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Review

Comprehensive Review on Development of Early Diagnostics on Oral Cancer with a Special Focus on Biomarkers

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Abstract: One of the most frequent head and neck cancers is oral cancer, with less than half of those diagnosed surviving five years. Despite breakthroughs in the treatment of many other cancers, the prognosis for people with OSCC remains dismal. The conventional methods of detection include a thorough clinical examination, biochemical investigations, and invasive biopsies. Early identification and treatment are important for a better chance of extending a patient's life. Early diagnosis may be possible by identifying biomarkers in biological fluids. Currently, the primary method for diagnosing oral lesions is a visual oral examination; however, such a technique has certain drawbacks, as individuals are recognized after their cancer has advanced to a severe degree. The first section of this review discusses several diagnostic techniques for cancer detection, while the second section discusses the present state of knowledge about known existing predictive markers for the timely identification of malignant lesions, as well as disease activity tracking. The aim of the paper is to conduct a critical review of existing oral cancer diagnostic processes and to consider the possible application of innovative technology for early detection. This might broaden our diagnostic choices and enhance our capacity to identify and treat oral malignant tumors more effectively.

Keywords: oral cancer; OPMD; OSCC; early diagnosis; biomarkers; treatment

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1. Introduction

Cancer can be caused by the uncontrollable multiplication of abnormal growth of cells, which can spread and cause damage to other organs. Oral cancer (OC) is a kind of cancer that can occur in any area of the mouth (oral cavity) or throat (back) (oropharynx). It generally begins as a tiny inexplicable growth or sore in the mouthparts that later extends to the oropharynx. OC is a severe problem that can affect people all over the world. In the last several years, it has climbed in global incidence and prevalence to be the sixth most frequent tumor around the globe and the third most prevalent in South Central Asia. It accounts for approximately a quarter of all cancers around the globe. It is a severe health concern for countries in economic transition and has the highest fatality and morbidity rates among all human malignancies [1]. In contrast to other human malignancies, OC can be usually detected due to the presence of a precursor lesion or the transparency of the oral mucosa, which can be identified with certain previous training and/or alertness.

Oral squamous cell carcinoma (OSCC) is caused by highly cancerous lesions or normal epithelial linings and accounts for 84–97% of oral cancer. Inflammatory oral submucosa, fibrosis, erythroplakia, leukoplakia, candidal leukoplakia, proliferative verrucous leukoplakia (PVL), dyskeratosis congenital, and lichen planus are the symptoms of OC at the preclinical stage and are considered to be Potential Malignant Disorders [2,3]. WHO defined OPMD (i.e., oral potentially malignant condition) as precancerous lesions which

lead to OC. OPMDs are a group of different oral mucosal lesions that are more likely to become cancerous. The lip, tongue, gums, palate, and other parts of the oral mucosa are the most pervasive sites of OC, as classified by the International Classification of Diseases (ICD) [4]. A lot of people who have oral cancer have a lot of different things going on in their mouths, including smoking, drinking too much alcohol, not brushing their teeth, and having long-term viral infections, such as the human papillomavirus (HPV) [5–9]. Tobacco use (in every form) is an important cause of cancer, particularly in underdeveloped nations. People who chew paan, which is made of piper betel leaves mixed with areca nut, lime, catechu, cinnamon, and other ingredients, are more likely to contract OC than people who smoke cigarettes. This is especially true in India, which has the highest cancer rate in the world [10]. Periodontal diseases are also a high-risk factor for OC, and they are more common in the Indian population, where it is mostly caused by the practice of chewing paan [11]. Chewing paan induces extended exposure of the oral mucosa, as well as abrasion of the epithelial linings. Smokeless tobacco consumption, both orally and nasally, has been linked to potentially malignant oral diseases and malignancies of the oral cavity. Tumorigenesis and inflammation caused by bacterial and viral infections, as well as inflammatory bowel illnesses, also play a major role in the development of cancer [11]. The exposure to harsh environmental circumstances, lack of information, and behavioral risk factors are the key indicators of this OC to be a worldwide incidence. The risk factors for OC vary by geographical area and the lifestyle habits of the people who live there. In Western nations, the primary risk factors are smoking and alcoholism. This is in contrast to South Asian countries, where betel nut eating and smoking are more prevalent [12]. This review paper provides a crisp view on conventional and recent advanced diagnostic methodologies used in the detection of OC. This paper also highlights recent molecular biomarkers used in diagnosis and detection.

2. The Pathogenesis

OSCC is a complex disease with a pathogenesis that involves interactions between a variety of carcinogenetic and genetic contributing factors to cancer's growth and progression. As previously stated, persistent consumption of alcohol and smoking of cigarettes are the primary contributors to the development OSCC. Simultaneously, chronic inflammation, viral infections (particularly HPV type 16 and 18), and hereditary factors all contribute to the disease's development. It is critical to understand the basic molecular processes that enable oral carcinogenesis [13], mechanisms that can lead to innovative treatment choices, in order to improve survival through improved preventive and therapy options.

