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Characterization Of Protein Expression In Oral Squamous Cell Carcinoma By MALDI-TOF- A Pilot Case Control Study

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ABSTRACT

Objectives: Oral squamous cell carcinoma makes up to 90% of the oral cancer cases. Proteomic profiling of oral squamous cell carcinoma will enable better understanding of the proteins expressed in oral squamous cell carcinoma. In this study, matrix assisted laser desorption/ionization- time of flight mass spectrometry (MALDI-TOF-MS) technology was used to analyse the proteins expressed in oral squamous cell carcinoma.

Materials and methods: In this study, serum and plasma samples of oral squamous cell carcinoma patients (n=5) and apparently healthy individuals (n=5) were collected. The samples were then subjected to MALDI-TOF analysis. Insilico analysis of the protein-protein interactions was performed by using STRING and CYTOSCAPE.

Results: The proteomic analysis of the plasma and serum of OSCC and control samples resulted in the differential expression of 62 proteins. Two of the plasma proteins FAM71B, Keratin Type II cytoskeletal 1 showed statistically significant expression in OSCC samples. The insilico analysis revealed that the proteins in plasma especially NCOR1, MED22 are known to have high degree of centrality.

Conclusion: The proteomic profiling of oral squamous cell carcinoma samples have showed involvement of various proteins that may be potential biomarkers for early diagnosis and targeted therapy for oral squamous cell carcinoma.

Keywords: oral squamous cell carcinoma, matrix assisted laser desorption ionization, time of flight, proteomic profiling.

INTRODUCTION

Oral cancer prevalence ranks eleventh in the world and it is the second most common cancer in India [(1)]. It is the third

most common disease in women and the most common disease in men [2]. Oral squamous cell carcinoma (OSCC) represents about 80-90% of the total malignancies of oral cavity [3].

It is the most common neoplasm of the oral cavity. The most common sites that gets involved include lips, buccal mucosa, floor of the mouth, tongue, gingiva-buccal sulcus, retromolar trigone, hard palate, soft palate, major salivary glands and minor salivary glands [4]. It is a multifactorial disease with most common etiological factors including smoking, alcohol usage, betel nut use, diet and nutrition, radiation exposure, genetic mutations, free radicals, chronic inflammation, oral microflora as well as occupational and dental variables [5]. Oral squamous cell carcinoma has a high chance of metastasis depending upon its staging, grading and anatomic site. The conventional treatment modalities of oral squamous cell carcinoma involves surgical resection of the tumor and/or radiotherapy and/or chemotherapy [6]. The treatment modalities have their own adverse effects like trismus which leads to difficulty in swallowing, eating, speaking, tiredness, xerostomia which all together will drastically reduce the quality of life of the treated patients [7].

Materials and methods

Ethical clearance:

The study was conducted in Saveetha dental college and Hospitals, Chennai, Tamil Nadu. The institutional ethical committee approval was obtained from Saveetha Dental College and hospitals, Chennai, Tamil Nadu (IHEC Ref. No: IHEC/SDC/OPATH-2001/22/017). All the procedures involving the human participants were in accordance with the ethical standards of the institution. Informed consent was receivedfrom the patients included in the study. After receiving the informed consent from the patients, the samples were collected.

Study population:

The study had two groups. A total of 10 participants were included in the study. Among the 10, 5 participants were oral squamous cell carcinoma patients and were considered as cases and another 5 participants were apparently healthy individuals considered as controls. All the oral squamous cell carcinoma cases were confirmed by clinical examination and histopathological investigations.

This study was approved by the institutional ethical committee of Saveetha Dental College and hospitals, Chennai and the sample collection was done by following the protocol. Informed consent was taken from the study participants.

Sample collection:

Blood samples were obtained from 5 oral squamous cell carcinoma patients and 5 apparently healthy individuals. 2ml of blood was collected in red top glass tube for serum isolation and 2ml of blood was collected in purple top glass tube (EDTA coated) for plasma isolation. After blood collection, the samples were allowed to clot or sediment at room temperature for 30 minutes after which the tubes were placed in a cooling centrifuge and were subjected to centrifugation at 3000 rpm for 5 minutes. The isolated serum and plasma were collected in a separate microcentrifuge tube and stored at -80°C until use.

