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ORIGINAL RESEARCH

# Assessment of MOB1A and B gene polymorphisms in oral lichen planus patients

**Fateme Askari[1], Leila Farhad Mollashahi[2], Mohammad Ayoub Rigi Ladiz[3], Dor Mohammad Kordi-Tamandani[4]**

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## Abstract

**Background: This study aimed to assess MOB1A and B gene polymorphisms in patients with oral lichen planus (OLP. Several of premalignant lesions such as, leukoplakia, erythroplakia, oral lichen planus (OLP), and submucosal fibrosis can undergo malignant transformation and lead to oral cancer and non-invasive screening of patients to find those susceptible to SCC before progression to cancer would be highly beneficial.**

**Materials and Methods: This case-control study was conducted on 35 OLP patients and 35 healthy controls presenting to the Oral Medicine Department of Zahedan Dental School 2015 -2017. Unstimulated saliva samples were collected from both groups, and MOB1A and B gene polymorphisms were assessed by polymerase chain reaction (PCR). The two groups were compared by the Chi-square test (alpha=0.05).**

**Results: Two groups had no significant difference in the frequency of T and G alleles for MOB1A gene polymorphisms. (P>0.05) and either two groups had no significant difference in the frequency of A and C alleles for MOB1B gene polymorphism's (P>0.05).**

**Conclusion: The present results showed no significant difference between the case and control groups regarding MOB1A and B gene polymorphisms; thus, MOB1A and B gene polymorphisms do not appear to play a role in the pathogenesis of OLP.**

**Keywords:** Genes; Polymorphisms; MOB1A, MOB1B, Lichen Planus, Oral

## Introduction

Squamous cell carcinoma is the most common malignant neoplasm of the oral cavity (1-3). It accounts for 90% of all oral malignancies (1, 2, and 4). It is an important cause of morbidity and mortality, and its incidence varies depending on the geographical location (1, 5).Oral cancer has a high prevalence in some Asian populations particularly in South Asian countries such as India and Pakistan. In such countries, oral cancer is the most common cancer in males, and the second most common cancer in both males and females (6, 7). Several of premalignant lesions such as, leukoplakia, erythroplakia, oral lichen planus (OLP), and submucosal fibrosis can undergo malignant transformation and lead to oral cancer (8). According to Mishra et al, (9) the risk of malignant transformation of such lesions varies from 0% to 20% based on the type of lesion (9). Since early diagnosis is the key to the treatment of oral cancer (10), non-invasive screening of patients to find those susceptible to SCC before progression to cancer would be highly beneficial.

Currently, intracellular pathways and the compounds involved in such pathways are targets for cancer treatment. Hippo signaling has a prominent role in several types of cancer. Recent investigations on mammals have suggested a hypothesis regarding Hippo signaling and tumorigenesis in rats. Gene mutations and altered expression of genes in Hippo signaling have also been detected in human cancers. For instance, neurofibromin 2 is the only gene in Hippo signaling that is inactivated by mutation in cancers. Hippo signaling includes a large network of proteins that control tissue growth and organ size in the process of development, regeneration, and also pathological conditions such as cancer. This signaling pathway can control the number of cells and size of organs by limiting cell proliferation and increasing apoptosis (11). The Hippo signaling pathway has a kinase core, which is composed of a pair of serine/threonine kinases including MST1, MST2, LATS1, LATS2, SAV1, Mps one binder 1a (MOB1A),

Corresponding authour: Dr Leila Farhad Mollashahi
Department of periodontics, Zahedan university of medical science, Zahedan, Iran

[1] Departemant of oral and maxillofacial pathology , school of dentistry, Islamic Azad university, Isfahan (Khorasgan)branch, Isfahan, Iran

[2] Department of Periodontics, Zahedan University of Medical Sciences, Zahedan, Iran

[3] Department of Oral Medicine, Oral & Dental Disease Research Center, School of Dentistry, Zahedan University of Medical Sciences, Zahedan, Iran.

