International Journal of Immunopathology & Pharmacology

Original Research Article

Macrophage migration inhibitory factor: A promising oncogenic serological biomarker for oral squamous cell carcinoma

International Journal of Immunopathology and Pharmacology
Volume 35: 1–6

DOI: 10.1177/20587384211038417
journals.sagepub.com/home/iji
SAGE

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Abstract

There are few reports in oral squamous cell carcinoma (OSCC) that indicate the expression of macrophage migration inhibitory factor (MIF) in tissues, serum, or saliva of patients with OSCC. The aim of this study was to evaluate the mRNA expression and protein of MIF in tissues and serum, respectively, in OSCC patients and its association with the TNM stage. A cross-sectional study was performed. Serum and tissues of 25 patients with OSCC and 25 healthy control subjects (HCS) were included to evaluate the MIF mRNA expression and protein serum levels by real-time PCR and ELISA, respectively. Serum MIF levels were significantly higher in OSCC compared with control subjects. Furthermore, in the OSCC group, MIF was significantly increased in accordance with tumor disease stage (TNM III–IV), as well as in poorly differentiated tumors. The mRNA showed significantly higher levels in HCS, as well as in more differentiated tumors. The results of this study suggest that MIF could be an indicator of severity and progression of OSCC. Further studies are required to explore the role of MIF as a serological biomarker for OSCC.

Keywords

oral squamous cell carcinoma, oral cancer, macrophage migration inhibitory factor

Date received: 24 March 2021; accepted: 22 July 2021

Background

Oral squamous cell carcinoma (OSCC) along with oropharyngeal carcinoma represents the most common malignant tumor of the head and neck region. In 2020, the estimated annual incidence of the OSCC was approximately 476,000 cases. The incidence of OSCC is increasing, together with the poor prognosis[1,2] and its important esthetic and functional sequel. This neoplasm represents a serious public health problem, and therefore, it is required to study the behavior of this neoplasm in order to elucidate the biological mechanisms of the development and progression of the OSCC.

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The immune system plays an important role in the development and promotion of cancer. Macrophage migration inhibitory factor (MIF) is a potent pro-inflammatory mediator, and this pleiotropic cytokine is involved in the regulation of innate and adaptive immunity. MIF can be used as a biomarker useful in cancer and in disorders with an inflammatory component.[3] The activation of MAPK/PI3K/Akt pathways by MIF promotes the proliferation and survival of tumor cells, protumorigenic immune evasion, and angiogenesis processes.[3,4] Moreover, there are reports that indicate the MIF overexpression in tissues, serum, and saliva of patients with OSCC;[5,6] however, the association with clinical factors remains unclear. Since there are no studies evaluating the expression of MIF mRNA in OSCC tissues and there are no reports comparing the serum levels of MIF in OSCC patients versus HCS, it was our aim to investigate the correlation of MIF mRNA expression in the tumor tissue and protein serum levels with the clinical–pathological parameters in patients with OSCC.

Patients and methods

Subjects. A cross-sectional study was performed. A total of 25 patients with OSCC histological diagnosis were recruited from the Head and Neck Cancer Surgery Service of the Jalisco Cancer Institute. None of the patients were taking corticosteroids and none of them reported a personal history of autoimmune disease or inflammatory diseases. Serum and tissue samples were obtained before undergoing radiation therapy or chemotherapy. Histological grade and staging were carried out according to the TNM classification of malignant tumors TNM system and the World Health Organization histological differentiation guidelines.[7] For comparison of mRNA expression and serum soluble MIF levels, age and gender-matched 25 subject controls with healthy non-inflamed oral cheek mucosa were included. We calculate the sample size based on the data reported in GLOBOCAN.[2] Oral cavity and lip cancer show a frequency of 0.76% in Mexico; with these data, a confidence interval of 99% was obtained with a minimum sample of 21 OSCC patients.

Ethical considerations

This study was approved by the Ethics Committees of Jalisco Cancer Institute (registration no. 021/2015). All participants signed the informed consent, and the experiments were performed according to the ethical principles of the Declaration of Helsinki.