Proteome fractionation:

The serum and plasma were subjected to fractionation using magnetic bead bases kit from Bruker Daltonics Inc, CA. 5 µl of serum / plasma were taken which were then mixed with 10 µl of binding solution in a 0.2 µl PCR tube. After this, about 5 µl of C8-hydrophobic interaction chromatography resin (C8) or Cu-IMAC resin was added and was mixed thoroughly by aspirating the reagents by using the pipette. Then the unbound solution was separated by using a magnetic bead separator. The solution was washed for three times. This led to the elution of the bound proteins from the magnetic beads. The eluted sample was collected and a portion of the eluted sample was taken and diluted with 1:10 solution containing CHCA. From the resulting mixture, around 0.5 µl was placed on the anchorchip target (Bruker Daltonics Inc, CA) and was allowed to air dry at room temperature for 5 minutes.

MALDI-TOF Mass spectrometry:

The fractionated samples were subjected to MALDI-TOF analysis (Bruker Daltonics Inc.) Nitrogen laser (337 nm) at 20 Hz was used to irradiate and perform the ionization procedure. A high gating factor with signal suppression upto 800 Da was used for matrix suppression. The mass spectra was detected in the linear positive mode. The caliberation mass range was set at 1000-25000 Da. The signals were recorded using flex tool (Bruker Daltonics Inc).

Results:

The proteomic profiling of the serum and plasma of oral squamous cell carcinoma and the control samples identified 62 differentially expressed proteins. Among the 62 proteins, 31 proteins were plasma proteins and the other 31 were serum proteins. Among the 31 plasma proteins, 21 plasma proteins were from oral squamous cell carcinoma patients and 10 plasma proteins were from controls. Among the 31 serum proteins, 10 serum proteins were from oral squamous cell carcinoma patients and 21 serum proteins were from the controls. The protein score is -10 Log (P), where P is the probability that the observed match is a random event. The protein score was considered significant only if the score is greater than 56 ($p<0.05$). The summary of the plasma proteins are given in Table 1. The mascot histogram of the plasma proteins from the cases and controls are given in Figure 1. The STRING analysis of the proteins in the plasma of OSCC samples is shown in Figure 2a and the interactome of the proteins in the plasma of OSCC protein interaction using cytoscape is shown in Figure 2b. The insilico analysis of the protein-protein interactions in the plasma revealed that the proteins NCOR1, MED22, have the highest degree of centrality followed by BCL6, GRIK5, ICOSLG, GRID2, SCN2A, UBR5 (Table 2). The summary of the serum proteins are given in Table 3. The mascot histogram of the serum proteins from the cases and controls are given in Figure 3. The STRING analysis of the proteins in the serum of OSCC samples is shown in Figure 4a and the interactome of the proteins in the serum of OSCC protein interaction using cytoscape is shown in Figure 4b. Table 4 shows the summary of the degree of centrality of the proteins in the serum of OSCC samples.

Table 1 : Plasma proteins and their mass, score in the OSCC and control samples.

Protein	OSCC/Control	Mass	Score	GN	PE	SV
CLEC1A C-type lectin domain family 1 member A	OSCC	31932	46	CLEC1A	1	2
LHPL6 LHFPL tetraspan subfamily member 6 protein	OSCC	21583	22	LHFPL6	2	1
FA71B Protein FA71B	OSCC	64716	64	FAM71B	1	2
SCN2A Sodium channel protein type 2 subunit alpha	OSCC	227827	35	SCN2A	1	3
K2C1 Keratin, type II cytoskeletal 1	OSCC	65999	100	KRT1	1	6
OR2D3 Olfactory receptor 2D	OSCC	37464	23	OR2D3	2	2
MCR Mineralocorticoid receptor	OSCC	107013	32	NR3C2	1	2
UBR5 E3 ubiquitin-protein ligase UBR5	OSCC	309158	53	UBR5	1	2
MED22 Mediator of RNA polymerase II transcription subunit 22	OSCC	22207	22	MED22	1	2
K2C3 Keratin, type II cytoskeletal 3	OSCC	64378	21	KRT3	1	3
ICOSL ICOS ligand	OSCC	33328	21	ICOSLG	1	2
TPPC3 Trafficking protein particle complex subunit 3	OSCC	20261	21	TRAPPC3	1	1
I27L2 Interferon alpha inducible protein 27 like protein	OSCC	12402	20	IFI27L2	2	1
FRAS1 Extracellular matrix organizing protein FRAS 1	OSCC	442928	42	FRAS1	1	2