OSCC helps people get a biopsy if they show signs of the disease; histological tests are then performed based on where the disease is located in their bodies [14]. OSCC is linked to changes in the oral mucosa that are caused by a variety of genetic alterations. These changes can lead to papillary hyperplasia, different stages of dysplasia (slight, minor, or major), and the growth of cancer (in situ or well-differentiated intrusive squamous cell). Most oral cancers exhibit stimulation of the extracellular environment and activity of melanoma fibroblasts. This highlights the link between tumor cell growth and basal lamina breakdown [15]. Cyclin D1 and Ki-67 have been found to be strong in OSCC that was well-differentiated [16].

3. Process Flow in the Molecular Diagnosis of OC

A molecular analysis can be performed in situ or via the separation of test sample from clinical specimens. A molecular analysis procedure is used to look at clinical specimens, such as biofluids and cell culture products. The tissue sample is preserved in formalin and afterward embedded in paraffin before being examined histopathologically in the conventional manner. In order to prepare for light microscopy, the implanted tissue is finely sliced and put on a glass slide. The tissue can then be stained with hematoxylin and eosin [17]. This method can be used to examine particular molecules in their natural topographical environment present in these tissues, often known as in situ. Antibodies

(immunohistochemistry) and nucleotide probes (in situ hybridization) can be used to analyze proteins and nucleic acids, respectively.

Biofluids (e.g., saliva or blood), tissue, and extracted specific or target molecules can be examined as additional molecular components in the preparation for cell-level-based research. As a result, analyzing by using the extracted target molecules was found to be a more sensitive and particular approach that can be employed. Moreover, the sample type should be taken into main consideration when choosing the target and techniques used for the analysis. The approaches employed may have either a low or a high throughput. Low-throughput approaches can only evaluate a small number of hits or samples at once, while high-throughput techniques can examine a large number of targets simultaneously. Multiplexing can also be used, which is when a single sample is analyzed for a lot of different things at the same time or at the same time, using the same method to find out about a lot of different things. Molecular "labels", such as fluorophores that emit light in different colors, are often used in this method. Each color is unique to a single target.

4. Need for Early Diagnosis

Overall, 15% to 50% of people who have oral cancer (OC) will live for at least five years. This is because most oral tumors are found at an advanced stage (stage III or stage IV) [3]. In contrast, if OC is detected and treated early, such as at stage I or stage II, the survival rate can surpass 80% [10,18]. In general, OC can be diagnosed by several clinical procedures, such as physical and histological examinations, biopsy, spectroscopic, radiographic techniques, and so on (Table 1). The early discovery of cancer is essential for averting additional psychological, physical, and financial losses for the patient. Numerous unique procedures have been developed as a result of advances in science and technology that offer benefits over currently used traditional diagnostic methodologies.

Table 1. Traditional methods for detecting oral cancer.

Method	Sample Type	Highlight
Physical examination (visual)	Oral cavity (in situ)	Low cost and decreased death and morbidity
Vital staining (visual)	Oral tissue	Low cost and non-invasive
Histopathology	Oral tissue	Large sample can be analyzed
	Biopsy	
Brush biopsy	Oral cells	Non-invasive, painless, and low cost
Liquid biopsy	Biofluids	Fast, less invasive, and complete sample profile
Incisional biopsy	Oral tissue	Less sample; specific and accurate
Exfoliative biopsy	Epithelial cells	Low cost and minimum skills
	Imaging Techniques	
CT scan	Tissue (in situ)	Rapid, good visualization
MRI scan	Tissue (in situ)	Non-invasive and high-resolution image
Optical coherence topography	Oral tissue	Non-invasive and high-resolution image
	Molecular method	
PCR	Biofluids, cells	High sensitivity and reproducibility
Mass spectroscopy	Tissue (proteins/lipids)	Accurate and high specificity

The molecular biology findings are useful in solving the many new issues that arise in the detection and diagnosis of OC. These methods can detect any changes at the molecular level considerably earlier than a microscope can detect them, and well before clinical changes occur. Oral lesions can also be classified based on their molecular characteristics. As a result, it is now possible to predict the toxic potential of ulcerations, meaning that the number of oral cancers will go down and that early detection and treatment will be better [19–23]. There has been a lot of progress in our understanding of the human genome, as well as different ways to study genetics and molecular biology. These tools can be used to quickly diagnose and treat oral lesions through molecular examination. In today's world, molecular biology helps us comprehend pathological progressions and development at a level beyond the human sight by revealing the characteristics and functional properties of macromolecules. The application of molecular diagnostic tools to determine disease risk,

their presence, and therapy efficacy is referred to as molecular diagnostics. Microarrays, deep sequencing methods, and proteomics methods are often used to study the molecular level of OC and for its early diagnosis. In the past, high-throughput methods were used to find biomarkers in saliva and plasma or predictive markers in tissue specimens that were used to find biomarkers [24].

