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Review Article

A Systematic Review of Salivary and Serum Metabolomics in Oral Squamous Cell Carcinoma

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Abstract

Background: Metabolomics is a branch of 'omics' sciences that utilises a couple of analytical tools for the identification of metabolites. It is increasingly being used to study metabolomics in various cancers. However, the field of metabolomics in OSCC has not been extensively reviewed or assessed.

Objective: To summarise the presently available metabolomic biomarkers and to discuss the various metabolites belonging to altered metabolic pathways such as carbohydrate, amino acids, lipids, nucleotides, and other metabolisms linked to OSCC in saliva and serum. Finally, to highlight the clinical significance of metabolomics in OSCC, we performed a systematic review that could facilitate developing an understanding of the disease mechanisms as well as new therapeutic measures.

Methods: A search was conducted in the Web of Science, PubMed, EBSCO Host, Science Direct, Springer, and Google Scholar academic journal databases between November 2021 and January 2022. The research strategy was constructed considering the "PICO" method.

Results: 43 articles were included in this review on metabolomics. It involved carbohydrates, proteins, nucleotides, lipids, and others. Results indicated upregulated metabolites such as lactate, pyruvate, choline, methionine, methylthioadenosine, S-adenosylmethionine, and sphinganine-1-phosphate and downregulated metabolites like glucose, valine, ornithine, guanine, and eicosane.

Conclusion: The identification of the number of serums, especially salivary metabolites, even when present in small quantities, and their close proximity to oral lesions have led to the understanding that salivary metabolomics is a promising tool in oral cancer diagnostics because it provides insights into new pathogenic pathways and therapeutics.

Keywords: Metabolomics; Oral Squamous Cell Carcinoma; Saliva; Serum; Plasma

Abbrevations

OSCC: Oral Squamous Cell Carcinoma; HMP: Human Metabolome Project; HMDB: Human Metabolome Database; FTIR: Fourier Transform Infrared Spectroscopy; NMR: Nuclear Magnetic Resonance; GC-MS: Gas Chromatography-Mass Spectroscopy; LC-MS: Liquid Chromatography-Mass Spectrometry; HPLC-MS: High Performance Liquid Chromatography

Introduction

Oral squamous cell carcinoma is the most common form, accounting for 90% of oral cancers [1,2]. In this complex and long journey of oral cancer, numerous tumour biomarkers have been studied to date in OSCC tissues and other body fluids, which are certainly categorised according to various "omics" techniques, namely genomics (genome), transcriptomics (transcriptome), proteomics (proteome), and metabolomics (metabolome) [3].

Metabolomics is a newly emerging field of 'omics' research concerned with the high-throughput, unbiased analytical method for the identification, quantification, and characterization of the small-molecule metabolites in the metabolome [4]. It detects metabolites of relatively low molecular weight (up to ~1000 Da or less) [5,6]. Metabolic alterations in cancer cells are numerous and include aerobic glycolysis, reduced oxidative phosphorylation, and the in-

creased generation of biosynthetic intermediates needed for cell growth and proliferation. These are the ultimate results of cellular pathways, taking into account alterations in the genome, transcriptome, proteome, and metabolic influences. These metabolic intermediates are not detectable by other proteomics, transcriptomics, or genomics [7].

To identify and quantify all detectable metabolites (>1 μM) in the human body, the Human Metabolome Project (HMP), launched in 2004, identifies and quantifies hundreds of metabolites. Also, this project was tasked with backfilling and validating the information on all previously identified metabolites and providing the information as a freely available electronic database called the Human Metabolome Database (HMDB) [8]. Hence, the discovery and qualification of new metabolic biomarkers are being routinely measured to improve the diagnosis and prognosis of OSCC [9].

