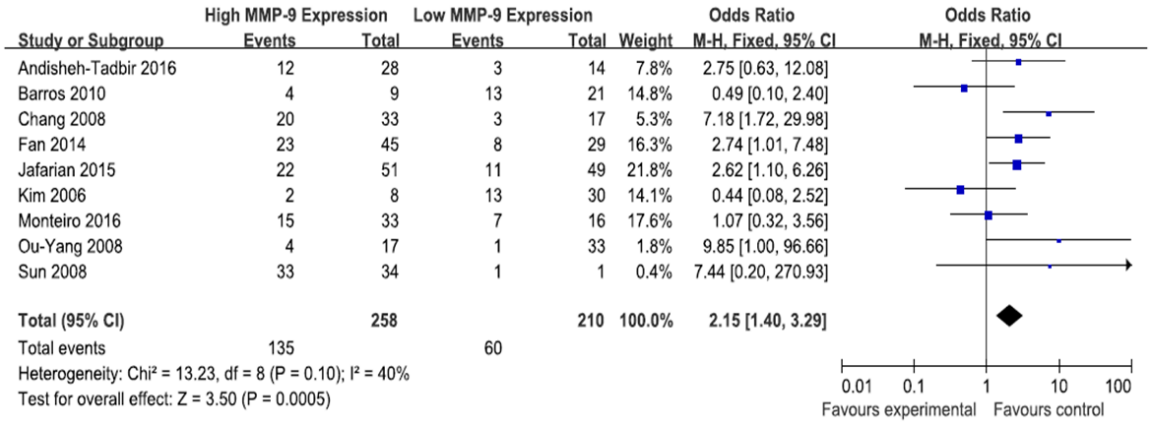

Figure 3. Forest plot of MMP-9 expression and differentiation grading in OSCC. The horizontal lines correspond to the study-specific OR and 95% CI, respectively. The area of the squares reflects the study-specific weight. The diamond represents the pooled results of OR and 95% CI. In this analysis, the fixed-effects model was used.

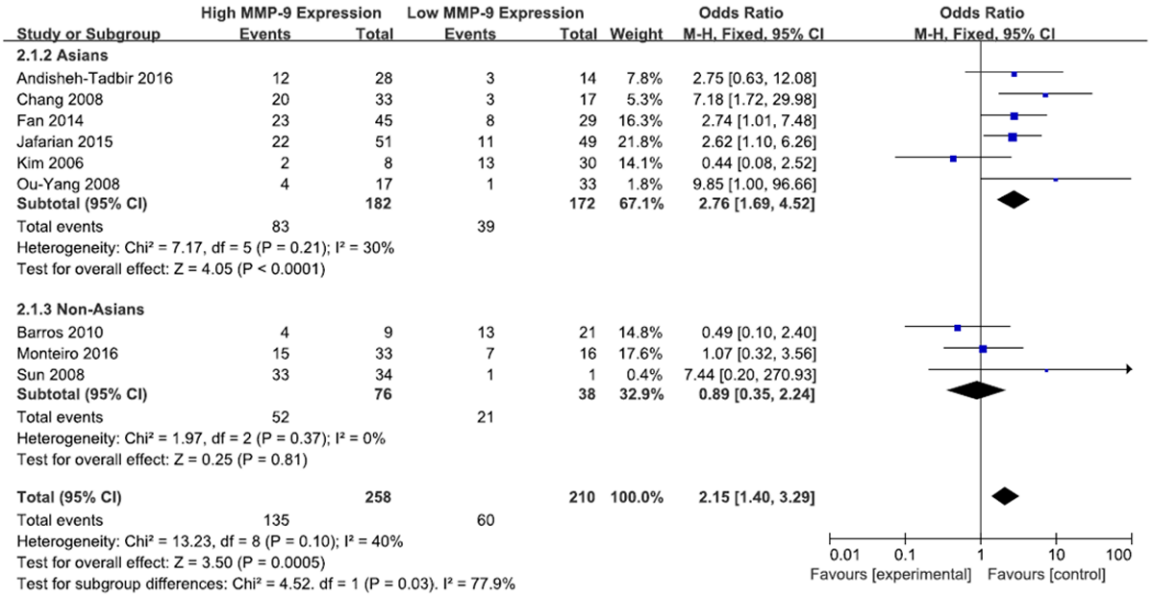

Figure 4. Forest plot of MMP-9 expression and differentiation grading in OSCC grouped according to the region. The horizontal lines correspond to the study-specific OR and 95% CI, respectively. The area of the squares reflects the study-specific weight. The diamond represents the pooled results of OR and 95% CI. In this analysis, the fixed-effects model was used.

which showed a significant association between high MMP-9 expression and poorer differentiation grade (OR=2.15, 95% CI: 1.40-3.29; P=0.0005) (**Figure 3**).