GRIK5 Glutamate receptor ionotropic, kainate 5	**OSCC**	**109195**	**22**	**GRIK5**	**2**	**2**
BCL6 B cell lymphoma 6 protein	**OSCC**	**78795**	**21**	**BCL6**	**1**	**1**
DEAF1 Deformed epidermal autoregulatory factor 1 homolog	**OSCC**	**59290**	**21**	**DEAF1**	**1**	**1**
LMA1L Protein ERGIC-53-like	**OSCC**	**57093**	**20**	**LMAN1L**	**2**	**2**
TBX20 T-box transcription factor TBX20	**OSCC**	**49201**	**20**	**TBX20**	**1**	**4**
CT141 Uncharacterized protein c20orf141	**OSCC**	**17328**	**19**	**C20orf141**	**1**	**1**
GRID2 Glutamate receptor ionotropic, delta-2	**Control**	**113284**	**19**	**GRID2**	**1**	**2**
PTPRT Receptor-type tyrosine protein phosphatase T	**Control**	**162031**	**38**	**PTPRT**	**1**	**6**
UBP22 Ubiquitin carboxyl-terminal hydrolase	**Control**	**59921**	**19**	**USP22**	**1**	**2**
MSD2 Myb/SANT-like DNA binding domain-containing protein	**Control**	**61281**	**41**	**MSANTD2**	**1**	**1**
SHLD1 Sheildin complex subunit 1	**Control**	**22924**	**41**	**SHLD1**	**1**	**1**
OR4F4 Olfactory receptor 4F4	**Control**	**34236**	**20**	**OR4F4**	**2**	**2**
OR4F5 Olfactory receptor 4F5	**Control**	**34307**	**20**	**OR4F5**	**3**	**1**
OR4M2 Olfactory receptor 4M2	**Control**	**35393**	**20**	**OR4M2**	**2**	**2**
ORM2B Olfactory receptor M2B	**Control**	**35380**	**20**	**OR4M2B**	**3**	**1**
TM192 Transmembrane protein 192	**Control**	**30903**	**44**	**TMEM192**	**1**	**1**
E4F1 Transcription factor E4F1	**Control**	**83444**	**42**	**E4F1**	**1**	**2**

Figure 1:

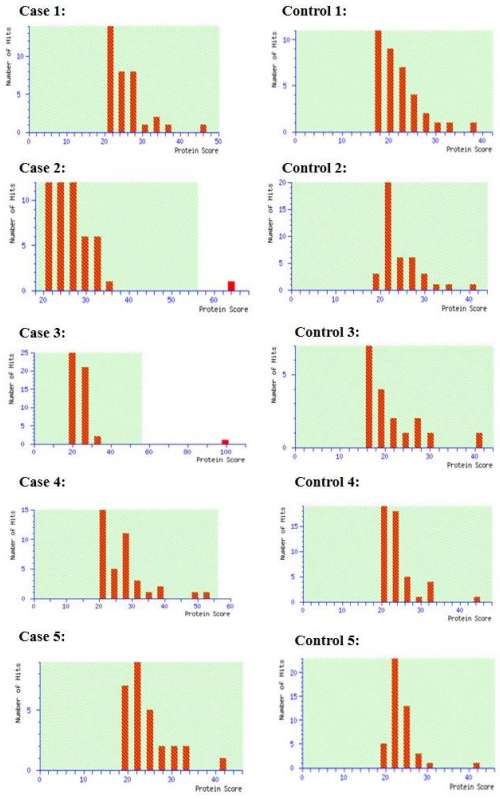

Figure 1 : Mascot score histogram of plasma profiling in OSCC and control samples using MALDI-TOF.

Figure 2:

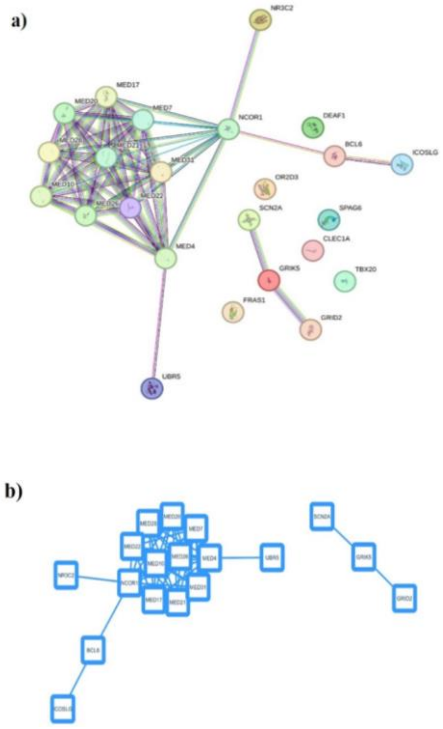

Figure 2: a) STRING analysis of the proteins in the plasma of OSCC samples. b) Interactome of the plasma proteins in OSCC demonstrating the protein interaction using cytoscape.

