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RT-qPCR Analysis of LAMP3 (CD208) Gene Expression in Oral Lichen Planus and Oral Squamous Cell Carcinoma

Farnaz Mohajertehran[1,2], Nooshin Mohtasham[1], Mojtaba Ahmadi[2], Mehdi Shahabinejad*[1], Maryam Mohammadi*[2]

Abstract

Background: Many new studies have been conducted on cellular proteins to use them as prognostic markers or in target therapy through determining the increase or decrease in their expression in the lichen planus and OSCC. LAMP3 protein is one of these proteins which has been recently considered. Thus, considering the unknown etiology of lichen planus, significance of their early diagnosis and treatment and lack of a suitable and final treatment for this disease and oral cancers, and preventing the progression of lichen planus, which can turn into OSCC, we decided to investigate the level of expression of this gene and its effect on the progression, study the connection between these two conditions and the probable factors contributing to their etiopathogenesis.

Methods: In this study, ninety-four paraffin blocks tissue samples of patients were obtained together with their demographic documents. LAMP3 expression was measured RT-qPCR method.

Results: The results show that there is not any significant difference between age and sex population of our study. in squamous cell carcinoma the amount of expression of LAMP3 was higher than lichen planus and healthy margin. Average LAMP3 Gene expression in grade III was higher than group grade I & II in which considering significant level of 5%, it is statistically significant.

Conclusions: According to the findings of this study, it can be concluded that the expression of the LAMP3 gene in SCC lesions is higher than in healthy tissue. Hence, LAMP3 gene expression can be used as a diagnostic biomarker.

Keywords: CD208 protein, LAMP3 protein, oral Lichen Planus, Oral Squamous Cell Carcinoma.

Introduction

Lichen planus is a relatively common autoimmune disease that frequently affects the oral mucosa. It can happen at any age. In this skin condition, the immune system attacks the patient's natural skin, mucosa, hair, or nails. The potential for malignancy, if present, associated with patients who have erosive lichen planus (1).

One of the most common cancers in the head and neck region is oral squamous cell carcinoma (OSCC). It also accounts for more than 9% of all oral cancers. Numerous factors, including genetics, smoking, alcohol, tobacco, and immune system dysfunction, all contribute to the etiology and progression of SCC in the mouth (1).

Many new studies on cellular proteins have been conducted in order to use them as prognostic markers or in target therapy by determining the increase or decrease in their expression in lichen planus and OSCC. LAMP3 (lysosomal associated membrane

1: Oral and Maxillofacial Pathology Department, Oral and Maxillofacial Diseases Research Center, Faculty of Dentistry, Mashhad University of Medical Sciences.
2: Oral and Maxillofacial Diseases Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.
***Corresponding author: Mehdi Shahabinejad; Tel: +98 9133991840; E-mail: shahabinejadm@mums.ac.ir & Maryam Mohammadi; Tel: +98 9032033132; E-mail: Maryam_mohammadi16@yahoo.com.**
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protein 3, XM 005247360.6) is one of these recently identified proteins (1-3).

Dendritic cells (DCs), the most powerful antigen-presenting cells, induce cytotoxic T lymphocytes in the tumour environment. After being exposed to antigens, DCs undergo maturation and MHC enhancement, as well as co-stimulation of their molecules, as well as lysosome-associated membrane glycoprotein 3 (LAMP-3), a specific marker of mature DCs. Mature DCs are generally thought to be immunostimulatory in the cancer microenvironment (4).

The expression of mature Dendritic Cells increases in oral lichen planus, which, along with the simultaneous expression of LAMP3, indicates the maturity of these cells and has an inflammatory effect in terms of the secretion of T lymphocyte cell-associated factors (1).

LAMP3 (CD208) is specifically expressed by activated human dendritic cells (DC) and thus serves as a marker of by RT-qPCR (Real-Time Quantitative Polymerase Chain Reaction). Human dendritic cell maturation, which is a molecular marker on chromosome 3q. LAMP3 was discovered as a protein in lung cells. Recent research indicates that this gene is overexpressed in patients with laryngeal SCC (LSCC) (2), head and neck SCC (HNSCC) (3), breast cancer (4), Sjögren's syndrome (5), and cervical cancer (6). It has been reported that LAMP3 influences tumour cell metastasis and invasion (7, 8).

Given the unknown etiology of lichen planus, the importance of early diagnosis and treatment, and the lack of a suitable and final treatment for this disease and oral cancers, as well as preventing the progression of lichen planus, which can lead to OSCC, we decided to investigate the level of expression of this gene and its effect on the progression and destruction of the epithelium, study the connection between these two conditions, and the probable factors contributing to their occurrence.

Given the prevalence of OSCC, studying the expression of the LAMP3 (CD208) gene, which is known as a marker of mature dendritic cell level, may lead to a better prognosis and understanding of lichen planus and OSCC treatment.