Real-time polymerase chain reaction

Total RNA was obtained from homogenized tissues using TRIzol reagent (Invitrogen, MA, USA) according to the Chomczynski and Sacchi[8] method. RNA purity and concentration were determined by spectrophotometry (NanoDrop 200c, Thermo Scientific, MA, USA). First strand complementary DNA was synthesized from 1 µg of total RNA by reverse transcription using the oligo(dT)15 primer (Promega Corporation, WI, USA) according to the manufacturer's instructions. The mRNA levels were determined by real-time PCR through UPL hydrolysis probes (Roche Applied Science, BY, Germany). The probes and primers were obtained with a program by Roche Applied Science (Universal Probe Library Assay Design Center), and the *MIF* mRNA sequence was used: NCBI ID number NM_002,415.1 (Cat. No. 04687990001). The *GAPDH* (glyceraldehyde 3-phosphate dehydrogenase) was used as a reference gen (Cat. No. 05190541001). The LightCycler Nano System (Roche Applied Science, BY, Germany) was used to perform the PCR reactions. For the analysis of *MIF* mRNA expression, the $2^{-\Delta\Delta Cq}$ and $2^{-\Delta Cq}$ method was used, and the efficiency of the reaction of the gene of interest (*MIF*) as well as the reference gene (*GAPDH*) was validated by running serial dilutions.[9]

Quantification of MIF serum levels

Peripheral blood samples (5 mL) were taken from both groups in Vacutainer tubes (BD, USA), between 9 and 10 am. The tubes were set at room temperature for 10 min and then centrifuged at 1500 r/min for 15 min. Serum was placed in 1.5 mL Eppendorf tubes (Thermo Fisher Scientific, MA, USA) and stored at −80°C until use. Quantitative determination of MIF levels in the serum of all study participants was performed using the enzyme-linked immunosorbent assay (ELISA) of the Legend Max™ commercial kit (Biolegend, CA, USA) according to the manufacturer's specifications. The MIF assay sensitivity was 1.7 pg/mL. The normal cut-off values of serum MIF reported for clinically healthy subjects are 2–6 ng/mL.[10]

Statistical analysis

SPSS software version 2.0 (SPSS, Inc., IL, USA) and GraphPad Prism version 5.0 (GraphPad Inc., CA, USA) were used. A one-sample Kolmogorov–Smirnov test was used to check for normality distribution. The analysis of the relationships and comparisons between clinical–pathological parameters was performed using the Mann–Whitney test. Data are shown as median and $p25^{th}$ and 75^{th}. Only p values <0.05 were considered significant.

Results

A total of 25 patients with OSCC histological diagnosis for this study were recruited. Clinical–pathological characteristics are listed in Table 1. The mean age of the patients

Table 1. Clinical and pathological parameters of patients with oral squamous cell.

Variable	N	(%)	MIF ng/mL median (p^{25th}–p^{75th})	P
Age, years				
≤50	7	(28)	22.94 (11.88–28.65)	0.97
>50	18	(72)	15.96 (9.91–41.41)	—
Gender				
Male	18	(72)	23.80 (9.61–46.43)	0.31
Female	7	(28)	15.09 (9.94–16.83)	—
Tumor size				
T1–T2	14	(56)	15.96 (9.48–32.54)	0.39
T3–T4	11	(44)	24.65 (10.12–52.23)	—
Lymph node metastasis*				
Positive	13	(52)	28.65 (16.25–48.36)	0.11
Negative	5	(20)	9.94 (6.20–28.05)	—
TNM stage				
I–II	8	(32)	10.54 (7.43–14.47)	0.01
III–IV	17	(68)	24.65 (13.74–48.36)	—
Histologic differentiation*				
Well and moderately	16	(64)	19.36 (11.33–34.72)	0.02
Poor	4	(16)	70.52 (37.80–84.00)	—

*Incomplete/not evaluable.

was 60.8 years old, with a range of 31–87 years. In the majority of patients presented in advanced stages of the disease (III and IV), while well and moderately differentiated tumors were the most common histological type, the main localization was tongue.

Interestingly, we found significant high levels of mRNA in tissues from HCS compared to patients with OSCC (p = 0.001), as well as in TNM stages III–IV (p = 0.007). Although there were no significant differences, it was found that the mRNA levels were higher than in stage I and stage II tumors, compared to more advanced tumors (III–IV) (p = 0.71). On the other hand, HCS exhibited significantly higher levels of mRNA compared to patients with well-differentiated tumors (p = 0.008) and moderately differentiated tumors (p = 0.02) (Figure 1(a)-(c)).

The serum levels of MIF were significantly higher in OSCC patients compared with HCS (p < 0.0001) (Figure 1(d)). Table 1 presents the correlations between the serum MIF concentrations and the main clinical and pathological variables. Statistical analysis did not reveal differences between some clinical variables such as age, gender, presence of lymph node metastasis, and tumor diameter nor did we find differences when we analyzed the TNM stage variables individually and the degree of histological differentiation. However, in grouping patients with early TNM stages (I–II) and more advanced stages (III–IV), we found significant associations (p = 0.01); similarly, we found differences when we subgroup the better differentiated tumors (well-moderately and poorly differentiated) (p = 0.02) (Figure 1(e)-(f)).