Table 2: CYTOSCAPE analysis of plasma proteins showing the degree of centrality, betweenness centrality, closeness centrality and the number of directed edges of the plasma proteins in the OSCC samples.

Name	Betweenness Centrality	Closeness Centrality	Degree	Number Of Directed Edges
NCOR1	0.384615	0.875	12	12
MED4	0.142857	0.777778	11	11
MED10	0	0.736842	10	10
MED31	0	0.736842	10	10
MED28	0	0.736842	10	10
MED17	0	0.736842	10	10
MED21	0	0.736842	10	10
MED7	0	0.736842	10	10
MED26	0	0.736842	10	10
MED22	0	0.736842	10	10
MED20	0	0.736842	10	10
BCL6	0.142857	0.518519	2	2
GRIK5	1	1	2	2
ICOSLG	0	0.35	1	1
GRID2	0	0.666667	1	1
SCN2A	0	0.666667	1	1
UBR5	0	0.451613	1	1
NR3C2	0	0.482759	1	1

Table 3: Serum proteins and their mass, score in the OSCC and control samples:

Protein	OSCC/Control	Mass	Score	GN	PE	SV
PDZ1I PDZK1-interacting protein 1	OSCC	12219	48	PDZK1IP1	1	1
ALG6 Dolichyl pyrophosphate Man9G1cNAc2 alpha-1,3 glucosyltransferase	OSCC	58082	24	ALG6	1	2
ZN843 Zinc finger protein 843	OSCC	37042	48	ZNF843	1	1
O10J6 Putative olfactory receptor 10J6	OSCC	31000	19	OR10J6P	5	1
LUR1L Leucine rich adaptor protein 1-like	OSCC	24397	50	LURAP1L	1	3
MOB2 MOB kinase activator 2	OSCC	26909	45	MOB2	1	1
TRI58 E3 ubiquitin-protein ligase TRIM58	OSCC	54732	41	TRIM58	2	2
OTUB2 Ubiquitin thioesterase OTUB2	OSCC	27196	24	OTUB2	1	2
LRC43 Leucine rich repeat containing protein 43	OSCC	72976	21	LRRC43	2	2
IST1 IST1 homolog	OSCC	39725	20	IST1	1	1
HNRDL Heterogeneous nuclear ribonucleoprotein D-like	Control	46409	53	HNRNPDL	1	3
BACE2 Beta-secretase 2	Control	56145	19	BACE2	1	1
FUT2 Galactoside alpha-1,2 fucosyltransferase 2	Control	38992	19	FUT2	1	1
MSLNL Mesothelin-like protein	Control	74493	19	MSLNL	3	3
GP135 G-protein coupled receptor 135	Control	51703	18	GPR135	1	2
PAMR1 Inactive serine protease PAMR1	Control	80146	31	PAMR1	1	1
FCN2 Ficolin-2	Control	33980	21	FCN2	1	2
EGFL8 Epidermal growth factor-like protein 8	Control	32241	20	EGFL8	1	1
IL1B Interleukin 1 beta	Control	30728	20	IL1B	1	2
LSM6 U6 snRNA associated Sm like protein LSm6	Control	9122	20	LSM6	1	1
PHAG1 Phosphoprotein associated with glycosphingolipid enriched microdomains 1	Control	46951	19	PHAG1	1	2
LY6H Lymphocyte antigen 6H	Control	14659	19	LY6H	1	1
OSBL7 Oxysterol binding protein related protein 7	Control	95372	19	OSBPL7	1	1

AKCL1 Putative aldo-keto reductase family 1 member C8	**Control**	**14578**	**18**	**AKR1C8**	**5**	**2**
CDX4 Homeobox protein CDX-4	**Control**	**30461**	**46**	**CDX4**	**1**	**1**
HLF Hepatic leukemia factor	**Control**	**33178**	**52**	**HLF**	**1**	**1**
TF3C6 General transcription factor 3C polypeptide 6	**Control**	**24033**	**20**	**GTF3C6**	**1**	**1**
UBAD1 UBA like domain containing protein 1	**Control**	**18942**	**18**	**UBALD1**	**1**	**1**
ABHEA Protein ABHD14A	**Control**	**29746**	**36**	**ABHD14A**	**2**	**2**
ZBT44 Zinc finger and BTB domain-containing protein 44	**Control**	**63807**	**18**	**ZBTB44**	**1**	**1**
PINK1 Serine/threonine protein kinase PINK1, mitochondrial	**Control**	**62729**	**17**	**PINK1**	**1**	**1**