Utilising high-resolution analytical methods such as Fourier transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR) spectroscopy, gas chromatography-mass spectroscopy (GC-MS), liquid chromatography-mass spectrometry (LC-MS), high-performance liquid chromatography (HPLC-MS), and many more for the quantitative analysis of hundreds of small molecules (less than ~ 1000 Da) present in biological samples is now available on a relatively routine basis in metabolomics studies [10].

Metabolic alterations of tumours are well-recognised and are considered one of the hallmarks of cancer. Cancer cells adapt their metabolic competences in order to efficiently supply their novel demands of energy to sustain cell proliferation. Metabolites in the human body that may undergo alteration in OSCC are merely termed endogenous metabolites [11]. These alterations are evident in the metabolite profiles of not only the tissues, but also the patient's body fluids such as saliva and serum/plasma. These metabolites present in biofluids or tissue provide detailed information on biological systems and their current status [12]. The quantification and characterization of such metabolites may aid in early diagnosis, patient selection strategies for clinical trials, and/or as biomarkers of treatment response in OSCC [13].

In this respect, the objective of the present systematic review was to identify the various salivary and serum metabolomic biomarkers and highlight the superlative vehicle serum or saliva for metabolite biomarkers in OSCC from the existing literature.

Material and Methods

Information sources and search strategies

Electronic searches in Web of Science, PubMed, EBSCO Host, Science Direct, Springer, and Google Scholar were undertaken from November 2021 to January 2022. The following search strategy was used for the databases: Predefined search strings using a few core keywords were included as follows: (Metabolites OR Metabolomics) AND (Oral cancer OR Oral squamous cell carcinoma) AND ("saliva" OR "serum" OR "plasma") The retrieved references were exported to Excel data, and all duplicates were manually removed upon identification.

Eligibility criteria

Inclusion criteria

- Studies include oral cancer and oral squamous cell carcinoma, with metabolite biomarkers in saliva and serum.
- Human metabolomic biomarkers, which are endogenous in origin.
- Downloaded literature for various databases.
- Study published in English in the periodical literature.

Exclusion criteria

- Reviews, case studies, and letters to the editor were excluded.
- Studies that only reported results from in-vitro, in-silico, or animal studies.
- Studies that were conducted as part of theses, or incomplete studies.
- Non-English literature.
- Articles without full text.
- Cancers of metastatic origin, recurrent OSCC, and those on therapy were excluded.

Study selection

Following the electronic search, the two authors independently reviewed the articles on the basis of title, abstract, and full text. Abstracts and full-text articles that met the inclusion criteria were included in this review by agreement between the two authors (M.S. and G.S.).

Data extraction

The two reviewers independently extracted data using a single Excel data extraction form from the study described.

The quality assessment

The modified Newcastle-Ottawa Quality Assessment Scale (NOS) for cross-sectional studies was used to assess the risk of bias for the included studies. The methodological quality score was calculated based on three domains: Selection, Comparability, and Exposure. For the assessment of each domain, a series of multiple-choice questions were answered after the reading of each study. A study could be classified with a maximum of one point for each numbered item within the Selection (four items), Exposure (three items), and Comparability (two items). Therefore, the scores could vary from a minimum of zero to a maximum of nine points [32].

Results

Study selection

An initial search of all the databases yielded a total of 5,551 articles to date. After the removal of duplicates, 5,457 articles were excluded, and 94 articles remained, which were subjected to the inclusion and exclusion criteria. A thorough screening of the papers based on their relevance through scrutiny of the information implied by the title alone excluded 49 articles and reduced the search results to 45. A further quality assessment excluded an additional 2 articles and furnished 43 articles. Figure 1 illustrates a flow chart with the various steps of the systematic review (along with the guidelines proposed by the Preferred Reporting Items for Systematic Reviews, PRISMA).

