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Review article

Metabolomics: a promising diagnostic tool for oral cavity cancer

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Introduction

Oral cavity cancer (OCC) is an important global public health problem, especially in Asia, and it is estimated to affect 354,684 people globally with 177,384 deaths in 2018 (1). Squamous cell carcinomas (SCC) represent 90% of OCC (2). OCC accounted for 9.75% of all cancers in Sri Lanka in the year 2015 (3). It is predominantly found in middle-aged and older individuals (4). OCC is considered a major health problem in Sri Lanka since it accounted for 8.2 and 2.5 percent of the age-standardized mortality rate per 100,000 population in the year 2015, in males and females, respectively (3). The stage of diagnosis is crucial for survival. It has been identified that oral squamous cell carcinoma (OSCC) has a high possibility of cure when detected at the pre-cancerous stage (5) and any delay in the treatment might result in considerable morbidity and mortality (6, 7). In most patients, OSCC is preceded by an oral potentially malignant disorder (OPMD) (8). OPMDs are known as a group of lesions of the oral mucosa with an elevated risk of malignant transformation (9). These disorders/conditions comprise a variety of diseases with various clinical appearances, histological subtypes and aetiologies/risk factors. They include oral submucous fibrosis (OSF), oral leukoplakia (OL), erythroplakia, oral lichen planus (OLP) and oral dysplasia (10,11) (Figure 1). The global prevalence of any OPMD is estimated to be 4.47% with a male preponderance (12). However, South and Southeast Asia are reported to have higher prevalence of OPMDs [i.e., Papua New Guinea 11.7% (13), Taiwan 12.7% (14)]. In Sri Lanka, the prevalence of OSF and OL was reported to be 1.5% and 2%, respectively (15). The major contributory factors for OPMD and OSCC in the South Asian region are betel quid chewing with/without tobacco, areca nut chewing, pan masala (a

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combination of areca nuts, cardamom, lime, catechu and natural perfumes) and gutka/ghutka/guṭkha (chewing tobacco preparation made of crushed areca nut). In Sri Lanka, OSF, OLP and OL are the most common OPMDs. Erythroplakia has a higher potential for malignant transformation, however, it is relatively rare (16). Non-squamous cell carcinomas of the oral cavity are uncommon. Minor salivary gland carcinomas account for less than 5% of carcinomas (17), and usually arise on the buccal mucosa (15%), lips (25%) and hard palate (60%). The most frequent type is mucoepidermoid carcinoma (54%), followed by low-grade adenocarcinoma (17%) and adenoid cystic carcinomas (15%) (18, 19). Mucosal melanomas are rare locally aggressive tumours, primarily occurring in gingiva and hard palate (20).

Every year, approximately 3.5% of Sri Lanka's gross domestic product is spent on the health budget. Of this amount, 5,945.5 million Sri Lankan Rupees (SLR) (2.3% of current expenditure) was spent on treatment of cancer (21). However, if a cancer is detected in the precancerous stage, the costs can be reduced by one-fifth of the overall expenditure per individual. Therefore, it will be beneficial if these cancer patients can be diagnosed at an early stage because Sri Lanka offers free healthcare to all citizens (16).

Metabolomics is a novel promising "omics discipline" in systems biology claiming to explore the entire set of metabolites that exist in a biological system (22). This encompasses the quantification, identification and detection of intermediary metabolites and reflects the biological alterations associated with tumorigenesis (23, 24). Cancer cells are renowned to have a highly inimitable metabolomic profile because of their altered metabolism. It has been suggested as the whole set of individual metabolites in a specific biological system (25). Therefore,