Figure 3:

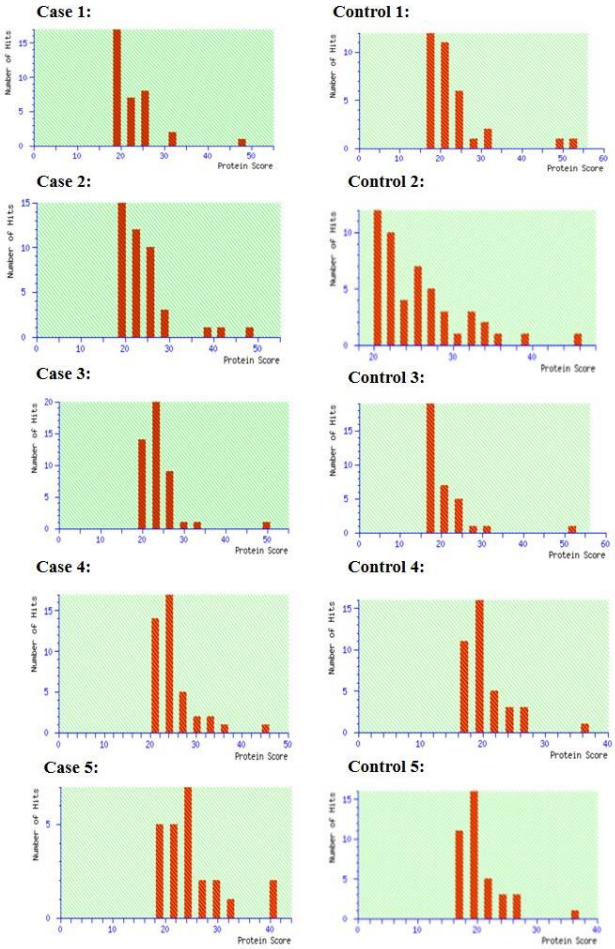

Figure 3: Mascot score histogram of serum profiling in OSCC and control samples using MALDI-TOF.

Figure 4:

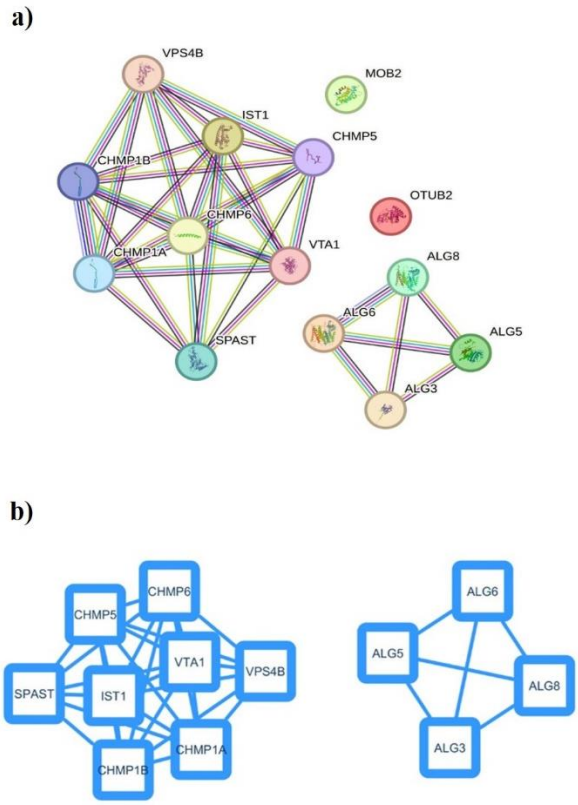

Figure 4: a) STRING analysis of the proteins in the serum of OSCC samples. b) Interactome of the serum proteins in OSCC demonstrating the protein interaction using cytoscape.

Table 4: Cytoscape analysis of the serum proteins showing the degree of centrality, betweenness centrality, closeness centrality and the number of directed edges of the serum proteins in the OSCC samples.