Risk of bias assessment tool

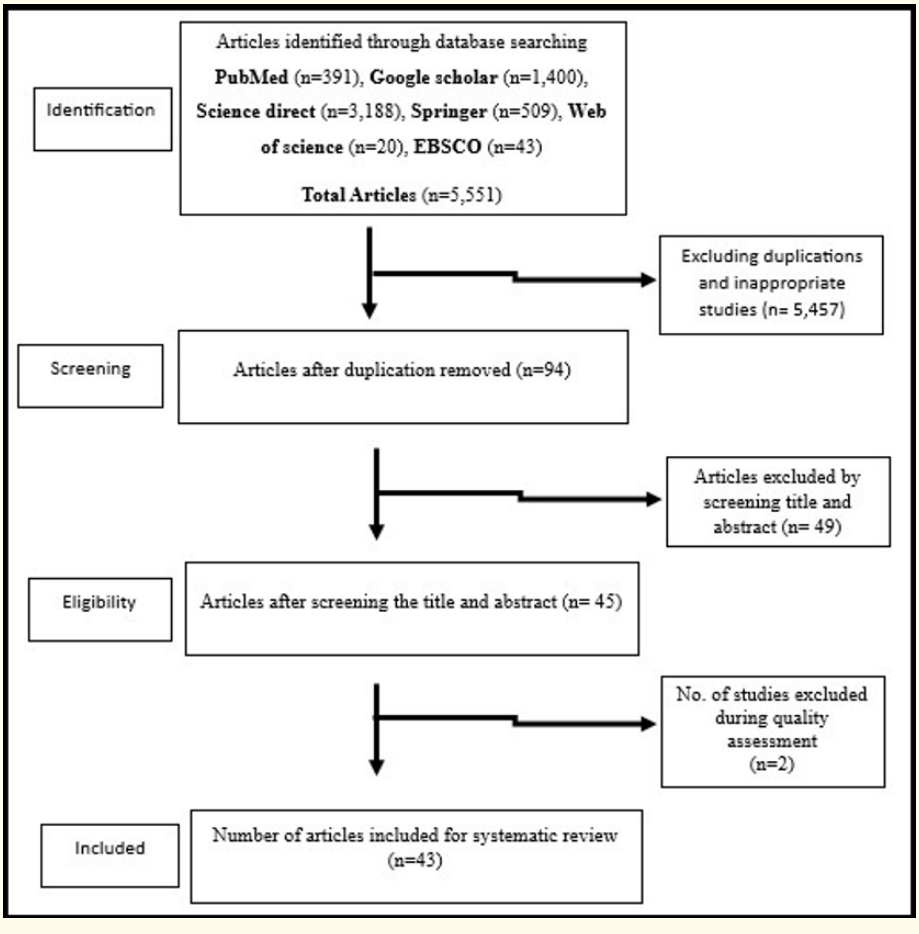

Figure 1: Flow diagram outlining the search strategy and the search results along various steps.

Based on the modified Newcastle-Ottawa Quality Assessment Scale Each article was scored based on its selection (0-4), comparability (0-2), and exposure (0-3) and was further categorised into three groups based on NOS scores: very high risk of bias (0-3), high risk of bias (4-6), and low risk of bias (7-9) [32]. From the total 45 articles after quality assessment, 38 are at low risk and 5 are at high risk (Table 1).

