Comparison of Methylation and Expression Profile of MOB-1A in Blood, Saliva and Tissue of Patients with Oral Squamous Cell Carcinoma (OSCC) and Precancerous Patients

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YAP remains unphosphorylated, translocates into the nucleus and activates transcription of target genes [10].

The aberrant DNA promoter methylation that influences gene expression is a common feature of many human cancers [11]. So, the present study is trying to compare the methylation and expression profile of MOB1A gene in blood, saliva and tissue samples of healthy controls as well as high risk (precancerous patients) and OSCC patients.

Materials and Methods

Samples and DNA preparation

In this study, subjects included healthy controls, high risk and OSCC patients. In control group, blood (3 cc) and saliva (5 ml) samples were collected from 20 persons who were free from any oral cavity diseases after explanation of study purpose and signing of consent form (Mean age: 58.70 ± 10.38). 20 patients with precancerous lesions of oral cavity (15 oral lichen planus and 5 oral leukoplakia patients) composed high risk group and blood as well as saliva samples were collected from them (Mean age: 48.95 ± 11.93). In OSCC group, 20 paraffin-embedded tissues were collected from patients referred to oral disease diagnosis department (Mean age: 60.55 ± 15.37). Since 8 patients of 20 investigated OSCC patients were dead, blood and saliva samples were collected only from 12 OSCC patients. Clinico-pathological data of the three investigated groups such as age, sex and clinical stage are shown in Table 1. Genomic DNA was isolated from blood, tissue and saliva samples by salting out and phenol-chloroform methods and then its quality was estimated by spectrophotometer.

		Control (N=20)	Precancerous (N=20)	OSCC (N=20)	P-value
Gender	Male	13	9	10	
	Female	7	11	10	1
Age average		58.70 ± 10.38	48.95 ± 11.93	60.55 ± 15.37	0.177
Sample	Blood	20	20	12	-
	Saliva	20	20	12	-
	Tissue	-	-	20	-
Precancerous lesion	OLP	-	15	-	-
	Leukoplakia	-	5	-	-
Grade(OSCC)	One	-	-	12	-
	Two	-	-	6	-
	Three	-	-	2	-
Addiction	Yes	-	8	11	-
	No	20	12	9	-
Familial history	Yes		2	2	-
	No	20	18	18	-

	R: GTTATTGTTTTTTTCGTAGGATCGT		
MOB1A-U	F: TCTCACAAACTAAAATTTCACTACAC C	156	55
	R: TTATTGTTTTTTTTGTAGGATTGT		

Gene expression analysis

Total RNA was extracted from saliva samples of control and high risk groups as well as saliva and tissue samples of OSCC patients using parstous total RNA extraction kit (Cat. No.A101231) according to the manufacturer's instructions. The cDNA synthesis kit (vivantis, Cat.No.RTPL12) was used to reverse-transcribe 1 µg of RNA in a final volume of 20 µl. As an internal standard, RNA18s was used. Real time PCR of MOB1A was performed using the primers and annealing temperatures according to Table 3. Cycle Threshold (CT) at which the fluorescence for the reaction well crosses was recognized in all samples and then, normalized CT (CT target gene/CT housekeeping gene) was used for comparison of gene expression between samples and groups.

MOB1A	F: CAGCAGCCGCTCTTCTAAAAC	134	58
	R: CCTCAGGCAACATAACAGCTTG		

[redacted]ata were analyzed using SPSS16 software. Analysis of relative gene expression between saliva
As it is clear from the results, there wasn't any significant di**f**erencebetween the comparisons of methylation status in any of t
value: 0.025) (T**a**ble 4). Unmethyl14 (70%)5 (25%)RefrenceMethyl2 (10%)12 (60%)0.025M

Discussion