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Value of Salivary Biomarker as a Diagnostic Tool in Oral Cancer- A Systematic Review

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Abstract

More than 90% of oral cancers are squamous cell carcinomas (OSCC). Most OSCCs are not diagnosed at an early stage even though it is easily accessible for direct visual examination. This event underscores the early and accurate detection by clinicians to reduce its morbidity and mortality rate. Anatomical proximity of the saliva to oral cancer makes it most accurate and specific diagnostic tools, while more than 100 salivary biomarkers have already been identified, including cytokines (IL-8, IL-1β, TNF-a), P53, transferrin, DUSP, MMP, LDH, and many more. However, further research is required for validating the best salivary biomarker. Current review aimed at finding the value of salivary biomarker as a diagnostic tool in oral cancer.

Keywords: Saliva, Biomarker, Oral Cancer, Oral squamous cell carcinoma

Introduction

6th most common cancer worldwide is cancers of the oral cavity and pharynx [1]. Oral squamous cell carcinomas (OSCC) seems to be the most incessant of every single oral neoplasm, and over 90% of every oral neoplasm are evaluated to be OSCC. They arise from the epithelial lining of the oral cavity. Most OSCCs are not diagnosed until an advanced stage though it's visible under direct visual examination, which is believed to be the major reason for the low survival rate [2]. More than 550,000 cases of head and neck cancers are detected in worldwide, with an annual death rate approaching 300,000/year [3]. This underscores the significance of timely and specific discovery by clinicians. In reacting to the call for early recognition of OSCC, a few indicative clinical indicators have been produced, or right now are being developed [4-6]. Biomarkers have developed as basically vital apparatuses to recognize infections in their different clinical stages by expanding the exactness to definitely

describe the ailment in a demonstrative or prognostic level.

Saliva has been found to reflect the diseased or physiological state of the human body, and hence could be utilized for diagnostic purpose [7-9]. Salivary testing, a non-invasive alternative option to serum testing, is a viable methodology for finding and forecasting expectation of different illnesses. Proteins, mRNA, catalysts, and chemicals extracted from salivation has been found to be at adequately better levels amongst OSCC & control tests, to be considered as potential biomarkers. They could be a prospect to fill in as a generally accessible screening apparatus that is autonomous of the limitation of an injury for analysis. This technique being conceivably better than other identification techniques, enable salivary biomarker screening to sort patients with harmful and conceivably threatening injuries. Although there are an extensive number of examinations on salivary biomarkers and OSCC, a precise survey is important to figure out which of the various accumulations of detailed biomarkers displays satisfactory indicative test exactness. Subsequently, the objective of this methodical survey was to answer an engaged inquiry, in particular: "Do salivary biomarkers have the capacity to precisely distinguish Oral SCC patients from non-oral SCC controls?"

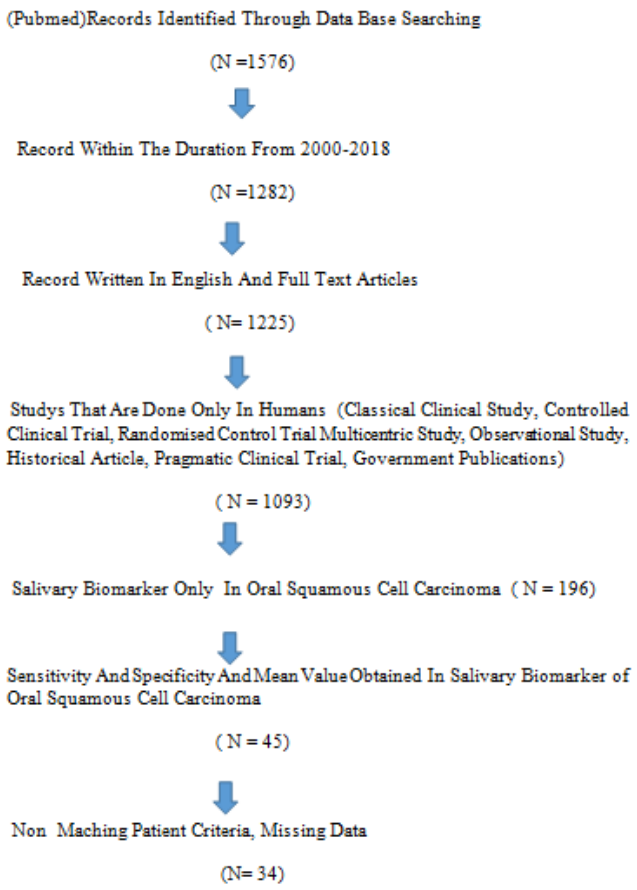

Figure 1: Image depicting the identification, the screening process and the eligibility criteria of those studies that were included in this review.