Discussion

Sr. No.	Author and Year	Selection (0-4)	Comparability (0-2)	Exposure (0-3)	Risk of Bias (0-9)
1	Almadori G., *et al*, 2001 [14]	4	2	2	8
2	Yan SK., *et al*, 2008 [15]	4	2	2	8
3	Zhou J., *et al*, 2008 [16]	3	2	2	7
4	Tiziani S., *et al*, 2009 [17]	3	2	2	7
5	Sugimoto M., *et al*, 2010[18]	4	2	2	8
6	Wei J., *et al*, 2011[19]	4	2	2	8
7	Reddy I., *et al*, 2012 [20]	3	2	2	7
8	Wang Q., *et al*, 2013 [21]	3	2	2	7
9	Wang Q., *et al*, 2013 [22]	4	2	1	7
10	Gupta A., *et al*, 2015 [23]	4	2	1	7
11	Wang Q., *et al*, 2015 [24]	3	2	2	7
12	Ishikawa S., *et al*, 2016 [25]	4	2	2	8
13	Rekha P., *et al*, 2016 [26]	3	2	2	7
14	Connolly JM., *et al*, 2016 [27]	3	2	2	7
15	Miranti EH., *et al*, 2016 [28]	2	2	2	6
16	Ohshima M., *et al*, 2017 [29]	3	2	2	7
17	Sridharan G., *et al*, 2017 [30]	3	2	2	7
18	Suzuki M., *et al*, 2017 [31]	4	0	2	6
19	Ishikawa S., *et al*, 2017 [32]	3	2	2	7
20	Adeeba., *et al*, 2018 [33]	3	2	2	7
21	Ishikawa S., *et al*, 2019 [34]	3	2	2	7
22	Hsu CW., *et al*, 2018 [35]	4	0	2	6
23	Heawchaiyaphum C., *et al*, 2018 [36]	4	2	2	8
24	Shahid N., *et al*, 2018 [37]	4	2	2	8
25	Lohavanichbutr P., *et al*, 2018 [38]	4	2	2	8
26	Taware R., *et al*, 2018 [39]	4	2	2	8
27	Enomoto Y., *et al*, 2018 [40]	4	2	2	8
28	Mikkonen JJW., *et al*, 2018 [41]	3	2	2	7
29	Sridharan G., *et al*, 2019 [42]	4	2	2	8
30	Shigiyama H., *et al*, 2019 [43]	3	2	2	7
31	Vsiansky V, 2019 [44]	3	2	1	6
32	Ishikawa., *et al*, 2020 [45]	3	2	2	7
33	Song X., *et al*, 2020 [46]	4	2	1	7
34	Falamas A., *et al*, 2021 [47]	3	2	2	7
35	Falamas A., *et al*, 2020 [48]	4	2	1	7
36	Tsai CK., *et al*, 2020 [49]	3	2	2	7
37	Ludwig N., *et al*, 2020 [50]	4	2	2	8
38	Supawat B., *et al*, 2021 [51]	3	2	2	7
39	Hurskainen MO., *et al*, 2021 [52]	2	2	2	6
40	de Sa alves., *et al*, 2021 [53]	3	2	2	7
41	Falamas A., *et al*, 2021 [54]	4	2	2	8
42	Yang X., *et al*, 2021 [55]	4	2	2	8
43	Ishikawa S., *et al*, 2022 [56]	4	2	2	7

Table 1: Risk of bias for the included studies, using the modified Newcastle-Ottawa Quality Assessment Scale.

The increasing worldwide incidence of OSCC urgently demands the discovery of new biomarkers [24,57]. Therefore, possible biomarkers are much needed to predict prognosis, help individualise therapy approaches, and overcome possible resistance mechanisms [58]. Hence, metabolomics, which enables the assessment of the levels of a broad range of endogenous and exogenous metabolites, has the advantage of being much more dynamic than proteomics or genomics, since metabolomics allows the detection of alterations in metabolites resulting from physiological and/or environmental events in shorter periods of time [59].

The findings of this review will be further discussed according to their upregulation and downregulation of metabolomics biomarkers under various metabolomics pathways, as follows

Carbohydrates

Alterations in the carbohydrate metabolomic pathway have been reported in the literature. The list of altered carbohydrate metabolites is tabulated in (Tables 2) (Table 3).

In 1920, Otto Warburg observed a common characteristic in the metabolism of tumour cells. This characteristic consisted of increased glucose uptake with high lactate release, signalling that the rate of glycolysis in tumour cells is high even in the presence of oxygen and perfectly functioning mitochondria. This process, known as the Warburg effect, was established as a form of energy generation in tumour cells [19,24].