Discussion

The immune system plays an important role in the oncogenesis of different types of cancer. In OSCC, the expression of different inflammatory mediators has been associated with the development and progression of the disease. There is evidence that indicates a dysregulation of MIF in various types of solid malignancies, which have a direct correlation between increased levels of MIF and a more aggressive cancer phenotype,[6] however the data for the OSCC are scarce. We found that OSCC patients had higher serum MIF levels than subject controls, which is consistent with results from de Souza et al., where they measured serum levels of patients with OSCC prior to treatment and significantly reduced after tumor resection.[4] Although most studies evaluating MIF expression in OSCC have been carried out on tumor tissue through immunohistochemistry, they agree that there is high immunostaining of MIF in tumor cells compared to controls.[5]

It is known currently that MIF is a potent cytokine that has a pivotal role linking inflammation and cancer, through a different MIF signals promotes angiogenesis, cell proliferation, and metastasis.[3,6] Although there is the consensus in the existence of high levels of MIF in patients with cancer, there are discrepancies in the association of MIF with clinical and histopathological classical factors.[4-6] Some authors point out that MIF represents a local regulator rather than a systemic mediator in this disease. It has even been pointed out that the increase in serum

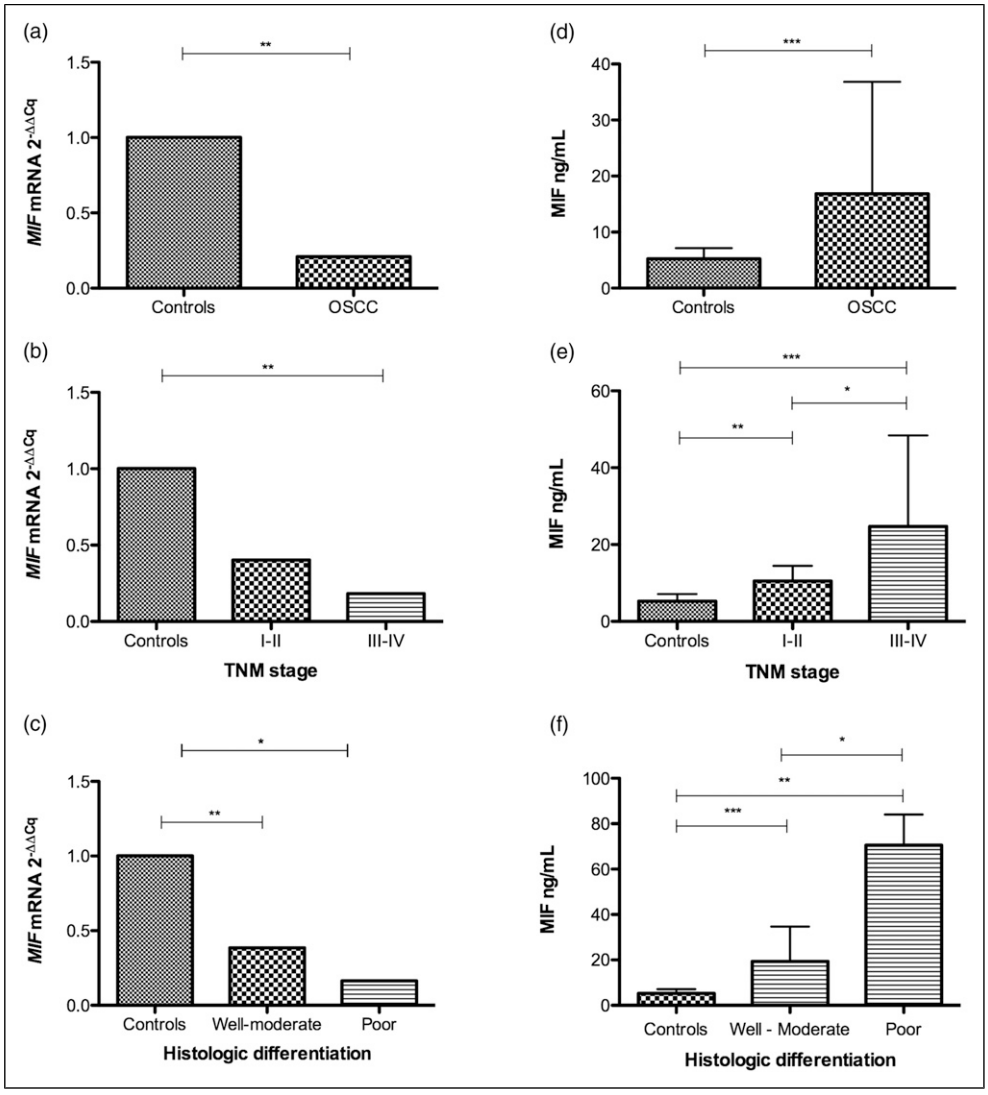

Figure 1. Serum levels and tissue mRNA expression of MIF (a) *MIF* mRNA expression in healthy controls and OSCC patient tissues, $^{**}p = 0.001$, (b) according to TNM stage, $^{*}p = 0.007$ controls versus III–IV; $p = 0.37$ controls versus I–II; $p = 0.71$ controls versus III–IV, as well as (c) according to degree of tumor differentiation, control versus poor, $^{**}p = 0.008$; control versus well-moderate $^{*}p = 0.02$; and well moderate versus poor $p = 0.94$. (d) The quantification of serum MIF levels showed significant differences between the control group (median: 5.25 ng/mL) with respect to OSCC patients (median: 16.81 ng/mL), $^{***}p < 0.0001$. (e) MIF serum levels of OSCC patients according to TNM stage. Early stages (I–II; median: 10.54 ng/mL) compared with the later stages (III–IV; median: 24.65 ng/mL) and controls (median: 5.25 ng/mL), $^{*}p = 0.0156$ I–II versus III–IV; $^{**}p = 0.0067$ controls versus I–II; $^{***}p < 0.0001$ controls versus III–IV. (f) Regarding the degree of cellular differentiation, the OSCC patients with well and moderately differentiated tumors exhibited significantly lower levels of MIF (median: 19.36 ng/mL) compared to the poorly differentiated tumors (median: 70.52) and controls (median: 5.25 ng/mL), $^{*}p = 0.02$ well-moderate versus poor differentiated; $^{**}p = 0.006$ controls versus poor differentiated; $^{***}p < 0.0001$ controls versus well and moderately differentiated. Each bar represents the median value. Results are expressed as nanograms/mL.