Results

Study selection and characteristics

The systematic review intended to test the value of salivary biomarker as a diagnostic tool in oral cancer. Our systematic search of PubMed resulted in 1576 unique papers. Articles that were recorded from the duration of 2000-2018 was around 1282. Articles that are written in English and full- text article were 1225. Those articles which were related only to humans and those that followed these study design including (classical clinical study, controlled clinical trial, randomised control trial multicentre study, observational study, historical article, pragmatic clinical trial, government publications) were 1093. Out of these 1093 articles, salivary biomarker in

OSCC was around 196. In this, about 45 articles had either sensitivity and specificity or mean values. 11 were excluded due to missing data. Hence, 34 articles were included in the systematic review [Figure1].

Individual characteristics of the included 34 studies are summarised in [Table 1]. Of 34 full texts assessed, the first observation was that not all studies presented with the sensitivity and specificity values. Only sixteen studies showed the sensitivity and specificity values [Table 2]. Majority of these studies were conducted in Asian Countries (INDIA, CHINA) and US (California). Most of the studies utilized quantitative polymerase chain reaction (qPCR) technique. Seven studies used ELISA as the detection method. The sample size of these studies ranged from 19 to 191.

Sensitivity and specificity

MASPIN, CYCD1 showed 100% sensitivity and specificity with ELISA as the detection method. Followed by MMP1 with sensitivity and specificity of 93.5% and 97.8% respectively, when detected by the RT-qPCR method. DUSP1 showed least sensitivity of 0.14 using PCR AND ELISA. Lactate dehydrogenase showed least specificity values when detected by ELISA, Kinetic spectrophotometry.

Table 1: Shows the Individual characteristics of the included 34 studies

Sl No	Year	Lead Authour	Study Design	Country	No Of Case	No Of Control	Salivary Biomarker	Detection Method	Sensitivity	Specificity	Mean Value Obtained
1	2004	Yang Li	A clinical study	US	Patients with primary T1/T2 OSCC(n=32)	Control =32 Healthy subjects	DUSP1, H3F3A,IL1B,IL8,OAZ1,S100P, SAT	RiboAmp RNA Amplification kit,Human Genome,Quantitative Polymerase Chain Reaction	DUSP1=59,H3F3A=53,IL1B=63,IL8=88,OAZ1=100,S100P=72, SAT=81	DUSP1=75,H3F3A=81,IL1B=72,IL8=81,OAZ1=38,S100P=63,SAT=56	NIL
2	2007	Elizabeth J. Franzmann	pilot study	US	102 patients with HNSCC	Control =69 healthy subjects	CD44	ELISA assay	CD44 =62% to 70%	CD44 = 75% to 88%	NIL
3	2008	ME Arellano-Garcia	A Clinical study	US	Patient with Oscc= 20	Control=Normal Healthy =20	IL-8 (single-plex) IL-8 (multiplex) IL-1β (single-plex) IL-1β (multiplex)	Bead-based assays, ELISA	OSCC (n = 20) Control (n = 20) Sensitivity IL-8 (single-plex) =75 IL-8 (multiplex) =75 IL-1β (single-plex) =75 IL-1β (multiplex) =80	OSCC (n = 20) Control (n = 20) Specificity (%) IL-8 (single-plex) =80 IL-8 (multiplex) =80 IL-1β (single-plex) =80 IL-1β (multiplex) =65	OSCC (n = 20) Mean value IL-8 (single-plex) 3313.2 ± 3759.8 IL-8 (multiplex) 2834.9 ± 3385.6 IL-1β (single-plex) 945.2 ± 1134.8 IL-1β (multiplex) 1013.5 ± 1221.1
4	2008	Alice Y. Chuang	A Cohort study	US	59 HNSCC patients, HPV-16 positive=20 , HPV -16 Positive =39	NIL	HPV DNA	Quantitative PCR	HPV-16 Positive Sensitivity = 50%	HPV -16 Positive Specificity = 100%	NIL
5	2008	Shen Hu1	A Clinical study	California	OSCC = 64	Control=Healthy subjects (n = 64	soluble CD44,, cytokeratin 19 fragment Cyfra21-1, tissue polypeptide	Reversed-phase liquid chromatography ,LC-tandem mass spectrometry,2D Quant kit,ELISA	sensitivity of 90%	specificity of 83%	NIL
6	2009	Benjamin Lallemant	A Case control study	France	case HNSCC=74	Control=Healthy control = 18	FNI	RT-qPCR	FNI= 58.7	FNI=76.1	NIL