Name	Betweenness Centrality	Closeness Centrality	Degree	Number Of Directed Edges
CHMP1A	0.007937	1	7	7
CHMP5	0.007937	1	7	7
CHMP6	0.007937	1	7	7
VTA1	0.007937	1	7	7
CHMP1B	0.007937	1	7	7
IST1	0.007937	1	7	7
VPS4B	0	0.875	6	6
SPAST	0	0.875	6	6
ALG3	0	1	3	3
ALG5	0	1	3	3
ALG6	0	1	3	3
ALG8	0	1	3	3

Discussion:

Serum and plasma are known to be very important sources of proteomic information. Proteomic profiling is one of the most important technology which pays vital role in understanding various cellular process. Oral squamous cell

carcinoma being a highly invasive and metastatic disease has a better prognosis if diagnosed and treated earlier. The use of proteomic profiling in oral squamous cell carcinoma will enable in the discovery of biomarkers which will aid in early diagnosis of the disease. In this study we used MALDI-TOF analysis for proteomic profiling of the serum and plasma of oral squamous cell carcinoma samples and the control samples.

Among the 21 differentially expressed proteins that were found from plasma profiling, 2 proteins Protein FAM71B and Keratin type II cytoskeletal 1 were found to be significant in oral squamous cell carcinoma patients. The identified plasma proteins included families of C-type lectin domain family 1 member A (CLEC1A), LHFPL tetraspan subfamily member 6 protein (LHPL6), Protein FA71B (FAM71B), Sodium channel protein type 2 subunit alpha (SCN2A), Keratin, type II cytoskeletal 1 (K2C1), Olfactory receptor 2D (OR2D3), Mineralocorticoid receptor (MCR), E3 ubiquitin-protein ligase UBR5 (UBR5), Mediator of RNA polymerase II transcription subunit 22 (MED22), Keratin, type II cytoskeletal 3 (K2C3), ICOS ligand (ICOSL), Trafficking protein particle complex subunit 3 (TPPC3), Interferon alpha inducible protein 27 like protein (I27L2), Extracellular matrix organizing protein FRAS 1 (FRAS1), Glutamate receptor ionotropic, kainate 5 (GRIK5), B cell lymphoma 6 protein (BCL6), Deformed epidermal autoregulatory factor 1 homolog (DEAF1), Protein ERGIC-53-like (LMA1L), T-box transcription factor TBX20 (TBX20), Uncharacterized protein c20orf141 (CT141), Glutamate receptor ionotropic, delta-2 (GRID2) in oral squamous cell carcinoma samples.

In our study, keratin type II cytoskeletal 1 is seen to be significantly expressed in oral squamous cell carcinoma patients. Cytoskeleton is a proteinaceous substance that helps the cell to maintain its shape and internal organization. Keratins are the intermediate filament forming proteins of the epithelial cells. It plays important role in the process of cell migration. The cancer cells gain motility by modulation of the cell shape and alteration in the expression of keratins. Tumor development involves various genetic, epigenetic and cellular changes that transforms the normal cell to tumor cell [8, 9]. The cellular level changes includes the epithelial mesenchymal transition during which the cells loses the cell-cell adhesion and cellular polarity [10]. The process of EMT is considered to the most important mechanism that accounts for enhanced motility and invasiveness of the tumor cells [11, 12, 13]. Keratin network alterations owing to sphingosylphosphorylcholine has increased association with cell deformability resulting in greater chances of the cancer cells to invade the surrounding tissue and permeate through the stroma resulting in the escape from the primary tumor[14]. A study in 2006 analyzed the tissue samples of SCC cervix which revealed elevated levels of keratin type 2 cytoskeletal 1.

E3 Ubiquitin ligase URB5 is found to be expressed in higher amounts in our study. The ubiquitin proteasome system is a very important system in cell signaling. The E3 Ubiquitin URB5 is a key regulator of the ubiquitin proteasome system. Ubiquitination is a posttranslational modification which is responsible for normal cellular functions [15]. It has been showed that deletion of URB5 in 4T1 mammary tumor suppressed metastatic lung colonization. The tumor growth was also blocked by URB5 knockout which was associated with decreased angiogenesis, increased apoptosis, necrosis and arrest of tumor growth [16]. It has been reported that E3 ubiquitin ligase URB5 promotes the metastasis of pancreatic cancer by destabilizing the F-actin capping protein CAPZA1 [17]. E3 ubiquitin URB5 is also considered as a potential biomarker in colorectal cancer [18].

Extracellular matrix organizing protein FRAS1 is also found be expressed in higher amounts in our study. FRAS1 is a protein that plays important role in the regulation of epidermal basement membrane adhesion and organogenesis. It has been proved that there is elevated expression of FRAS-1 related extracellular matrix 1 in breast cancer [19]. It has been reported elevated FRAS1/FREM expression is being correlated with cancer progression, poor survival, immune infiltrations and DNA methylations in kidney renal clear cell carcinoma [20]. It has also been proved that FRAS1 contributes to the malignant phenotype of gastric cancer especially liver metastasis and serves as a predictive marker for treating liver metastasis [21].