[4] Department of Biology, University of Sistan and Baluchestan, Zahedan, Iran



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and Mps one binder 1b (MOB1B). These proteins limit tissue growth by LATS1 and LATS2-dependent YAP1 phosphorylation. YAP 1 and TAZ phosphorylation inhibits their activity by binding to 14-3-3 protein binding receptors, resulting in their cytoplasmic accumulation, and subsequent ubiquitination and degradation by casein kinase 1 (12). Mechanical stimuli such as tension or contraction of cell attachments can increase the expression of YAP and activate the Hippo pathway or decrease its expression and deactivate this pathway. In case of deactivation of this pathway, YAP/TAZ enters the nucleus and enhances cell bioactivity and tissue growth by regulation of the activity of different transcription factors such as TEADs, SMADs, and RUNX. The activity of other signaling pathways also affects the activation/deactivation of the Hippo signaling pathway. For instance, mutation in WNT pathway genes increases β-catenin, which subsequently increases the entry of YAP into the nucleus because the released catenin binds to YAP, and serves as a transcription factor.

Uncontrolled gene expression is the main characteristic of neoplasia. Mutations in proteins involved in the Hippo pathway that result in hyper-activity of YAP and TAZ in mammals and YKI in birds result in ectopic cell proliferation (11).

MOB1A and MOB1B are among the key components of the Hippo signaling pathway, which are deactivated and undergo mutation in many cancer types. These two tumor suppressor genes exert their effect by controlling the down-stream components of the Hippo pathway. Seven MOB homologues (hMOB1A-B, 2A-C, 3, and 4) have been identified in humans (13). The hMOB1A (MOBKL1B and hMATS1), and hMOB1B (hMATS2 and MOBKL1A) are 95% similar in terms of amino-acid sequence. Although no second function has yet been identified for hMOB1A/B, only these two types of MOB can bind to LATS1/2, and activate it (13).

Under in vitro conditions, upregulation of hMOB1A inhibits cell proliferation while inhibition of hMOB1B or hMOB1A with the help of shRNA or siRNA increases cell proliferation, and impairs mitotic exit (13).

Considering all the above, this study aimed to assess MOB1A and B gene polymorphisms in OLP patients.

## Materials and Methods

After this study was approved by the ethics committee of Zahedan University, this case-control study was conducted on 35 OLP patients and 35 healthy controls presenting to the Oral Medicine Department of Zahedan Dental School 2015 to 2017.

The inclusion criterion for the OLP group was definite diagnosis of OLP. The control group participants were selected among the age- and sex-matched healthy individuals. The exclusion criteria were unwillingness for participation in the study, systemic diseases, tobacco use, presence of oral pathologies, xerostomia, alcohol consumption, using chewing tobacco, and history of radiotherapy or chemotherapy. The participants were selected by convenience sampling.

*Sample size:*

The sample size was calculated to be 35 in each group (11).

*Saliva collection:*

Written informed consent was obtained from all participants. All participants in both the case and control groups were requested to brush their teeth 1 hour prior to saliva collection. Next, 1 cc of unstimulated saliva was collected by the spitting method in Falcon tubes containing 17 mM Tris/HCL (pH 8.0), 3 cc of protective and 11 cc EDTA diluted with TNE; 11% ethanol was also added.

*Purification:*

The tubes were centrifuged at 3000 rpm for 11 minutes at room temperature in order for the epithelial cells and debris to deposit. The supernatant was discarded, and 1 mL of TNE solution was added to the cell sediment. The tubes were the centrifuged for 2000 rpm for 1 minute, and the supernatant was discarded. The cell sediment was vortexed in 1.3 mL of proteinase K lysis solution (pH of 2, 10 mM Tris (20 mg/mL), 20 mg/mL), SDS, and 5 mmM of 0.5% EDTA). Next, Falkon was added to 10 µL of (Sigma Chemical Co., St. Louis, MO, USA) solution. The obtained mixture was vortexed for 1 second and incubated overnight at 55°C. After incubation, 1.4 mL of the solution was transferred to a micro tube, and proteins and other impurities were deposited by addition of 500 µL of 8 M solution of ammonium acetate in 1 mM EDTA. The micro tube was vortexed for 1 second and then centrifuged at 17000 g for 11 minutes; 111 µL of the supernatant was added to two 1.71 mL micro tubes containing 121 µL of 2-propanol.