Materials and Methods

From November 2014 to November 2020, 94 paraffin block tissue samples of patients with their corresponding demographic (Age, sex, social habit) records were taken from the oral and maxillofacial pathology archive department of Mashhad Dental School, Mashhad University of Medical Science. Our healthy control group is the healthy margin of the samples that the pathologist determined to be healthy and disease-free. Patients with a definitive diagnosis of OSCC and oral lichen planus, whose disease was proven through biopsy taken from their lesions, who had no previous radiotherapy or chemotherapy, and who currently had no other skin or oral diseases, were chosen for the study. Following that, these patients' paraffin blocks were sectioned by microtome to obtain sections with a thickness of 5 microns. The samples were deparaffinized after the sections were placed in 1.5 mL microtubes. The sample size and adequacy were determined using H&E staining. Following that, total RNA was extracted from the sample cells.

Following RNA extraction, a Nano drop device is used to determine the amount of RNA and its purity percentage, as well as whether the amount of RNA purity is appropriate. RT-qPCR was used to quantify the relative expression of LAMP3 on an ABI thermocycler (One Step, USA) with a SYBR Green master mix kit (Thermos Scientific, Germany) and appropriate primers (Table 1). RT-qPCR (real-time reverse transcription-PCR) has become the gold standard for detecting and quantifying RNA targets and is increasingly being used in novel clinical diagnostic assays. In this method, the expression of the LAMP3 gene is decreased or increased in comparison to the reference gene and the control sample. All procedures were performed in duplicate in 20 µL volumes in separate wells. Each reaction contained 0.5 µL of each primer (10 pM), 10 µL of SYBR Green master mix, 7 µL of DEPC

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water, and 2 µL (4 ng) of cDNA extract. The PCR program started with 1 cycle at 94 ºC for 10 min followed by denaturation, annealing, and replication processes including amplification cycles, respectively, at 94 ºC for 30 secs, 60 ºC for 30 secs, and 72 ºC for 30 sec. The final amplification followed at 72 ºC for 35 sec. Differential expression was analyzed by the ΔΔCT method. The housekeeping GAPDH (NM_001289745.3) gene was used as the reference gene. Fold change above two was regarded as over expression, and below 2 as low or no expression (16-18). Finally, patient information on LAMP3 gene expression status in lichen planus and OSCC patients is analyzed by SPSS software using statistical methods.

Table 1. Specific primers for expression of LAMP3.

Primers		Sequences
LAMP3	Forward	5'-CCTTCAAGTGCGTGAGTGAA-3'
	Reverse	5'-CCATAAGGCAGAGACCAACC-3'
GAPDH	Forward	5'-CCCATCACCATCTTCCAGG-3'
	Reverse	5'-CATCACGCCACAGTTTCCC-3'

Results

Overall, 94 patients including women with average age of 50.9±16.2 ranging from 22 to 84 years and men with average age of 53.7± 16.2 ranging from 22 to 84 years have been analyzed. Age range in healthy margin was 22 to 84 however, in LP group were 22 to 81 years and in SCC group 30 to 84 years. The gender distribution between groups was not statistically significant (P=0.953).

The healthy margin group has the lowest range of LAMP3 gene expression, while the SCC group has the highest. In terms of LAMP3 gene expression, there was a statistically significant difference between groups. (P <0.001). The association between LAMP3 gene expression is inverse. The average LAMP3 Gene expression in grade III was higher than in group grades I and g II, which is statistically significant at 5% significance level.

As shown in Table 2, the LAMP3 gene expression range attributed to the advanced stage was greater than the early stage and the SCC group LAMP3 gene expression range attributed to the mouth was greater than that of the lips and larynx.

Table 2. Demographic feature and Correlation between LAMP3 gene expression and clinicopathologic of OSCC patients.

Demographic feature		Count (%)	LAMP3 expression mean ± SD		P value
Sex	Men	48 (51.1)	Healthy margin	1.43 ± 0.58	>0.05*
			LP	3.38 ± 0.47	
			OSCC	8.23 ± 5.19	
	Women	46 (48.9)	Healthy margin	1.19 ± 0.51	
			LP	3.14 ± 0.28	
			OSCC	7.76 ± 3.67	
Age	Healthy margin	30 (31.9)	50.8 ± 18.95		< 0.05**
	LP	29 (30.9)	48.4± 15.01		
	OSCC	35 (37.2)	56.8 ± 13.7		
Clinicopathologic feature					
Clinical stage in OSCC	Early	22 (62.9)	5.81 ± 2.35		< 0.05*
	Advanced	13 (37.1)	11.82 ± 4.58		
Histological grade in OSCC	I	12 (34.3)	6.72 ± 3.12		<0.05**
	II	12 (34.3)	5.48 ± 1.29		
	III	11 (31.4)	12.29 ± 4.88		
Location in OSCC	Lip	7 (20)	8.49 ± 6.42		>0.05**
	Mouth	21 (60)	8.26 ± 4.21		
	Larynx	7 (20)	6.95 ± 2.87		

*: Mann-Whitney Test, **: Kruskal Wallis test.