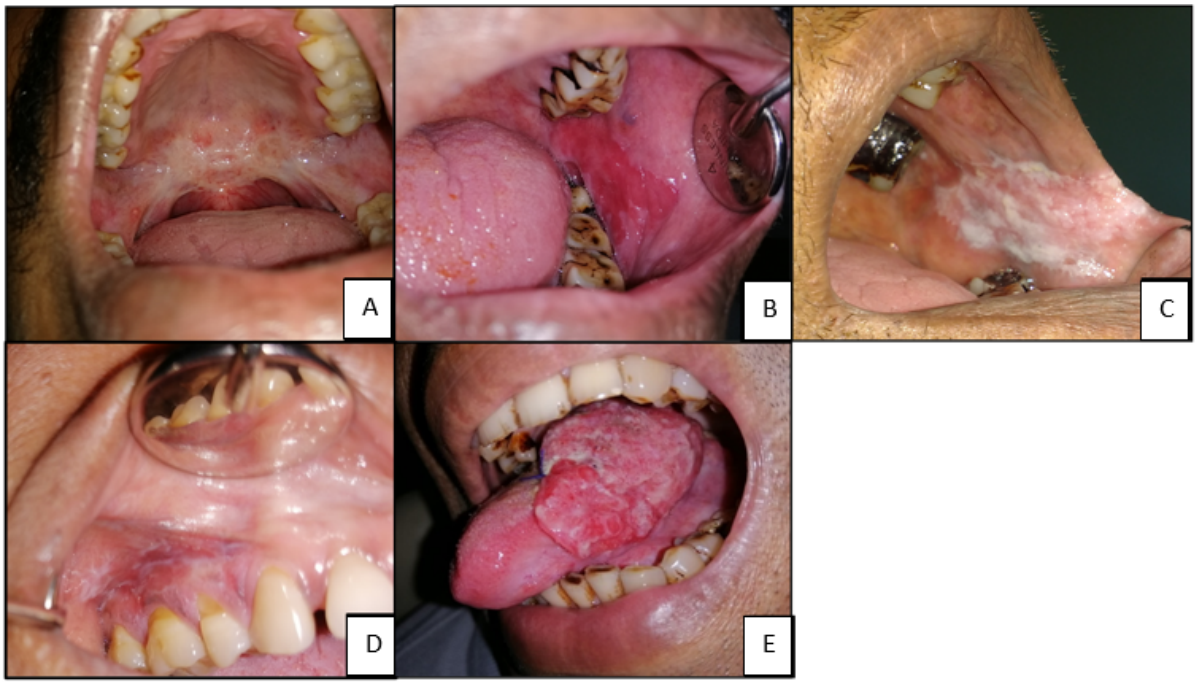

Figure 1. Clinical presentations of OPMDs; **A**. OSF is characterized by reduced fibro-elasticity which causes inflammation in the oral cavity, limiting the opening of the mouth and leading to stiffening of oral mucosa, **B**. Erythroplakia presents as a bright red velvety patch, **C**. OL presents with whitish patches or plaques in the buccal mucosa, **D**. OLP may appear as white, lacy patch, red, swollen tissues or open sores causing burning sensation, pain or other discomforts, **E**. OSCC is characterized by lumps, swellings/thickenings, rough spots/crusts or eroded areas on the lips, gums or other areas in the oral cavity.

metabolomic profiling may provide the opportunity to recognize the molecular mechanisms of carcinogenesis, and to detect a specific biomarker for early cancer detection, the prognosis of the therapy and even cancer treatment (26). Cancer is distinctively well-suited for metabolomic analysis because a majority of the cancer cells are metabolically active and thus develop alterations in several metabolomic pathways including glycolysis, amino acid metabolism and fatty acid oxidation to fulfil their high energy requisites to continue rapid cell growth (27). Therefore, theoretically, cancer cells can be differentiated easily from healthy ones by examining their metabolomic profile (28), which is the signature profile of altered metabolites (29). Considering these aspects, this non-systematic review will be focused on metabolomics as a promising biomarker in cancer detection.

Metabolomics as a promising biomarker for OSCC detection

The most definitive way for OCC diagnosis is a scalpel biopsy followed by careful histopathological evaluation by a qualified pathologist (30). However, there are novel approaches that can be employed together with histopathological assessment for diagnosis. Attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy is currently being used to diagnose numerous diseases by determining the chemical and molecular alterations (31). Over the past few years, several specific genes and proteins have been identified through salivary transcriptome and proteome analyses and serve as biomarkers (32-35). The two most accepted methods, nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry (MS) are employed in the measurement of metabolites. These methods have ameliorated the sensitivity and spectral resolution of analytic assays on metabolomic samples in attempts to accomplish an inclusive biochemical assessment (36) (Figure 2).