							IL1RN		IL1RN=93.5	IL1RN = 95.7	
							KRT13		KRT13=75.0	KRT13= 95.5	
							KRT4		KRT4=89.1	KRT4= 91.3	
							MAL		MAL=95.7	MAL=91.3	
							MMP1		MMP1= 93.5	MMP1= 97.8	
							PLAU		PLAU=80.5	PLAU=89.1	
							SPARC		SPARC=71.7	SPARC=93.5	
							TGM3		TGM3=84.8	TGM3=91.3	
7	2009	T Shpitzer	A Clinical Study	Israel	Case =19	Control =19 Healthy subjects	MMP-9 Carbonyls OGG1 phospho-Src Ki67 Maspin LDH CycD1	ELISA , LDH activity was detected by kinetic spectrophotometry using a commercial kit	MMP-9 = 100 Carbonyls = 90 OGG1 = 77 phospho-Src = 77 Ki67 = 58 Maspin =100 LDH = 79 CycD1 = 100	MMP-9 = 79 Carbonyls = 80 OGG1 = 75 phospho-Src = 75 Ki67 = 67 Maspin = 100 LDH = 42 CycD1= 100	NIL
8	2010	Yu-Jen Jou	A Clinical study	China	OSCC = 41	Controls=30 healthy subjects	salivary transferrin levels	Two dimentional gel electrophoresis, (2DE) and mass spectrometry (MS),Western blotting and ELISA	Salivary transferrin-based ELISA=100% inT1 group, overall OSCC=95%	salivary transferrin-based ELISA was 100% in T1 group and overall OSCC=100%	The mean plasma transferrin 216.3mgdL−1 in the T1 group, 235.0mgdL−1 in the T2 group,and 203.6mgdL−1 in the T3/T4 group
9	2010	Jie Wei	A Clinical study	China	37 OSCC patients, 32 oral leukoplakia (OLK) patients	Control =34 healthy subjects	Lactic acid		Lactic acid=73.0(healthy/oscc) Lactic acid=73.0(oscc/olk)	Lactic acid=70.6(healthy/oscc) Lactic acid=75.0(oscc/olk)	NIL
							c-Aminobutyric acid		c-Aminobutyric acid=61.8(healthy/oscc) c-Aminobutyric acid=75.0(oscc/olk)	c-Aminobutyric acid= 62.2 (healthy/oscc) c-Aminobutyric acid=70.3(oscc/olk)	
							Valine		Valine=82.4(healthy/osc	Valine=75.7(healthy /oscc)	NIL

									c) Valine=78.1(oscc/olk)	Valine=75.8(oscc/olk)	
							Phenylalanine		Phenylalanine=52.9(healthy/oscc) Phenylalanine=71.9(oscc/olk)	Phenylalanine=56.8(healthy/oscc) Phenylalanine=75.7(oscc/olk)	
							n-Eicosadienoic acid		n-Eicosadienoic acid=51(healthy/oscc) n-Eicosadienoic acid=70.3(oscc/olk)	n-Eicosadienoic acid=73.5(healthy/oscc)	
10	2011	Yu-Jen Jou	Clinical study	China	OSCC =47,	Control =30Healthy subjects	ZNF510 peptide	Two dimentional gel electrophoresis, (2DE) and mass spectrometry (MS),Western blotting and ELISA	Salivary transferrin-based ELISA=100% inT1 group, overall OSCC=95%	salivary transferrin-based ELISA was 100% in T1 group and overall OSCC=100%	The mean plasma transferrin concentration was 216.3mgdL−1 in the T1 group, 235.0mgdL−1 in the T2 group,and 203.6mgdL−1 in the T3/T4 group
11	2011	Ole Brinkmann	A Clinical study	Europe	OSCC= 35	Control = 51 Healthy subjects	DUSP1, IL8, IL1B, OAZ1, SAT1, S100P	PCR and ELISA	Maximum Sensitivity Protein markers OSCC total, T1-T2, T3-T4 OSCC total, T1-T2 ,T3-T4 IL1B 0.83 0.83 0.76 0 IL8 66 0.61 0.71 0. M2BP 0.37 0.83 0.29	Maximum specificity Protein markers OSCC total T1-T2 T3-T4 OSCC total T1-T2 T3-T4 IL1B = 76 0.78 0.84 IL8 = 80 0.80 0.80 M2BP= 0.90 0.59 0.92	NIL
12	2013	Juliana Schussel	prospective study	US	Total Case=191 Benign=113 ,Mild DysplasIa=.27 Moderate Dysplasia= 10 Severe	NIL	*DAPK*	Quantitative Methylation Specific PCR	CRC – 56%	66%	NIL
									EDNRB & DCC- 46%	72%	