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Discussion

In this study, we compared LAMP3 expression to GAPDH as a control in 94 tissue embedded paraffin-block samples, including 35 OSCC, 29 OLP, and 30 healthy margins.

The purpose of this study was to see if there was a difference in the expression of the LAMP3 gene between patients with oral lichen planus and oral squamous cell carcinoma. SCC patients had a higher mean age than lichen planus patients. Time of diagnosis is the most important predictor of prognosis in the treatment of squamous cell carcinoma.

The mean expression of the LAMP3 gene was higher in the SCC group than in the lichen planus group, and it was higher in the lichen planus group than in healthy margins. Due to the precancerous nature of lichen planus lesions, increased LAMP3 expression in these lesions can be used to predict malignant potential.

The marker's specificity and independence from gender can provide greater confidence and certainty in diagnosis. Another important feature of a diagnostic marker is its ability to predict disease progression. LAMP3 expression was higher in grade III SCC than in grades I and II. Furthermore, the expression of the LAMP3 gene was higher in the advanced stage than in the early stage.

Finding a new and reliable marker for faster diagnosis of this malignancy can help with screening and definitive diagnosis. The LAMP3 gene is one of the most recently studied cases, and its prevalence has been observed in several cancers.

Lysosomes are transported to different cell areas and secrete several enzymes as cancer progresses. LAMP family proteins are a group of glycosylated proteins that are related to lysosomal membranes and are secreted at various levels in various tissues. This family's five members (5-LAMP1) have been linked to a variety of cancers (15). LAMP1 and LAMP2 expression levels have been found to be higher in colorectal cancer cells, and this has been linked to cancer metastasis (16). LAMP3 protein was initially identified as a lung-specific protein. Nonetheless, it is now recognized as a marker of adult dendritic cells (17).

Increased LAMP3 expression in gastrointestinal cancer tissues was higher than in healthy margins and was associated with poor disease survival (18). LAMP3 expression was found to be higher in cervical cancer samples compared to healthy margins (13). LAMP3 overexpression has been linked to breast cancer recurrence (19). Tamoxifen resistance is associated with LAMP3 expression, which increases tamoxifen sensitivity by stimulating autophagy in breast cancer cells (20).

LAMP3 overexpression is also linked to a poor prognosis in cervical cancer and esophageal squamous cell carcinoma (13, 14). The expression of the LAMP3 gene in the SCC was higher than in healthy margins and lichen planus in the current study. LAMP3 expression was also higher in lichen planus than in healthy margins. According to Liao et al. (14), the expression of LAMP3 mRNA and protein was higher in esophageal tissues with squamous cell carcinoma than in healthy margins, and LAMP3 expression was significantly associated with the amount of DNA transcription. An increase in LAMP3 expression was observed in OSCC tissues compared to healthy tissue in a study conducted by Lu et al (17).

In the present study, LAMP3 gene expression in SCC and lichen planus groups had a weak inverse correlation with age, and no significant relationship was found with gender. In a study by Qiu et al. similar to our study, no association was found between gender, age, and LAMP3 expression (9). In the study of Liao et al. the expression of the LAMP3 gene was higher in samples over 57 years old than in under 57 years old (14). Lamp3 gene expression was not correlated with gender. LAMP3 gene expression in SCC and lichen planus groups had a weak inverse correlation with age, but no significant

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relationship with gender was found in the current study. In a study similar to ours, Qiu et al. discovered no link between gender, age, and LAMP3 expression (9). The expression of the LAMP3 gene was higher in samples over 57 years old than in samples under 57 years old, according to Liao et al (14). Gender had no effect on Lamp3 gene expression.

According to the findings of this study, the expression of the LAMP3 gene is higher in SCC lesions than in healthy tissue. As a result, LAMP3 gene expression can serve as a diagnostic biomarker. LAMP3 gene expression in SCC lesions is unrelated to age or gender, and thus can be used confidently in a wide range of patients. LAMP3 gene expression was higher in SCC lesions than in lichen planus, allowing researchers to distinguish between malignant and precancerous lesions and assess the risk of precancerous lesions. LAMP3 expression was higher in more advanced stages and SCC grades, suggesting that LAMP3 gene expression can be used to predict the course and severity of the disease. Given that LAMP3 was more common in advanced lesions than in early ones, LAMP3 may be involved in squamous cell differentiation.

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Conflicts of Interest

None.

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