5. Current Diagnostic Methods for Detection of OC

5.1. Visual Oral Examination

Doctors need to know as soon as possible if an OPMD has a chance of becoming cancerous so that they can keep an eye on it, treat it, and improve survival rates. Currently, the usual screening approach for detecting oral mucosal lesions is a visual oral examination (VOE). Because white or red lesions and long-term ulcers can be hard to tell apart at first glance, VOE is very dependent on the physician's knowledge [25,26]. It is very important to distinguish lesions with a greater risk of malignant transformation because this will have a big impact on the treatment success. Though histological evaluation acts as the gold standard for clinical diagnosis and therapy, it may be insufficient to distinguish lesions that require active treatment, particularly if they are subtle or in a non-dysplasia stage.

A quick diagnosis is very important for preventing oral premalignant disorders from becoming cancerous and increasing the chances that the patients will live long lives. For the right diagnosis, many different procedures need to be performed, such as swabbing the exterior of the abscess and looking at the biological data of oral precancerous lesions. It is hard to tell, but specialists need to be capable of distinguishing between the features of ulcerations just by looking at them, without changing the cells in the area [27–38]. While incisional biopsy with histopathology is the gold standard for identifying dental pathology, it is hurtful for patients and results in a late diagnosis, due to the completion of histology. The autofluorescence approach is a new noninvasive tool for analyzing a soft-tissue injury. It can be utilized to locate oral precursor malignant lesions, as well as the proper place for biopsies within the changed mucosa. The procedure's biggest drawback is the risk of false-positive outcomes [27,28,39]. Identifying the potential biomarkers will improve the ability to exactly analyze and forecast the likelihood and danger of OPMDs evolving into an OC lesion, which requires active treatment if found.

5.2. Physical Examination

The physical examination is the first and most important stage in diagnosing oral cancer. Typically, it is processed in two stages: a comprehensive visual evaluation, followed by palpation. External body components such as lymph nodes, salivary glands, and lips were inspected initially, followed by an inspection of the buccal cavity's interior. In the superficial anatomy, abnormalities, irregularities, edema, and fluctuation are observed. Soft-tissue thickening, lumps, discomfort, difficulty moving the jaw, chewing and swallowing, earache, and other symptoms are typical. The parotid gland (biggest salivary gland) is palpated both within and outside the mouth, as well as the submandibular and sublingual glands [40]. Physical observations are compared to the patient's clinical picture by the examiner. Additionally, morphological changes, as well as texture and color aberrations, are described [41].

5.3. Histopathological Examination

OC can range from benign tumors to extremely aggressive tumors with a high proclivity for invasion. Histological examinations demonstrate how oral carcinogenesis progresses from benign dysplasia to highly invasive malignancies. Histological investigation is essential to identify cell proliferation and development difficulties, cellular and cytoplasmic atypia, and abnormalities in the surface epithelium or deep tissue cytoarchitecture [42]. The crucial stage in a histological examination is identifying the correct region and proper sampling of the oral lesion, as the histopathological alterations can also develop in places where there is no indication of oral lesions upon physical examination. Thus, for the

diagnosis of benign or malignant tumors, a method that recognizes both histopathological and molecular changes is desirable, as genomic changes can occur in normal tissues prior to the emergence of microscopic and clinical morphological changes [43].

5.4. Vital Staining Techniques

The vital staining technique includes labeling cells or tissues in their living state. This method of optical tissue staining for cancer detection provides an alternative to physical examination [44]. Toluidine blue staining is used to detect oral mucosal abnormalities. Toluidine blue is an acidophilic metachromatic dye that has a good attachment for acidic tissue components, coloring the nuclear material of DNA and RNA-rich tissues. This approach, when used in conjunction with Lugol's iodine, aids in the differentiating between inflammatory lesions. Such a combination accurately predicts the stage of tumors, ranging from benign to malignant lesions, making it an advantageous visual staining technique for evaluating OC before treatment [45].

5.5. Biopsy

Biopsy is a process where part of tissue or sample of cells is surgically extracted from the suspicious area from the body and submitted to a pathology facility for microscopic analysis. This is the sole approach to determine if an oral cavity or oropharyngeal carcinoma is present [46]. For a reliable histological diagnosis after a biopsy, proper treatment of the tissue is essential. Improper sample handling might lead to a faulty biopsy, which necessitates repeating the process. Exfoliative cytology and incisional biopsy are two types of biopsies that are performed depending on the individual need. Brush biopsy involves scraping the surface mucosa from the oral lesion to extract the cells from transepithelial region. This technique is a straightforward, safe, painless, cost-effective, and highly sensitive method. Small white and red lesions in the mouth can be checked to make sure that they are not dysplastic. Compared to other biopsy procedures, brush biopsy holds 90% of sensitivity and specificity [47].