					Dysplasia =6 Cancer=35						
13	2013	David Elashoff	A Cohort Study	US	Cohort 1 (oscc=48)Cohort 2 (oscc=24)Cohort 3 (Oscc=30))	Cohort 1 (control=48),Cohort 2 (Control=24),	Cohort 1 (control=48),Cohort 2 (Control=24),	(qPCR)			
					Cohort 4 (oscc= 36)Cohort 5(Oscc=31	Cohort 3 (Control=30),Cohort 4 (controi=54,Cohort 5(control=70	DUSP1		DUSP1= 0.60	DUSP1= 0.56	NIL
							H3F3A		H3F3A= 0.61	H3F3A= 0.56	
							IL1B		IL1B = 0.65	IL1B = 0.60	
							IL8		IL8 = 0.68	IL8 = 0.64	
							OAZ1		OAZ1=0.62	OAZ1=0.58	
							S100P		S100P= 0.60	S100P= 0.56	
							SAT		SAT= 0.66	SAT= 0.63	
							B)Proteins				
							IL8		IL8 = 0.8	IL8 = 0.43	
							M2BP				
14	2014	Yu-Jen Jou	A clinical Study	China	OSCC=100	Control =35 Healthy subjects	S100A8	nanoLC–MS/MS,ELISA	Sensitivity for T1= (95%)	Specificity for T1=95%	NIL
									Sensitivity for T2= 0.68	Specificity for T2= 0.68	
									Sensitivity for T3=0.99	Specificity for T3=0.99	

									Sensitivity for T4 =0.98	Specificity for T4 =0.98	
15	2014	Rajkumar. K	A Clinical study	India	Premalignant condition (50 in each group, leukoplakia & OSMF) =100 , Oscc =100	Control =100 healthy subjects	IL -8	ELISA	Sensitivity = 85%	Specificity=93%	NIL
16	2014	Qihui Wang	A Clinical study	China	oscc =30	Control=30 Healthy subjects	Lactic acid	Mass spectrometry	Lactic acid=100.0	Lactic acid=73.3	NIL
							Hydroxyphenyllactic acid		Hydroxyphenyllactic acid=82.4	Hydroxyphenyllactic acid=60.0	
							N-nonanoylglycine		N-nonanoylglycine=52.9	N-nonanoylglycine=73.3	
							5-hydroxymethyluracil		5-hydroxymethyluracil=47.1	5-hydroxymethyluracil=96.7	
							Succinic acid		Succinic acid=88.2	Succinic acid=66.7	
							Ornithine		Ornithine=82.4	Ornithine=73.3	
							Hexanoylcarnitine		Hexanoylcarnitine=70.6	Hexanoylcarnitine=60.0	
							Propionylcholine		Propionylcholine=64.7	Propionylcholine=80.0	
							Carnitine		Carnitine=94.1	Carnitine=46.7	
							4-hydroxy-L-glutamic acid		4-hydroxy-L-glutamic acid=94.1	4-hydroxy-L-glutamic acid=56.7	
							Acetylphenylalanine		Acetylphenylalanine=82.4	Acetylphenylalanine=70.0	
							Sphinganine		Sphinganine=70.6	Sphinganine=83.3	