The increased levels of glucose may be linked to its unique behaviour, which interferes with the ability of insulin to modulate the uptake of glucose and thus regulates consequent energy metabolism, favouring the accumulation of carbohydrates and the process of ketogenesis and it may also be due to surgical stress, which is attributed to increasing the levels of hormones such as glucagon, cortisol, and growth hormone and enhancing the sympathetic nerve [17]. Galactose metabolism shows the conversion of galactose into glucose and other sugar intermediates that may be used for a range of metabolic processes. The involvement of this pathway in OSCC is to meet the increasing metabolic needs and for the proliferation and rapid growth of cancer cells, as has also been reported. Therefore, alteration of galactose metabolism can be directly linked to the development of cancer [37]. In a few studies, upregulation of L-fucose was noted. Thus, suggesting a fucosylation of L-fucose at the terminal end of the oligosaccharide chain is one of the most important features that mediate several specific biologic functions. Tumour cells alter their surface by increasing fucosylation levels to escape recognition, which contributes to numerous abnormal characteristics of tumour cells, such as decreased adhesion and uncontrolled tumour growth [60].

Proteins

Numerous studies have been published focusing on the variation of proteins, more specifically amino acids, in the metabolism of oral cancer. The amino acid metabolic profiling is tabulated in (Table 2)(Table 3).

Alterations in amino acid metabolism in patients with neoplasms are usually the result of reduced protein synthesis, elevated breakdown of muscle protein, increased production of protein in the liver, increased whole-body protein turnover, gluconeogenesis, and semistarvation [15]. The significance of increased concentration is due to regulated protein synthesis in tumors, owing to their need for more protein synthesis caused by rapid cell proliferation. Increased activity of various amino acid transport systems may enhance protein synthesis in tumors. Furthermore, accessory pathways of protein synthesis may become activated in order to meet the demands of rapid cell proliferation in tumours [20].

Choline is an important constituent of the phospholipid metabolism of cell membranes; its upregulation can be directly related to membrane synthesis and degradation, which changes indicate the apoptotic activity of OSCC [18,21,29,41]. Cysteine is a non-essential sulphur-containing amino acid in humans. Cancer cells, through cysteine, maintain high levels of intracellular glutathione, a reactive oxygen species scavenger [32,40,44,61]. β-alanine is a non-essential amino acid responsible for reducing fatigue and increasing muscle strength. From the literature, it is found to be related to free radicals and the reduction of both cell migration and proliferation without acting in a cytotoxic fashion [53,62]. Less evidence of proteinogenic amino acid proline might be due to the role of proline catabolic enzymes in cellular redox control, superoxide generation, apoptosis, and cancer [26,41,52,63]. Trimethylamine N-oxide (TMAO) is a colourless amine oxide produced after the digestion of animal-sourced foods. N-Nitroso compounds, the carcinogenic component of trimethylamine N-oxide (TMAO), are responsible for DNA damage [34,64]. L-carnitine is an essential factor in the β-oxidation of long-chain fatty acids synthesised from lysine and methionine. In cancer, it indicates increased membrane synthesis and cellular turnover through lactate accumulation in response to the higher energy demand [33,49], while polyamines related to the cadaverine product putrescine were found to be associated with regulation of tumour growth [22]. L-phenylalanine and L-leucine were related to enhanced energy metabolism or upregulation of the appropriate biosynthetic pathways. L-leucine, frequently noted with increased metabolic utilisation by the TCA cycle, may also be associated with cancer cachexia and enhanced protein synthesis [22]. Tyrosine upregulation is responsible for transforming functions due to mutation, overexpression, and autocrine paracrine stimulation, leading to malignancy [27,65]. Methionine plays a key role in antioxidant processes and has a protective effect against cancer. Low levels of methionine lead to DNA hypo-