Oral exfoliative cytology (OEC) is a simple, non-aggressive method which is comfortable for the patient and can be used to detect OC early. OEC concentrates on the individual cell's morphological and staining features, thus necessitating the involvement of expert cytopathologists [48]. Despite its benefits, OEC is neither specific nor sensitive, and as a result, it is mostly utilized for screening from a large population, regular monitoring of precancerous lesions, and identification of appropriate biopsy sites in large lesions [49]. Once the cancer is detected by OEC, incisional biopsy is performed, in which a small portion tissue sample is carefully chosen for diagnosis. Though it does not cover the whole lesion, the incisional biopsy is reasonably accurate [50]. In circumstances when it is impossible to remove the entire lesion, e.g., a broad white patch or lichen planus, an incisional biopsy is employed. In addition, this approach is favored when the clinical diagnosis is unknown [51].

5.6. Imaging Techniques

OC is diagnosed by using a variety of technological imaging methods, such as computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET) [52].

5.6.1. Magnetic Resonance Imaging

The structures in the mouth cavity, as well as surrounding regions, may be seen in detail by using MRI. Discrimination of soft tissues by MRI assists in determining the degree of the tumor's site dissemination, invasion depth, and lymphadenopathy [53]. This is because it has a better contrast resolution and can look at things from many different angles. This makes it easy to see how far OC has spread into the soft tissues next to it. Thus, an MRI is needed to check for advanced oral and oropharyngeal cancers before they can be treated.

5.6.2. Computed Tomography

The CT scan uses X-ray radiation and a computer to make pictures of the body. It looks for the cancerous lesion and sees if it has spread to many other body parts. Because CT scans are readily available and very inexpensive, they are frequently used as a routine imaging tool for the identification of cancers. However, it has been noted that CT scans cannot detect lesions in their early stages.

5.6.3. Positron Emission Tomography

PET scans have been used to determine whether tumor cells have spread to lymph nodes or other organs. After administering a radioactive dye intravenous or oral, gamma rays emitted by positron decays are analyzed. It is a precise approach for determining the lymph node staging. Even if more lymph node metastases are identified away from the afflicted region, it does not necessitate any changes in therapy [54]. A PET scan utilizing fluorodeoxyglucose can be used to evaluate the condition of lymph nodes prior to surgery (FDG). As a result, PET scanning is crucial for detecting oral cancer in its early stages [55].

6. Biomarker Detection

Biomarkers are cell components that are highly expressed in body fluids or tumor cells at the beginning of illness. In the context of OC, the biological cells exhibited in blood or saliva act as cancer-detection indicators. Specifically, the progression of cancer consists of three stages: beginning, promotion, and advancement. All three steps are associated with substantial modifications in the cell's processes, including metabolome, transcriptome, and proteome processes [56]. These changes occur as a result of mutations in certain genes or proteins, which result in the modification or stopping of important metabolic and structural pathways. Some of the most promising biomarkers related to the progression of OC are Ki-67 antigen, TSG p16, TSG p53, and DNA ploidy. Early identification of OSCC is aided by salivary biomarkers such as circulating tumor DNA, miRNAs, and extracellular vesicles [57]. In OSCC, p53 expression is regarded as a key indicator of carcinogenesis. The advent of the p53 mutant gene is confirmed by using immunohistochemical staining [58]. Apart from that, many advanced techniques and high-throughput molecular methodologies are used to detect the biomarkers (Figure 1 and Table 2).