							Phytosphingosine		Phytosphingosine=76.5	Phytosphingosine=83.3	
							S-carboxymethyl-L-cysteine		S-carboxymethyl-L-cysteine=88.2	S-carboxymethyl-L-cysteine=90.0	
17	2014	Qihui Wang	A Clinical study	China	oscc =30	Control=30 Healthy subjects	Salivary L-phenylalanine, L-leucine	Ultra performanceliquidchromatography–electrosprayionization–mass	L-Leucine sensitivity = 84.6%	L-leucine specificity = 81.7%	NIL
18	2014	Chih-Ching Wu	A clinical Study	China	86 OPMD ,131 OSCC patients	controls =131 healthy subjects	anti-p53,anti-survivin, anti-CK-8, anti-Hsp60, and anti-RPLP0	multiplexed bead-based system	sensitivity with the fluorescence emission spectra=88.9	Specificity of 94.0 % has been achieved from fluorescence emission spectra. specificity of 90%	NIL
									LR-OPMD (42) 5 (11.9%) 5 (11.9%) 3 (7.1%) 6 (14.3%) 4 (9.5%)		
									HR-OPMD (44) 11 (25.0%) 9 (20.5%) 9 (20.5%) 11 (25.0%) 10 (22.7%)		
									OSCC (131) 31 (23.7%) 27 (20.6%) 23 (17.6%) 31 (23.7%) 38 (29.0%)		
									Well-differentiated OSC (60) 18 (30%) 19 (31.7%) 14 (23.3%) 20 (33.3%) 23 (38.3%) The sensitivity of utilizing anti-p53,anti-survivin, anti-CK-8, anti-Hsp60, and anti-RPLP0 for detection of well-differentiated OSCC were 30.0%, 31.7%, 23.3%, 33.3%, and 38.3%,		
19	2014	Manoharan Yuvaraj	A Clinical Study	India	OSCC = 67	Normal subjects =27	ethidium bromide	Fluorescence spectroscopic characterization was carried out using spectroflurometer	Sensitivity with the fluorehscence emission spectra=88.9	Specificity of 94.0 % has been achieved from fluorescence emission spectra	NIL
20	2015	Niranzena Panneer Selvam	A Clinical study	India	group I= 25 oral leukoplakia GroupII=25	Group III = 25 normal controls	IL-6	ELISA	NIL	NIL	groups I 43.00 ± 52.143 pg/Ml group II 132.88 ± 59.098 pg/mL group III9.68 ±

					OSCC						12.838 pg/mL.
21	2015	Salman Aziz	A Cross Sectional Study	Pakistan	OSCC patients = 30	Healthy Controls =33	Salivary IL-4, IL-10, IL-13, and IL-1RA Healthy Controls =33	Millipore's MILLIPLEX (4-plex) Human Cytokine/ Chemokine assay kit	NIL	NIL	control =82 ± 38.9 and for OSCC = 280± 146.86,
22	2015	Jasdeep Kaur	A clinical study	India	40 oral leukoplakia ,40 osmf, 40 oral squamous cell carcinoma,	Normal healthy controls =40	Salivary 8-hydroxy-2-deoxyguanosine, malondialdehyde, vitaminC, and Vitamin E	Lipid peroxidation products (MDA) were analyzed by the thiobarbituric acid (TBA) reaction . Salivary levels of 8-OHdG in supernatant were determined using a competitive ELISA kit.Vitamin C and vitamin E were estimated by HPLC	Diagnostic values of sensitivity of combination of salivary 8-OHdG (A), MDA (B), and vit. C (C) and E (D) determination in distinguishing oral precancerous,and cancer patients from healthy individuals Diagnostic values Oral squamous cell carcinoma versus vs. normal healthy were (AB)=82,(ABC)=83,(ABCD)=85 Oral pre-cancerousvs. normal healthy were(AB)=81,(ABC)=82,(ABCD)=83 Oral squamous cell carcinoma vs.pre-cancerous lesions were(AB)=80,(ABC)=80,(ABCD)=80	Diagnostic values of specificity of combination of salivary 8-OHdG (A), MDA (B), and vit. C (C) and E (D) determination in distinguishing oral precancerous,and cancer patients from healthy individuals Diagnostic values Oral squamous cell carcinoma versus vs. normal healthy were (AB)=81,(ABC)=81,(ABCD)=83 Oral pre-cancerousvs. normal healthy were(AB)=80,(ABC)=82,(ABCD)=81 Oral squamous cell carcinoma vs.pre-cancerous lesions re(AB)=79,(ABC)=80,(ABCD)=80	Mean (SD) salivary levels of oxidative stress markers in patients and healthy controls 8-OHdG (ng/ml) MDA (µmol/l) Vitamin E (µg/l) Vitamin C (µg/l) Control 0.07 (0.07) 0.08 (0.07) 1.4 (0.6) 1.2 (0.6) Oral leukoplakia 0.36 (0.07)a 0.33 (0.07)a 0.57 (0.16)a 0.55 (0.13)a Oral lichen planus 0.47 (0.07)b 0.43 (0.007)b 0.56 (0.13)b 0.56 (0.12)b Oral submucous fibrosis 0.49 (0.08)c 0.43 (0.07)c 0.56 (0.11)c 0.53 (0.12)c Oral squamous cell carcinoma 1.19 (0.19)d 1.00 (0.21)d 0.37 (0.08)d 0.27 (0.07)d