The contents of the tubes were centrifuged at 17111 rpm for 1 minute. The supernatant was removed and excess material was absorbed by an absorbent paper; 1 mL of 71% ethanol was added to the tubes. Each tube was agitated several times in order for the DNA to appear. After centrifugation at 17111 rpm for 1 minute, ethanol was carefully removed and the tube contents were transferred on an absorbent paper. They were then allowed to air dry for 11-21 minutes. The deposited DNA was dissolved in TE buffer and frozen at -20°C.

The concentration and purity of DNA were assessed by spectrophotometry (Epoch, USA). The concentration of DNA was read at 111 nm, and its purity was estimated by reading its wavelength at 111-181 nm. The sequence of primers used and the length of amplified segments



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**Table 1**. Sequence of primers used for assessment of MOB1A and MOB1B gene polymorphisms by tetra-ARMS PCR

| Gene polymorphism | Product size | Sequence of primers | |
|---|---|---|---|
| MOB1A | T allele size: 192 G allele size: 247 | 476 GCCTGGATCAGGATTCGGAGCTGACT 501 | Internal F |
| | | 526 GAGCTAAGGACGGTCCGCTCCCTTTC 501 | Internal R |
| | | 238 CTTCATCTCTGTCGGCCTCTCCCAACAT 265 | External F |
| | | 678 CAGCGAAACCTTAGCTCGCGAGATTTTG 651 | External R |
| MOB1B | A allele size: 118 C allele size: 132 | 143 TTTCAGATCCTGTCTTTCTAACTTACTAGC 172 61 | Internal F |
| | | 200 TTTCATTTTAAACTTAGGGAAGTTCTCAT 172 61 | Internal R |
| | | 83 CCAATTTTTTAGTTTTAAGCTTAGGGAA 110 61 | External F |
| | | 272 TAGGGTGCTTCAAATGTAATTTACTCAT 245 61 | External R |

are shown in Table 1.

After optimization of polymerase chain reaction (PCR) conditions, the genotype of the case and control groups was evaluated as such. For the PCR, each 11 µL of PCR Mix containing genomic DNA (11 ng), 1 µL of each primer, and 1.11 µL of Taq DNA polymerase Master Mix Red (Pishgam) were mixed; distilled water was also added to reach the volume to 11 µL. PCR was conducted according to the following protocol: Initial phase of denaturation at 12°C for 1 minute followed by 31 cycles (12°C for 11 seconds, 11°C for 21 seconds, and 71°C for 11 seconds). Extension was performed at 71°C for 1 minute. Finally, the PCR products were electrophoresed on 1% agarose gel, and the genotype was determined as such. MOB1A and MOB1B gene polymorphisms were compared between the two groups of case and control.

*Statistical analysis:*

Data were analyzed by SPSS 16 using the Chi-square test at 0.05 level of significance.

## Results

Three specimens from OLP group were lost during the experiment. Table 2 presents the frequency distribution and percentage of polymorphism of MOB1A gene in the case and control groups. As shown, no significant difference existed between the case and control groups regarding the frequency of T and G alleles (P>0.05). Thus, OLP had no significant correlation with MOB1A polymorphism.