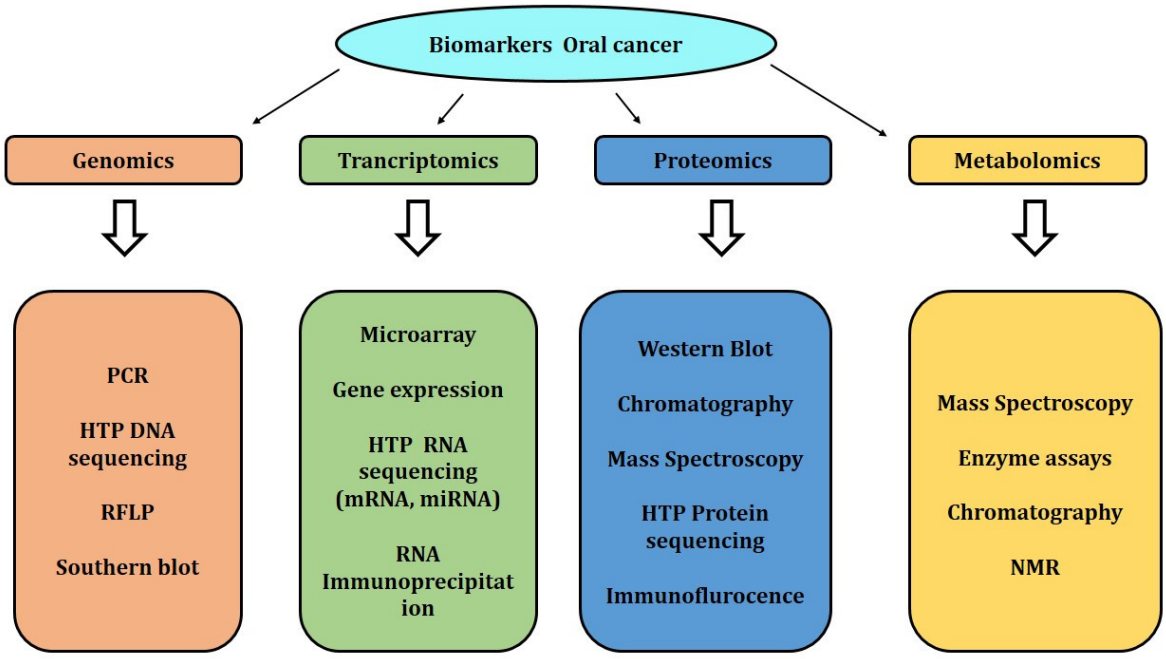

Figure 1. Various molecular methods involved in the detection of biomarkers.

Table 2. Biomarkers associated with the data source and technologies used to identify oral cancer and OSCC.

Sl. No.	Biomarker	Sample Type	Technique Used
	Protein biomarkers		
1	Collagen	Oral tissue	Diffuse reflectance spectroscopy
2	Elastin		
3	Keratin		
4	FAD		
5	NADH		
6	Cytokeratin-19	Oral tissue	Incisional biopsy
7	Secretory leukocyte protease inhibitor	Oral cells	Brush biopsy
8	Epidermal growth factor receptor (EGFR)	Oral epithelial cell linings	Exfoliative biopsy
9	Serum proteins	Serum	HPLC
10	Proteases	Saliva	ELISA
11	ANXA2		
12	CA2		
13	CD44		
14	CRNN		
15	CST3		
16	CSTA		
17	DSG3		
18	FLNA		
19	FSCN1		
20	GANAB		
21	GSTP1		
22	HMGCS1		
23	HSPA5		
24	IGFBP3		
25	ISG15		
26	KNG1		
27	LDHA		
28	LGALS3BP		
29	MMP1		
30	MMP3		
31	MMP9		
32	PRDX2		
33	S100A9		
34	SPARC		
35	STAT1		
36	TIMP1		
37	TYMP		
38	YWHAB		
39	Interleukin-6 (IL-6)		

Table 2. *Cont.*

Sl. No.	Biomarker	Sample Type	Technique Used
	Protein biomarkers		
40	Interleukin-8 (IL-8)		
41	Interleukin 1a (IL-1a)		
42	Interleukin 1b (IL-1b)		
43	TNF-a		
44	Tissue polypeptide antigen (TPA)		
45	Cyfra 21-1		
46	Cancer antigen 125 (CA 125)		
47	Telomerase		
48	Mac-2 binding protein (M2BP)	Saliva	ELISA, shotgun proteomics
49	CD44	Saliva	Immunoblot analysis
50	CD59		
51	Profilin		
52	MRP14		
53	Glutathione	Saliva	HPLC
54	Squamous cell carcinoma antigen 2	Saliva	ELISA, shotgun proteomics
55	Involucrin		
56	Calcyclin		
57	Cathepsin-G		
58	Azurocidin		
59	Transaldolase		
60	Carbonic anhydrase I		
61	Calgizzarin		
62	Myeloblastin		
63	Vitamin D–binding protein		
64	Immunoglobulin heavy-chain constant region gamma (IgG)	Saliva	LC/MS
65	S100 calcium-binding protein		
66	Cofilin-1		
67	Transferrin		
68	Fibrin		
69	Alpha-1-antitrypsin (AAT)	Saliva	2DE
70	Secretory leukocyte peptidase inhibitor (SLPI)	Saliva	MS-based approaches
71	Cystatin A		
72	Keratin 36		
73	Thioredoxin		
74	Haptoglobin (HAP)		
75	Salivary zinc finger		
76	Protein 510 peptide		
77	A-amylase		
78	Albumin		