23	2015	Shishir Ram	A Clinical study	India	GROUP I =50 oral leukoplakia, GROUP II=50 oral submucous fibrosis (OSMF),GROUP III=50 (OSSC),	GROUPIV= 50Healthy controls	copper, zinc and iron	GBC Avanta atom absorption spectrophotometer	NIL	NIL	Mean salivary **Cu level**s in group HC, OSMF, OL and OSCCwere 46.07 ± 4.56 µg/dL, 87.45 ± 2.67 µg/dL, 55.54 ± 2.57 µg/dLand 57.87 ± 4.98 µg/dL,mean salivary **Cu level**s in group HC, OSMF, OL and OSCCwere 46.07 ± 4.56 µg/dL, 87.45 ± 2.67 µg/dL, 55.54 ± 2.57 µg/dLand 57.87 ± 4.98 µg/dL,
24	2016	Ryan C. Chai	A Pilot study	Austalia	Total HNSCC = 82, Tumor specimen of 42 patient out of 82 were p16INK4 positive ,40 with p16INK4 negative	NIL	p16INK4a, HPV-16 DNAp16INK4a, HPV-16 DNA		p16INK4a positive =60%, using end-point(RT-PCR)and p16INK4positive using quantitativeRT-PCR =55%	p16INK4a positive both using end point RT-PCR and quantitative PCR=100%	NIL
25	2016	Basavaraj N	A Clinical study	India	Total Case= 60 subjects, (Group I)OSMF=25, Oral Cancer=25	Control=10	Lactate dehydrogenase	ERBA-CHEM 5 Semi Auto analyzer	NIL	NIL	The mean LDH levels were Group I= 608.28 Group II (Oral Cancer) = 630.96 Group III (control)= 39.80
26	2016	Evangelia Michailidou	A Clinical study	Greece	Patients with leukoplakia with dysplasia=20, Patients with OSCC=34	Healthy Control = 31	OAZ	Saliva RNA was performed using the QIAmpViral RNA Mini Kit and then Quantitative real-time PCR	NIL	NIL	OAZ in Normal Healthy control=35.44 ±2.07, Patients with leukoplakia with dysplasia=36.11±1.68, Patients with

											oscc=36.69±2.19
27	2016	Jau-Song Yu	A Clinical study	China	103 low-risk OPMDs,130 high-risk OPMDs, and 131 OSCC subjects	Healthy controls=96	ANXA2 CA2 CD44 CRNN CST3 CSTA DSG3 FLNA FSCN1 GANAB GSTP1 HMGCS1 HSPA5 IGFBP ISG15 KNG1 LDHA LGALS3BP MMP1 MMP3 MMP9 PRDX2 S100A9 SPARC STAT1	Multiplex LC-MRM-MS	ANXA2 80.2 CA2 76.3 CD44 76.3 CRNN 77.1 CST3 36.6 CSTA 93.1 DSG3 90.1 FLNA 71.8 FSCN1 61.8 GANAB 60.3 GSTP1 84.0 HMGCS1 67.9 HSPA5 90.1 IGFBP 68.7 ISG15 52.7 KNG1 84.0 LDHA 93.1 LGALS3BP 71.0 MMP1 69.5 MMP3 62.6 MMP9 75.6 PRDX2 92.4 S100A9 69.5 SPARC 56.5 STAT1 67.2	ANXA2 68.3 CA2 60.8 CD44 57.8 CRNN 52.3 CST3 77.9 CSTA 9.5 DSG3 47.2 FLNA 67.3 FSCN1 81.9 GANAB 73.4 GSTP1 45.2 HMGCS1 67.3 HSPA5 39.7 IGFBP 72.4 ISG15 74.9 KNG1 75.4 LDHA 46.7 LGALS3BP 50.8 MMP1 95.0 MMP3 76.9 MMP9 60.3 PRDX2 44.2 S100A9 69.3 SPARC 86.4 STAT1 68.3	NIL
28	2017	Yi -Ting Chen	A Clinical study	China	Total case=119,group I=32 OSCC Group II=29 OSCC	Group I =29 Healthy Controls,GROUP II=29 Healthy Control	A1AG1 AACT ANGT ANT3 APOB APOH C1 CERU	BCA Protein Assay Kit	A1AG1=0.869 AACT=0.639 ANGT=(82.41) ANT3=0.672	A1AG1= 0.793 AACT= 0.759 ANGT= 0.672 ANT3= 0.776	NIL