methylation, which is typical in malignant cells since methionine is the primary source of methyl groups [44]. Folate is crucial for normal DNA synthesis and repair deficiency because of its reduction of intracellular S-adenosylmethionine and alteration of cytosine methylation in DNA, leading to activation of proto-oncogenes and repression of tumour suppressor genes [14,66]. Homocysteine is a non-proteinogenic amino acid used to make protein. Hyperhomocysteinemia promotes inflammatory processes via oxidative stress, which may contribute to the biology of cancer [14,66]. High arginase activity around the tumour enables the malignant cells to escape the immune response [15,34,67]. Ornithine, a non-proteinogenic amino acid of the urea cycle, acts as a substrate to produce polyamine via the enzyme ornithine decarboxylase, which promotes the progression of cancer [35,68]. Acetylphenylalanine, known as N-acyl-alpha amino acid, reveals an abnormal phenylalanine and disturbance of glycine N-acyltransferase in OSCC [24].

Nucleotides

Nucleotides are required for an extensive variety of biological processes and are constantly synthesised *denovo* in all cells. When cells proliferate, increased nucleotide synthesis is necessary for DNA replication and for RNA production to support protein synthesis at different stages of the cell cycle, during which these events are regulated at multiple levels. Nucleotide synthesis is considered to be an energy-intensive process that uses multiple metabolic pathways across different cell compartments and several sources of carbon and nitrogen. It involves three amino acid donor reactions: the serine to glycine reaction for methyl donation, the aspartate to fumarate reaction for amine donation, and the glutamine to glutamate reaction for amine donation. The processes are regulated at the transcription level by a set of master transcription factors but also at the enzyme level by allosteric regulation and feedback inhibition [69].

Numerous metabolites of nucleotide metabolism, found to be upregulated or downregulated, are summarised in (Table 2)(Table 3). Significant upregulation of guanosine, pseudodouridine, and 5,6-dihydrouridine was seen, which could be attributed to rapid RNA degradation because of the tumour-host metabolic interactions [30,34,70]. Elevation of hypoxanthine revealed an increased catabolic flux of purines, where the activity of xanthine oxidase has a very significant effect on hypoxanthine [40,71]. A high concentration of MTA interferes with cell proliferation, tumour development, and invasiveness and increases intracellular cyclic adenosine monophosphate levels [30]. Inosine and S-adenosylmethionine (SAM) were elevated due to the deamination of cytosine, resulting in mutagenic U: G mispairs [53,72] and DNA hypomethylation on promoters of oncogenes, respectively [25,73].

Lipids

Lipids are an essential component of the homeostatic function of the human body. Lipids contribute to some of the body's most vital processes. Of particular interest, lipids also act as mediators in intra- and intercellular communications. Therefore, the lipidome is a crucial source of biomarkers for identifying a number of human diseases, including cancer. There are numerous lipid metabolomic biomarkers identified in the literature [11,74]. These biomarkers are summarised according to the lipid metabolomic pathway in (Table 2)(Table 3). Products of phosphatidylethanolamine lipolysis provide ethanolamine phosphate (Etn-P), which are independent growth-promoting signalling molecules found in most cancers [25,75]. Sphingosine-1 phosphate (S1P) is another lipid metabolite that is degraded to ethanolamine phosphate by S1P lyase and is actively involved in tumour progression [45]. Other pro-inflammatory and chemotoxic lipids showed increased levels are associated with tumorigenesis. It exhibits its actions through interaction with various receptors, such as epidermal growth factor, transforming growth factor beta, etc [22]. A review showed the estrogen-derived metabolite Estrone-3-sulphate's mechanism is direct action on DNA by forming adducts and causing oxidative damage [31,76]. Ketones such as 2-hydroxybutyrate, 3-hydroxybutyrate, acetone, acetate, and acetoacetate normally replace glucose as the main fuel in situations of glucose scarcity and promote tumour growth without any increase in angiogenesis. They can be linked to lipolysis as a backup mechanism for energy production [34]. S-adenosylmethionine is considered a regulator of the methylation process in cancer, inhibiting the growth of cancer cells by reversing the hypomethylation status [34]. Malic acid is involved in the Krebs cycle, which in cancer aids in high energy production that provides a favourable environment for disorderly growth [53]. 3-Hydroxybutyric acid is synthesised in the liver from acetyl-CoA and can be used as an energy source [29], and palmitic acid, linoleic acid, stearyl alcohol, hippuric acid, and adrenic acid play important roles in building blocks of fat in our body and are found to be associated with a higher metabolic turnover and the demand for membrane biosynthesis for cell proliferation, leading to a higher utilisation rate of lipids in cancer [37,46]. The increased level of succinic acid is probably due to the increased metabolic utilisation of the TCA cycle in oral cancer cells [23]. Sphinganine and phytosphingosine are involved in ceramide synthesis and metabolism; they prevent the apoptosis of tumour cells due to the downregulation of ceramide [23].