C-type lectin domain family 1 member A (CLEC1A) is one of the member of C-type lectin/C-type lectin-like domain (CTL/CTLD) superfamily. The members of this family play important role in cell adhesion, cell-cell signaling,

glycoprotein turnover, inflammation, immune response and dentritic cell function [22]. It is also known that the C-type lectins play important role in tumor metastasis. A crucial role of CLEC1A is its interaction with podoplanin, a glycoprotein expressed on the surface of several cell types, including lymphatic endothelial cells. A factor in the formation of blood clots is the binding of CLEC1A to podoplanin, which causes platelets to activate and aggregate. Concerning the preservation of vascular integrity and the avoidance of excessive bleeding, this connection is very crucial. It is believed that the CLEC1A-podoplanin relationship contributes to metastasis by improving platelet adherence to tumor cells. Through this platelet-tumor cell contact, circulating tumor cells may be shielded from immune monitoring and encouraged to adhere to the endothelium at distant locations, which could aid in the formation of metastatic colonies [23].

It was initially discovered that HMGA2's translocation partner in benign lipomas was LHFPL6, a member of the LHFP family [24]. The incidence and malignant development of stomach cancer have been linked to HMGA2, according to a number of research [25, 26]. DNA methylation and mutation have been demonstrated to be intimately linked to carcinomas, and methylation of the LHFPL6 promoter is essential for the advancement of gastric cancer [27].

The protein FAM71B is a member of the protein family linked to the Fanconi anemia (FA) pathway. The Fanconi anemia pathway is a multifaceted protein network that helps repair DNA damage, namely breaking down interstrand crosslinks. Fanconi anemia is a rare genetic disease marked by bone marrow failure and an elevated risk of malignancy. It is thought to be caused by mutations in genes linked to the Fanconi anemia pathway [28].

A crucial component in controlling RNA polymerase II-mediated transcription is the Mediator complex, of which MED22 is a component. Facilitating the initiation and regulation of transcription, the Mediator complex functions as a molecular bridge between RNA polymerase II and transcriptional regulators [29]. MED22, as a component of the Mediator complex, is primarily involved in transcription control. It affects the transcription of genes involved in a variety of cellular functions by interacting with different transcription factors and co-regulators to modify the activity of RNA polymerase II [30]. Uncontrolled cell division, a defining feature of cancer, may be facilitated by dysregulation of transcriptional regulatory systems. Gene expression related to growth control and cell cycle progression may be affected by modifications in the Mediator complex or any of its members, including MED22 [31]. The Mediator complex affects the epigenetic landscape of genes by orchestrating the recruitment of chromatin-modifying enzymes. Variations in the epigenetic control of particular genes may have an impact on the onset and spread of cancer [32]. The carcinogenic signaling pathways that involve growth factor receptors and signal transduction cascades may interact with mediator subunits, including MED22. Certain genes that are important in processes connected to cancer may have their transcriptional output affected by these interactions [33].

The primary function of ICOSL, a cell surface protein also referred to as B7-H2 or CD275, is immunological control and T-cell activation. It engages in interactions with activated T cells through its receptor, Inducible T-cell Costimulator (ICOS) [34]. The development of cancer can be impacted by immune cell activity, including Tumor Infiltrating Lymphocytes (TILs), and their presence in the tumor microenvironment. Tumor cell or antigen-presenting cell expression of ICOSL may influence TIL recruitment and activation in the tumor microenvironment [35]. Influencer T-cell responses and the maintenance of immunological responses in tissues are both aided by the ICOS-ICOSL signaling axis. When it comes to cancer, this axis' regulation may have an impact on the immune system's capacity to identify and eradicate cancer cells [36].

The trafficking protein particle (TRAPP) complex, of which TPPC3 is a component, is involved in membrane fusion events and intracellular vesicle trafficking [37]. The control of vesicular transport between the Golgi apparatus and the endoplasmic reticulum (ER) is mediated by TRAPP complexes. Changes in vesicle trafficking pathways have been linked to the onset and spread of cancer [38]. Dysregulated vesicle trafficking may be utilized by some oncoproteins and signaling pathways, or it may have an impact on them. Certain proteins connected to oncogenic or tumor suppressor pathways are also engaged in vesicle trafficking. One strategy to learn more about TPPC3's function in cancer biology is to look into possible interactions between it and these pathways [39].