concentrations of MIF is due to the inflammatory response against cancer, rather than being produced by the tumor itself.[4] In the present study, we found that patients in late stages III–IV showed significantly higher serum MIF levels than patients in stages I–II, which is consistent with previous studies. On the other hand, we found that OSCC patients with poorly differentiated tumors showed significantly higher plasma levels of MIF than well and moderately differentiated tumors. Thereby, some authors have reported that MIF is involved in the promotion of both epithelial–mesenchymal (EMT) of some epithelial neoplasms; de-differentiation can occur when neoplastic cells migrate toward new tissues and later form a metastatic focus to survive and proliferate.[11]

Analysis and quantification of mRNA expression can be a good indicator of gene regulation; however it is known that due to multiple regulatory mechanisms, post-transcriptional and post-translational modifications, as well as protein turnover, mRNA abundance does not always correspond to protein levels. In this sense, post-transcriptional regulation is orchestrated by RNA regulons, which are controlled by RNA binding proteins (RBPs) and noncoding RNAs (ncRNAs). In recent years it has been described that many RNA regulons are remodeled during tumorigenesis, playing a central role in cancer progression through the regulation of many mRNAs encoding proto-oncogenes, growth factors, cytokines, and cell cycle regulators.[12] Furthermore, MIF can be stored and exert its biological actions both in the cytoplasm or be secreted into the interstitial space. Currently, the knowledge of the transport mechanisms of MIF towards the cytosol or the extracellular space is very limited.[3] After translation, MIF is deposited in the cytoplasm constituting a "cytosolic storage" awaiting secretory signals. It has been shown that under inflammatory and stress conditions, MIF is upregulated mainly at the level of release rather than transcriptional induction, which suggests that the release of the MIF protein into the extracellular space is regulated by inflammatory stimuli.[13]

There also some limitation in our study, sample size is small, specially the few cases with poor differentiation and early stages disease (I–II), as well as the lack of association between elevated serum levels of MIF with the expression of MIF in OSCC tissues by immunohistochemistry in each OSCC case. To our knowledge, this is the first study where *MIF* mRNA levels were evaluated in tissues of patients with OSCC and HCS, it is interesting in future studies to know the basal expression of *MIF* mRNA in healthy tissues, mainly from surfaces more frequently affected by OSCC such as the tongue, floor of the mouth, or the alveolar process.

Conclusions

The findings of our study suggested that MIF is correlated with progression of OSCC, so it may play an important role in the pathophysiology of OSCC. Future studies are required to assess locally and systemically the role of MIF play in carcinogenesis, as well as to analyze the mechanisms involved in gene expression, translation, transport, and effects of MIF in OSCC tumor cells and the tumor microenvironment.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Ethics approval

Ethical approval for this study was obtained from ethics comittees of Jalisco Cancer Institute (approval number 021/2015).

Informed consent

Written informed consent was obtained from all subjects before the study.

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