1.	Total no. of studies		43
2.	Total samples	Oral cancer patients	2,595
		Control patients	1,477
3.	Total metabolomics biomarkers according to pathway	Carbohydrate Metabolites	25
		Protein metabolites	78
		Nucleotide metabolites	21
		Lipid metabolites	76
4.	Total studies according to vehicle	Saliva	31
		Serum/Plasma	12
5.	Total studies according to analytical platform	UPLC-MS	3
		LC-MS	5
		HPLC-MS	5
		CE-TOF-MS	4
		CE-MS	3
		GC-MS	5
		NMR	7
		Raman spectroscopy	3
		Others	8

Table 4: Summary of metabolomic biomarkers in OSCC from the result (Table 2)(Table 3).

Conclusion

On systematic analysis of various studies in the literature regarding metabolomic biomarkers in OSCC, several limitations were evident, which are as follows:

- Untargeted metabolomics analysis was the mode used to identify metabolites, which resulted in a wide range of metabolites, some of which may or may not have significance in OSCC.
- Qualitative analysis of the obtained data was reported in many studies, which resulted in an accurate comparison. The exact upregulation or downregulation was not clearly mentioned. Measures of absolute count were not provided in many studies.

This review identified several metabolites of various pathways, such as carbohydrate, protein, nucleotides, and lipids, in the saliva and serum of oral squamous cell carcinoma cases. Carbohydrate metabolites such as lactate, pyruvate, fucose, and maltose showed upregulation, while glucose was downregulated in the OSCC group. Protein metabolism was the predominant pathway that showed differential regulation in OSCC. Metabolites such as choline, methionine, threonine, alanine, cysteine, putrescine, and asparagine showed upregulation, while valine, ornithine, and arginine showed downregulation predominantly. In the nucleotide metabolomic pathway, methylthioadenosine and hypoxanthine were upregulated and guanine was downregulated. In the lipid metabolomics pathway, upregulated metabolites included S-adenosylmethionine, sphinganine-1-phosphate, 2-hydroxybutrate, 3-hydroxybutrate, and 3-hydroxybutyric acid, and downregulated metabolites were eicosane, ubiquinone, and 9,10-dihydroxyoctadecanoic acid. While both saliva and serum showed differential regulation of metabolites from various pathways, it was found that the metabolites themselves were not always similar. A common pitfall in the use of salivary diagnostics is the absence of technologies that help in the identification of various metabolites, which are found in minimal quantities in saliva. Currently, this has been overcome by the use of advanced technologies for the detection of salivary metabolites. As evidenced by the literature analysis, a range of metabolites were identified using advanced technologies such as NMR spectroscopy and mass spectrometry analysis. The identification of the number of salivary metabolites even when present in small quantities, along with the non-invasive nature of saliva collection and its close proximity to oral lesions, has led to the understanding that salivary metabolomics is a promising tool in oral cancer diagnostics and therapeutics.

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