These studies were conducted in different geographical regions, so we grouped them according to their origin. As shown in **Figure 4**, the OR for the differentiation grade in OSCC with high expression of MMP-9 was 2.76 in the Asian population (95% CI: 1.69-4.52, *P*<0.0001), and 0.89 in the non-Asian population (95% CI: 0.35-2.24, *P*=0.81).

Publication bias analysis

We analyzed publication bias with RevMan 5.3 software. The funnel plot in **Figure 5** shows that the points were evenly and symmetrically distributed. A majority of the points fell within 95% CI indicating no publication bias, and the study was credible.

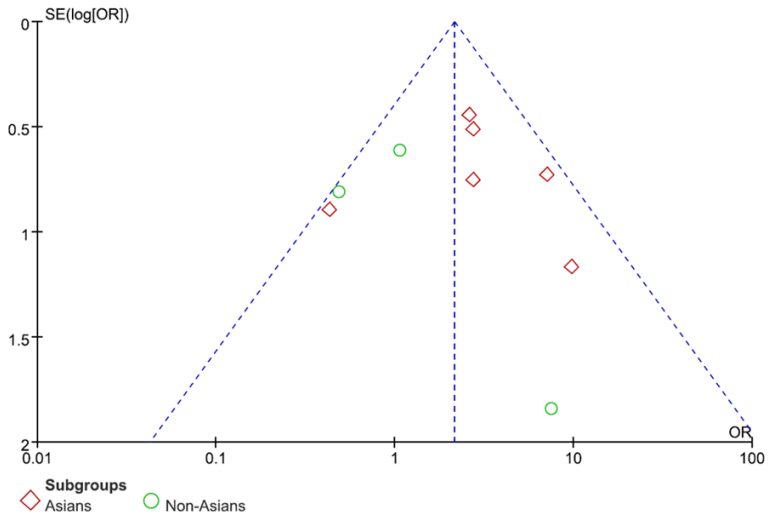

Figure 5. Begg's funnel plot for the publication bias test. Each point represents a separate study for the indicated association. Log OR represents the natural logarithm of OR. The vertical line represents the mean effects size.

Discussion

The main analysis was based on the MMP-9 expression levels and the differentiation status of OSCC, including 468 individual effect estimates from nine studies conducted between 2006 and 2016. In this study, patients with an increased expression of MMP-9 appeared to have a poorer differentiation grade of OSCC than those with lower MMP-9 expression.

Several studies have investigated the expression of MMP-9 in human OSCC and its implication in tumor invasion. MMP-9 is up-regulated in OSCC, and the expression levels are related to tumor invasion, lymph involvement and metastases, indicating that it plays an important role in tumor progression [26]. However, there are no meta-analyses on the association between MMP-9 expression levels and the clinical and/or pathological parameters of OSCC.

This is the first meta-analysis on the association between MMP-9 expression levels and the pathological differentiation grades of OSCC. Three of the nine reports included in this analysis had limitations [21-23], such as small sample sizes, lack of clinical information, lack of categorization by cancer site as well as an unclear description of the inclusion and exclusion criteria. These limitations, particularly the expression levels of MMP-9 with the specific clinical type of OSCC, for example, the severe dysplasia of OLK (in situ carcinoma) was excluded in a study [27], which might have influenced the analysis.

The OR value was a statistical indicator to assess the impact of the different expression levels of MMP-9 on the pathological differentiation grade in OSCC. We enrolled studies with full texts and also included an unpublished study [24]. There was no significant heterogeneity among the studies. After subgrouping the studies into Asian and non-Asian populations, we did not find heterogeneity in either population. Publication bias is a major concern for all forms of meta-analysis; positive results might tend to be accepted by journals, while negative results might often be rejected or not submitted. Although the analysis does not support publication bias, yet the obtained summary statistics likely approximate the actual average, and it should be noted that our meta-analysis could not completely exclude biases. For example, the study was restricted to publications in English and Chinese, which could have introduced bias. Therefore, the results may be impacted by the selection of baseline data.

This meta-analysis of nine studies showed that increased MMP-9 expression was associated with a poorer differentiation grade of OSCC in the Asian population. No significant associations were found in the non-Asian populations due to small sample sizes. It is necessary to conduct studies with large sample size studies in the future.

In summary, despite the study limitations, OSCC with high expression of MMP-9 appears to present poorer tumor differentiation grades as compared to low MMP-9 expression.

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Disclosure of conflict of interest

None.

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