Table 2. *Cont.*

Sl. No.	Biomarker	Sample Type	Technique Used
	Protein biomarkers		
79	OPN (osteopontin)	Human biopsy	Western blot
80	EZM2	Tissue-bank sample	
81	DEPDC1B	Human biopsy	Immunoprecipitation, Northern blot, Western blot
82	P63	Human biopsy	Microarray gene expression
83	DeltaNp63	Human biopsy	Cell membrane immunoreactivity, microscope
84	EIC		
85	Podoplanin		
86	EGFR	Human biopsy	FISH
	DNA-based biomarkers		
87	DNA (promoter hypermethylation)	Saliva	PCR, qPCR, microarrays
88	Histone family 3 (HA3)		
89	S100 calcium-binding protein P (S100P)		
90	Spermidine/spermine N1- acetyltransferase EST (SAT)		
91	Ornithine decarboxylase antizyme 1 (OAZ)		
92	P53 gene codon 63		
93	CDH1	Oral tissue	DNA Microarray
94	MMP3		
95	SPARC		
96	POSTN		
97	TNC		
98	TGM3		
99	HMGA1		
100	PABPC1		
101	NT5E		
102	FOS		
103	FASN		
104	P53		
105	Cytochrome co-oxidase I	Saliva	PCR, qPCR, microarrays
106	Cytochrome co-oxidase II		
107	DAPK		
108	DCC		
109	TIMP-31		
110	TIMP-3		
111	MGMT		
112	CCNA1		
113	MINT-31		
114	DNMT3B	Human biopsy	Microarray gene expression

Table 2. *Cont.*

Sl. No.	Biomarker	Sample Type	Technique Used
	RNA-based biomarkers		
115	miR-21	Tissue-bank sample	mirVana™, microarray gene expression, qPCR
116	Has-miR-101	Human biopsy	Microarray gene expression
117	miR-155-5p	Tissue	Gene expression, micro-RNA expression
118	miR-216a		
119	miR-21-3p		
120	miR-96-5p		
121	miR-141-3p		
122	miR-130b-3p		
123	miR-21-5p		
124	miR-483-5p	Serum	Gene expression, micro-RNA expression
125	miR-31-5p	Saliva	Gene expression, micro-RNA expression
126	miR-31	Human tissue	qRT-PCR
127	miR-21		
128	miR-92b		
129	miR-34a	Human tissue	Human biopsy, TaqMan miRNA assays
130	miR-139-5p	Saliva	Agilent miRNA microarray, qPCR
131	miR-203	Saliva	qPCR
132	IL-1b, IL-8	Saliva	ELISA
133	Dual-specificity phosphatase 1 (DUSP1)	Saliva	qPCR and microarrays
134	H3 histone family 3A(H3F3A)		
135	Long noncoding HOTAIR		
136	miR-125a, miR-200a, miR-31		
	Metabolomics-based biomarkers		
137	Cadaverine	Saliva	Capillary electrophoresis–time-of-flight mass spectrometry (CE–TOF-MS) and HPLC with quadrupole/TOF-MS.
138	Alanine		
139	Serine		
140	Glutamine		
141	Piperidine		
142	Taurine piperidine		
143	Choline		
144	Pyrroline hydroxycarboxylic acid		
145	Beta-alanine		
146	Alpha-aminobutyric acid betaine		
147	Tyrosine		
148	Leucine þ isoleucine		
149	Histidine		
150	Tryptophan		

Table 2. *Cont.*

Sl. No.	Biomarker	Sample Type	Technique Used
	Metabolomics-based biomarkers		
151	Glutamic acid		
152	Threonine		
153	Carnitine		
154	Pipercolic acid		
155	Lactic acid		
156	Phenylalanine		
157	Valine		
158	Hypoxanthine		
159	Guanine		
160	Guanosine		
161	Trimethylamine N-oxide	Saliva	Capillary electrophoresis–time-of-flight mass spectrometry
162	Spermidine		
163	Pipecolate		
164	Methionine		

6.1. Next-Generation-Sequencing-Based Biomarkers

Squamous cell carcinoma progresses because of changes at the genomic level. Next-generation sequencing (NGS) technology is a good way to find the mutations and copy number changes that cause these changes [15]. Stransky et al. performed whole-exome investigations on head and neck SCC from 74 patients and observed matched DNA pairs between tumors and peripheral whole blood [59]. This team had identified 130 coding mutations per tumor. Given that 14 percent of all cancers were HPV-positive, results from sequencing research show how these would have a genetic variation that was half that of HPV-negative tumors. This supports the idea that there are molecular differences among the two types of SCC. With the help of NGS which possess high sensitivity, the molecular characterization among the two SCCs may go even further, subdividing HPV-negative tumors not only by their precise location, but also by the frequency of specific nucleotide alterations. Aside from these findings, the presence of mutant NOTCH1 in 11% of all tumors examined was noteworthy, since it would be the first study linking genetic alterations in this gene to the genesis and progression of SCC. This research helps us better understand how this kind of cancer grows. It shows that the changes that happen at different stages of squamous differentiation have a big impact on the many genetic processes that drive SCC development [15,60]. Another interesting finding is that a mutation at TP53 had a key influence in the development of this disease for the younger patients who are preferably non-smokers [61]. Pickering and his colleagues did a very detailed genomic study of OSCC in order to find out what causes the disease to become malignant and what biomarkers can be used to classify, predict, and treat it [62]. Pickering and his team used fresh-frozen malignant cells and nonmalignant cells from 38 patients with OSCC to study genome-wide copy number changes. They found strong correlations between gene expression and comparative copy numbers for 1721 genes.