Table 3 presents the frequency distribution and percentage of polymorphism of MOB1B gene in the case and control groups. As shown, no significant difference existed between the case and control groups

**Table 2.** Frequency distribution and percentage of polymorphism of MOB1A gene in the case and control groups

| Genotype | T | | G | | TG | | Total | |
|---|---|---|---|---|---|---|---|---|
| | Frequency | Percentage | Frequency | Percentage | Frequency | Percentage | Frequency | Percentage |
| OLP patients | 13 | 40.6 | 6 | 18.8 | 13 | 40.6 | 32 | 100 |
| Healthy controls | 9 | 25.7 | 12 | 34.3 | 14 | 40.0 | 35 | 100 |
| P value* | P=0.298 | | P=0.178 | | P=1.00 | | | |

***Chi-square test**



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**Table 3**. Frequency distribution and percentage of polymorphism of MOB1B gene in the case and control groups

| Genotype | C | | A | | CA | | Total | |
|---|---|---|---|---|---|---|---|---|
| | Frequency | Percentage | Frequency | Percentage | Frequency | Percentage | Frequency | Percentage |
| OLP patients | 12 | 37.5 | 8 | 25.0 | 12 | 37.5 | 32 | 100 |
| Healthy controls | 10 | 28.6 | 13 | 37.1 | 12 | 34.3 | 35 | 100 |
| P value* | P=0.603 | | P=0.307 | | P=0.804 | | | |

regarding the frequency of A and C alleles (P>0.05). Thus, OLP had no significant correlation with MOB1B polymorphism.

## Discussion

This study assessed MOB1A and B gene polymorphisms in OLP patients. MOB1A and B genes are involved in the Hippo signaling pathway, which is a tumor suppressor pathway. Its mechanism of action is through inhibition of YAP and TAZ factors that control the size of organs and tumorigenesis (14). This pathway creates a balance between cell proliferation, cell death, and cell differentiation, and controls tissue growth as such. The main core of this cellular pathway includes MST1/2 and LATS1/2 serine/threonine kinases, AGC kinase family members, and Salvador and MOB1 adaptor proteins. The main function of Hippo signaling pathway is the negative control of the activator of YAP and TAZ transcription. The components of the aforementioned kinase core (MST1/2, SAV, MOB1, LATS1/2, YAP, and TAZ) are required to prevent disorders other than tumor development. The Hippo pathway can play a role in coordination between two processes in mitosis, namely (I) coordination in timing and location of mitotic chromosomes, and (II) cytokinesis.

The present results regarding MOB1A and B genes in the Hippo signaling pathway revealed that the two groups had no significant difference in the frequency of T and G alleles for MOB1A gene polymorphism, although the frequency of G allele was insignificantly lower in the OLP group.

The difference in the frequency of A and C alleles for MOB1B gene polymorphism was not significant either, although the frequency of an allele was insignificantly lower in the OLP patients. No previous study has been conducted on MOB1A and B gene polymorphisms in OLP patients to compare our results with However, the role of MOB1A and B gene polymorphisms in many malignancies has been previously confirmed (15-20). Nishio et al. (17) reported mutation of MOB1A in breast cancer and melanoma. Also, impaired expression of this gene has been confirmed in colorectal, lung, and skin cancers (15). A defect in MOB1A has been reported in 81% of hepatic cancers (16).

It has been demonstrated that MOB1B can compensate for a defect in MOB1A and vice versa. However, absence of both of these genes is fatal during the fetal development and results in tumor development and particularly skin cancer (17). Sasaki et al. (18) reported significant down-regulation of MOB1 genes in tumor cells of patients with lung cancer, compared with the control group. This down-regulation is probably the initial phase of tumor development, which leads to impairments in mitotic checkpoints, genomic instability, and chromosomal rearrangement (18).

According to Kim et al, (19) low expression of MOB3B was correlated with prostate cancer such that expression in patients with prostate cancer was significantly lower than that in the control group. Thus, it appears that this gene serves as a tumor suppressor gene in prostate cancer. Yuan et al. (20) showed that some genetic variants of the key genes in the Hippo pathway particularly YAP1 rs11225163, TEAD1 rs7944031, and MOB1A rs1990330 play a key role in diagnosis and lowering the risk of cutaneous melanoma.

Similar studies are required on a larger sample size and also on patients with oral squamous cell carcinoma.

## Conclusion

The present results showed no significant difference between the case and control groups regarding MOB1A and B gene polymorphisms; thus, MOB1A and B gene polymorphisms do not appear to play a role in the pathogenesis of OLP.

## Conflicts of interest: none

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