The gene known as interferon alpha-inducible protein 27 (IFI27) or interferon alpha-inducible protein 27-like protein 2 (I27L2) is elevated in response to interferon signaling [40]. The interferon pathway has been connected to cancer and is essential for the immune system's reaction to viral infections. IFI27/I27L2 appears to play a complex and context-dependent role in cancer [41]. There are studies that point to a possible tumor-suppressive activity and others that point to a tumor-promoting effect. The precise effect of IFI27/I27L2 on the behavior of cancer cells may alter depending on the type of cancer [42]. It is possible that IFI27/I27L2 interacts with different signaling pathways involved in the development of cancer. Deciphering these connections will be essential to understanding the molecular pathways through which IFI27/I27L2 may affect the biology of cancer [43].

Deformed epidermal autoregulatory factor 1 homolog (DEAF1) is a transcription factor that has been connected to a number of biological functions, including as chromatin organization and transcriptional regulation [44]. By attaching itself to particular DNA sequences, the transcription factor DEAF1 is known to control the expression of genes. Numerous physiological functions, including as cell differentiation, death, and immune response, are mediated by its target genes [45]. Although DEAF1's precise function in cancer is unknown, some cancer types have been linked to mutations in the DEAF1 gene or changes in the protein's expression levels. This implies that DEAF1 might be implicated in the development of tumors, possibly through affecting the expression of genes associated to cell division, apoptosis, or other processes connected to cancer[46].

BCL6, or B Cell Lymphoma 6, is a transcriptional repressor that is essential for maintaining healthy B-cell growth and controlling the immune system. It belongs to the zinc finger BTB/POZ transcription factor family and plays a role in the germinal center response that occurs during the maturation of antibody affinity. Despite being necessary for healthy immune function, BCL6 expression dysregulation has been linked to the etiology of a number of malignancies [47]. BCL6 has been shown to express aberrantly in a number of cancers, including breast and prostate cancer, suggesting that it plays a role in the etiology of non-hematologic malignancies. The most well-known association between BCL6 and B-cell lymphomas is DLBCL, or diffuse large B-cell lymphoma. The BCL6 gene frequently experiences chromosomal translocations in DLBCL, which leads to constitutive activation and abnormal expression of the gene. This adds to the B-cells' unchecked proliferation [48]. BCL6 dysregulation has also been linked to B-cell malignancies other than DLBCL, including follicular lymphoma and Burkitt lymphoma [49]. BCL6 overexpression aids in the formation of tumors by promoting cell survival and preventing apoptosis [50].

Though the study has been conducted in a comprehensive manner, future researches should consider a larger sample size.

CONCLUSION

To conclude, the level of expression of FAM71B and Keratin type II cytoskeletal 1 reported in this study using MALDI-TOF proteomic technology might provide valuable information towards serving as a potential biomarker of oral squamous cell carcinoma. Expression of E3 ubiquitin ligase URB5 and extracellular matrix organizing protein FRAS1 reported in this study shows that extensive research on E3 ubiquitin ligase URB5 and extracellular matrix organizing protein FRAS1 should be done to understand their importance in oral squamous cell carcinoma. The MALDI-TOF technology being a very useful option, it can be used in further researches to explore and understand more about the proteomics of various diseases.

ETHICAL COMMITTEE APPROVAL

The institutional ethical committee approval was obtained from Saveetha Dental College and hospitals, Chennai, Tamil Nadu (IHEC Ref. No: IHEC/SDC/OPATH-2001/22/017).

CONFLICTS OF INTEREST

The authors declare no conflicts of interest related to the study.

AUTHOR CONTRIBUTIONS

Author 1: Conceptualization, Data Curation, Methodology, Formal analysis, Investigation, Visualization, Writing - Original Draft. **Author 2:** Conceptualization, Software, Validation, Writing - Review & Editing, Visualization, Supervision. **Author 3:** Conceptualization, Resources, Project administration, Visualization, Validation, Writing - Review & Editing.

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Figure legends:

Figure 1 : Mascot score histogram of plasma profiling in OSCC and control samples using MALDI-TOF.

Figure 2: a) STRING analysis of the proteins in the plasma of OSCC samples. **b)** Interactome of the plasma proteins in OSCC demonstrating the protein interaction using cytoscape.

Figure 3: Mascot score histogram of serum profiling in OSCC and control samples using MALDI-TOF.

Figure 4: a) STRING analysis of the proteins in the serum of OSCC samples. **b)** Interactome of the serum proteins in OSCC demonstrating the protein interaction using cytoscape.

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