The NOTCH1 gene had many missense and truncating mutations in around one-tenth of the tumor samples, and this was confirmed by using a panel of HNSCC cell lines. With the previously described study group, they found comparable results. The absence of protein expression, along with other tests, such as in vivo experiments using mouse models, supports the hypothesis that the Notch signaling system had a major role in tumor-suppressive mechanisms in SCC [16]. This new but widely used method can help us understand not only the main molecular devices that play a role in SCC emer-

gence and development, but also other possible participants that play a role in the tumor transformation process.

Pushalkar and his study team sought to investigate how bacteria might play a role in tumorigenesis, especially with SCC [63]. They collected saliva samples from three people who had OSCC and two people who were healthy (control). Then the bacterial gDNA was extracted, processed, and pyro-sequenced. After analyzing the data, the researchers discovered several bacterial species related to eight distinct taxonomic phyla, with the Firmicutes phylum being the best defined in samples compared to controls. At the species level, NGS analyses revealed how microorganisms are expressed variably in the dental environments of SCC individuals versus healthy participants, suggesting that these microbes might contribute to the course of the disease. The same research group then looked at matched pairs of cancerous and non-cancerous tissue from SCC patients. This shows how important it is to use unique techniques such as this to understand the many and complex aspects of OSCC transition [64].

6.2. Transcriptomics-Based Biomarkers

MicroRNA is a group of functional non-coding RNA molecules that have about 22 nucleotides each. They contribute to gene control after transcription, and they have about 22 nucleotides in each. Many diseases start and get worse when there are problems with the way microRNAs are made. This is because microRNAs control so many important biological processes, such as advancement, divergence, and cell cycles [14,15]. Tus miRNAs might be used as biomarkers for a variety of illnesses, including neurodevelopmental disorders (Ohnishi et al. 2014), cancer, and cardiovascular disease [24,65]. A microarray analysis of 20 human whole-blood specimens on 1200 miRNAs revealed OSCC-specific signature biomarkers. Then qRT-PCR was used to validate the most important miRNAs, which showed a two-fold upregulation for miR-494 and miR-3651 and a two-fold downregulation for miR-186. Another study with microarray data using Affymetrix U133A platform revealed that 51 genes were upregulated and found that most of genes were related to OSCC [66]. Their findings also showed a new set of OSCC-related genes (RHEB, SOD2, SKP2, IFI16, IFI44, STAT1, and GREM1) and three genes (MMP1, SOCS3, and ACOX1) that differentiate the normal tissue from the cancer [67]. Microarray and qPCR were used to examine a group of 23 CXC-chemokine ligands and receptors that were then connected to therapeutic response by using a logistic regression technique. CXCL10 expression was found to be substantially associated with radiotherapy response, with the group with CXCL10 overexpression having a poor outcome [68]. Similar study was also observed where miRNAs showed different levels of expression between normal tissue and OSCC tissue. In this study, miR-375 showed the greatest inhibition, whereas miR-31 recorded overexpression. Moreover, 61 miRNAs were found to establish a 93 percent accurate molecular categorization of OSCC [65]. Lajer and his group found that HPV causes changes to 21 miRNAs, the most important of which are miR-127-3p and miR-363 [69]. The influence of HPV might explain why HPV-infected malignancies have variable clinical outcomes on miRNA profile.

MiR-375 downregulation, miR-127 overexpression, and miR-137 hypermethylation were discovered in another study of OSCC. Another interesting biomarker which is epigenetically activated in tumor tissue was observed with miR-200 and miR-205. Observing changes in miR-375 and miR-200a methylation, and also changes in miR-200c methylation, is a useful non-invasive biomarker, as shown by a study that compared the saliva of OSCC patients and healthy people. It has been suggested that salivary miRNA profiles could be used as a biomarker for initial evaluation of patients in OSCC [70]. The first lncRNA heat map for usual oral cavity vs. a pre-malignant abscess was made and publicly disclosed [71]. Many recent research studies have shown that new lncRNA can be used as non-invasive saliva biological markers for prediction and diagnosis. HOTAIR identified in saliva was shown to have considerable predictive relevance in a recent research study, with the expression level associated with lymph node metastases.

6.3. Proteomic-Based Biomarkers

Proteomics is a promising technique for identifying new biomarkers with prognostic and diagnostic potential [72,73]. The identification of new biomarkers in OSCC may be aided by analyzing cellular entire protein complements or biofluids. A key feature of various research studies [74] is a saliva proteome profiling study based on patient categorization. Tests have been performed to see how much protein is found in samples of OSCC and healthy tissue, as well as in other in vitro systems for this disease [75]. A lot of proteins that affect cellular functions and structure, cellular adhesion, or cell migration, and proteins that make cancer more likely were found to be changed in studies [76,77]. When researchers looked at the plasma proteome of mice with OSCC, they found that haptoglobin and the precursor of apolipoprotein A1 have both been upregulated. The expression of haptoglobin plasma in humans showed that there was a strong connection between increasing levels of haptoglobin and the clinical stages of OSCC. This suggests that haptoglobin could be used as a plasma diagnostic marker for identification of OSCC patients [78].