							CFAH CRP FA12 FETUA FIBB FINC HEMO HEP2 HPT HRG ITIH1 KNG1 PLMN SAA4 SAMP VTNC		APOB=0.738 APOH=0.803 C1 =0.639 CERU=0.705 CFAH=0.869 CRP=0.541 FA12=0.787 FETUA=0.705 FIBB=0.721 FINC=0.787	APOB= 0.776 APOH=0.845 C1 =0.914 CERU=0.897 CFAH= 0.845 CRP = 0.931 FA12= 0.741 FETUA= 0.897 FIBB =0.81 FINC= 0.81	

									HEMO=0.754 HEP2=0.803 HPT=0.721 HRG=0.689 ITIH1=0.754 KNG1=0.738 PLMN=0.738 SAA4=0.639 SAMP=0.770 VTNC=0.721	HEMO= 0.81 HEP2= 0.914 HPT=0.741 HRG= 0.897 ITIH1= 0.828 KNG1 0.793 PLMN= 0.793 SAA4= 0.793 SAMP= 0.897 VTNC= 0.914	
29	2017	Tahereh Nosratzehi	A clinical Study	Iran	25 had OLP(oral Lichen Planus) and 25 had OSCC	Normal Healthy Control=25	Salivary endothelin-1	Enzyme-linked immunosorbent assay	NIL	NIL	Control=137.19, OLP=160.90,SCC=163.98

30	2017	Lutécia H. Mateus Pereira	A Case control study	US	oral cancer patients = 150	Control=150	CD44 and Total protein	Sandwich ELISA assay	80.7%	48.7%	control (CD44 was <2.22 ng/ml and protein was <1.23 mg/ml) and in case (CD44 was ≥2.22 & <5.33 ng/ml and protein was ≥0.558 mg/ml)
31	2017	Andre Peisker	A Clinical Study	Germany	OSCC =30	Healthy controls=30	MMP-9	ELISA	100%	26.7%	NIL
32	2018	Shrikant Patel	A biochemical study	India	Oral leukoplakia Group II =25, Oral cancer GroupIII=25	Healthy control G roup I=25	Lactate dehydrogenase enzyme	BiovisionLactate Dehydrogenase activity colorimetric assay kit	NIL	NIL	Group I, II, and III were 261.16 ± 75.851, 497.00 ± 100.404, 686.40 ± 81.752
33	2018	Tharun Varghese,Jacob	A Clinical Study	India	20 Precancer GRPOUPII,20 Oral cancer GROUPIII	GROUP I = 20 Healthy controls	Sialic Acid	UV-spectrophotometer	NIL	NIL	Healthy control =21.65,Oral precancer=59.75, OSCC=204.85
34	2018	Nidhi Awasthi	cross-sectional study	India	30 OSCC (Group I) and 9 PML (Group II)	Healthy controls (Group III)=25	CYFRA 21-1	ELISA ,standard kit method			CYFRA 21-1 GROUP I (17.5±15.2), GROUP II (5.9±2.4,) GROUP III (3.9±2.2)
							CA 19-9				CA 19-9 GROUP I (20.1±9.0),GROUPII (19.5±3.8),GROUPIII (20.4±5.4)
							LDH				LDH GROUP I (425.4±158.2), GROUPII (19.5±3.8) GROUP III (

											20.4±5.4)
							Amylase				Amylase GROUPI (628.8±445.4), GROUP II (1115.3±275.6),GROUPIII (1287.5±289)
							Total proteins				Total proteins GROUP I (192.5±59.6),GROUP II (134.7±18), GROUP III (94.7±18.4)

Discussion

The aim of this review was to test the value of salivary biomarker as a diagnostic tool in oral Cancer. Saliva is a unique fluid both in its source and composition. Saliva has got the following functions including lubrication, digestion, and antimicrobial activity, facilitating remineralisation of the tooth enamel, and maintaining normal taste sensation [10]. These functions are achieved by the various components of saliva including water, inorganic and organic compounds, protein/polypeptides, and hormone [10]. So far, more than 2300 proteins and peptides have been found in human saliva [11]. The most abundant proteins in saliva are α-amylase, albumin, secretory-IgA, lactoferrin, mucins, lysozymes, proline-rich proteins, and transferrin [12]. These protein and peptides are used as a biomarker for the detection of oral cancer.