A SELDI-TOF Protein Chip technology was used to screen saliva proteins from pre- and post-treatment OSCC tests to find 26 candidates with different patterns in their saliva that could be linked to the disease. The investigation found a shortened cystatin SA-I of 14 kDa in pre-treatment saliva samples, with a deletion of three amino acids at the N-terminal end [79]. Protein Chip analysis could be a good way to screen for OSCC at an early stage, and truncated cystatin SA-I could be a good tumor prognostic marker for OSCC. Transglutaminase 3 (TGM3) downregulation was linked to OSCC histological differentiation loss in another study [80]. There were 52 proteins that had statistical significance, so a comparative proteomics study found a group of eight proteins that were more or less common in different people. Annexin A8, Peroxiredoxin-2, and Tyrosine Kinase have been observed in both diabetes and OSCC, and they were thought to be possible biomarkers for OSCC diagnosis because they were found in both [81]. A mass spectrometer study of saliva from people with cancer and healthy people found 213 new proteins. The salivary biomarkers Profilin, Cofilin, S100A9, and MMP9 have all been found to be linked to head and neck cancer. Vimentin has been linked to the epithelial–mesenchymal transition and metastasis of head and neck cancer [82].

Exosomes are endocytic membrane bound vesicles with nucleic acids (DNA and RNA) inside their lumen that originate from the cell's cytoplasm. They are secreted in large amounts and are found abundantly in bodily fluids. Exosome surface proteins can function as antigens for certain antibodies, which can then be utilized to isolate desired exosomes via affinity-based approaches. Exosomes generated from cancer are said to reflect the tumor microenvironment and can thus be employed as a biomarker for tumor identification [83,84]. The lipid bilayer of exosomes is made up of cholesterol, phosphatidylserine, and ceramide, as well as trans-membrane receptors, proteolytic enzymes, tetraspanins, and adhesion molecules. Some proteins that are linked to cancer are also very common on the exterior of exosomes, thus making them useful as markers for differentiating between different types of cancer. Proteins such as HER2, LMP1, and MUC18 can be used to make biosensors that can detect total exosomes.

6.4. Advantage of Use of Biomarker over the Traditional Technique

The diagnostic and predictive biological markers of cancer are the future of clinical cancer management. These biomarkers are critical because they will be utilized to make therapeutic decisions that will save lives. Biomarkers will help doctors make decisions about cancer treatment in the future. It is necessary to discover biomarkers that accurately predict the prognosis of a particular disease and allow clinicians and patients to make correct treatment decisions. In the absence of viable treatments, the consensus about cancer diagnostics has been that early detection, followed by surgical surgery before the tumor had progressed, was the best approach to cancer. Few novel diagnostic biomarkers have evolved, despite the success of traditional approaches, such as biopsy, vital staining, and

imaging techniques, in the early identification of cancer. A noninvasive saliva or serum biomarkers that properly predicts cancer prognosis is still needed. Patients' cancer is still being detected too late and treated without knowing if their tumor has progressed beyond its initial stage. Biomarkers will not only assist screen, detect, diagnose, aid in prognostic assessment, monitor therapy, and forecast recurrence over the next few decades, but they will also play an important role in medical decision making. Markers that indicate responsiveness to a specific therapy are, in fact, required.

7. Future Implications

Oral cancer candidate biomarkers should be identified and classified in the fields of screening, diagnostic testing, recurrence prediction, progression, therapeutics, and metastasis in future studies. These prospective markers will aid in the prediction of outcome measures and the development of dental public health strategies. The creation of biomarkers aimed at assessing the success of oral cancer medication therapy would be extremely helpful in determining therapeutic efficacy. Researchers should apply innovative research methodologies that adhere to the requirements of analytical authenticity, clinical validity, and clinical utility, in addition to biomarker reporting and evaluation systems.

8. Conclusions

In light of the vast amount of R&D in the field of OC diagnostics, the diagnostic procedures presented in this review paper are largely those that are already in clinical use or commercially accessible and show significant potential in clinical use. There are various other approaches for diagnosing OC that were not included in this paper. Each of these diagnostic approaches reveals its own technological originality. In the near future, it is anticipated that new innovations with enhanced detection sensitivity will emerge. The availability of these very sensitive methods (next-generation sequencing, mass spectrometry, and microarray technologies) will allow for reliable diagnosis of even lower amounts of sample analytes. To aid in the efficient detection of OPMDs and OC, further efforts to strengthen and enhance clinical performance during OC diagnosis are strongly urged.

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