Most of the potential OSCC salivary biomarkers are listed in (Table 1). A wide range of salivary biomarkers was analysed in this systematic review including IL8, IL6, IL1B, DUSP1, H3F3A, OAZ1, S100P SAT, LDH, Fe, OAZ, Lactic acid and MMP1. Challenges in the detection of the sensitivity and specificity values of the salivary biomarker could be due to very low concentration in saliva and lack of standardization of conditions and methods of saliva sample collection, processing, and storage.

Shen Hu1 et al [13]. Demonstrated a subtractive proteomics approach to profile proteins in pooled saliva samples from 16 OSCC and 16 healthy subjects with very well matched in terms of gender, ethnicity, and age to minimize potential bias. The study concluded that the target proteins with soluble CD44, cytokeratin 19 fragment Cyfra21-1, tissue polypeptide showed a sensitivity of 90% and specificity of 83%.

T Shpitzer et al [14]. Showed that the sensitivity values of the eight analysed markers were in the range of 58–100% whereas the specificity values were in the range of 42–100%. The sensitivity and specificity values were especially high for the CycD1 and Maspin markers, 100% for each value of each marker. These were also quite high for the carbonyls 90% and 80%, respectively, and for the MMP-9 100% and 79%, respectively. This suggests that the all eight biomarkers analysed in OSCC patients is highly desirable and beneficial if salivary tumour marker analysis could be performed on a routine basis. Furthermore, salivary biomarkers being noninvasive, and an effective alternative to serum testing, helps in detecting the OSCC at the earliest stage which will then further reduce the morbidity and mortality rate.

Ole Brinkmann et al [15]. Showed that three proteomes (IL1B, IL8, M2BP) and Four transcriptomes (IL8, IL1B, SAT1, S100P) were significantly elevated ($p<0.05$) in OSCC patients. The sensitivity/specificity for OSCC total was 0.89/0.78, for T1-T2 0.67/0.96, and for T3-T4 0.82/0.84. These salivary biomarkers are highly promising and recommended for OSCC detection.

Rajkumar et al [16]. Showed that the sensitivity and specificity of IL8 are 85% and 93% respectively. The study supports the utility of salivary IL-8 as a marker for routine diagnosis of OSCC and it also suggests that salivary IL-8 can be used as a screening marker of oral cancers.

Qihui Wang et al [17]. Showed the sensitivity of 84.6% and specificity of 81.7%. The possibility of salivary metabolite biomarkers for OSCC diagnosis is successfully demonstrated in this study as it is non-invasive, simple, reliable, and also provides lower detection limits and excellent precision and a simple clinical tool for the early diagnosis of OSCC.

Andre Peisker et al [18]. Showed that the sensitivity value of MMP-9 was 100% whereas the specificity value was 26.7%. The data indicate that the elevation of salivary

levels of MMP-9 may be a useful adjunctive diagnostic tool for detection of OSCC. However, the specificity values were much reduced due to lack of the standardization and methods used for the detection of the biomarker.

Most studies have investigated the potential salivary biomarker levels only in OSCC patients and non-OSCC controls, without regard for other inflammatory conditions that might have been present, so this would result in a high false positive rate. The oral cavity is commonly subjected to inflammation from a variety of causes including trauma, dental plaque, infection and certain mucocutaneous inflammatory diseases and periodontitis. So ruling out the inflammatory condition prior to the salivary sample collection is of utmost importance, in order to establish the reliability of that salivary OSCC biomarker. However, the current review demonstrates that MASPIN, CYCD1 showed 100% sensitivity and specificity with ELISA as the detection method. This is because of following a proper standardization procedure for saliva sample collection, processing, and storage. Followed by MMP1 with sensitivity and specificity of 93.5% and 97.8% respectively, when detected by the RT-qPCR method. DUSP1 showed the least sensitivity of 0.14 when PCR AND ELISA were used as the detection method. Lactate dehydrogenase showed least specificity values when detected by ELISA, Kinetic spectrophotometry due to lack of standardization for the saliva sample collection, processing and storage.

Conclusion

Salivary biomarkers represent a promising highly sensitive, reliable, specific and non-invasive method for oral cancer detection. However, certain challenges include a lack of standardization for saliva sample collection, processing, and storage & wide variability in the salivary biomarkers in both healthy individuals and OSCC patients. Further studies are needed in this field, to obtain an eventual standardization especially concerning biological variance and physiological changes affecting the potential salivary biomarkers for the detection of oral cancer. This review served as an important reference in salivary diagnostics including identifying, validating, and applying salivary biomarkers for detection of OSCC.

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