Table 1 summarizes selected salivary biomarkers for OSCC. Previous studies suggested that Gamma-Aminobutyric acid or γ-aminobutyric acid (GABA) should be considered as an important marker, such as a tumour signalling molecule in the periphery to control the proliferation of tumour cells (37) and a potential tumour suppressor for small airway-derived lung adenocarcinoma (38) and colon cancer cell migration (39). Further, the reduced levels of GABA are associated with

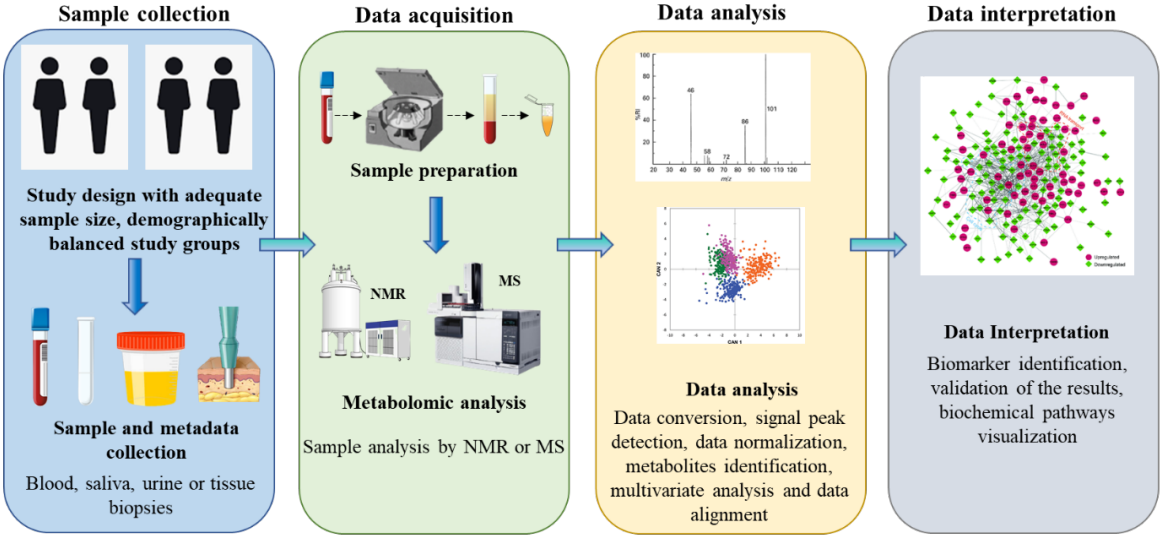

Figure 2. General workflow for metabolomic analysis consists of four steps: (1) sample and metadata collection (2) data acquisition which involves sample preparation, analysis and quality control (3) data analysis, which includes data normalization and identification of metabolites using specialized statistical software, and (4) data interpretation, which need to be integrated and modelled to raise novel hypotheses.

Table 1. Relative expression of selected salivary biomarkers and their function

Biomarker	Relative expression	Function	Reference
Lactic acid	Significantly upregulated in OSCC	Tumor cells are generally in a hypoxic condition and prone to utilize energy via glycolysis, thus, produce an elevated level of lactate, evince a higher level of lactic acid in many tumors.	46
BCAAs, valine, leucine, and isoleucine	Significantly downregulated in OSCC	Reduction in valine, leucine and isoleucine is due to the elevated metabolic utilization by the tricarboxylic acid (TCA cycle) in cancer cells. The intermediates of BCAAs are involved in the TCA cycle when there is a shortage in energy supply.	47, 48
Glutamine	Significantly downregulated in OSCC	Glutamine is lysed into glutamate and other TCA intermediates to fortify the energy production via the TCA cycle, a process known as glutaminolysis.	37
GABA	Significantly downregulated in OSCC	Together with the reduction of α-ketoglutarate (a TCA intermediate), more glutamate is transformed to 2-oxo-glutarate, resulting in an elevated utilization of GABA and thus GABA is found in lower levels in extracellular fluids such as saliva.	44, 37, 39
sphinganine-1-phosphate (S1P)	Significantly upregulated in OSCC and OLK	The elevated levels of S1P are correlated with tumorigenesis and poor survival.	49, 50
4-nitroquinoline-1-oxide	Significantly upregulated in OSCC and OLK	A powerful carcinogen and produce DNA adducts after metabolized into 4-hydroxyaminoquinoline 1-oxide forming 8-hydroxydeoxyguanosine (8OHdG) which is an essential free radical resulting in oxidative damage.	51
Ubiquinone (coenzyme Q-10)	Significantly downregulated in OSCC and OLK	An essential lipophilic antioxidant restricting the production of free radicals, oxidative modification of proteins, lipids, and DNA.	52
Estrone-3-glucuronide	Significantly upregulated in OSCC and OLK	Direct action on DNA by causing adducts and oxidative damage.	53
Inositol 1,3, 4-triphosphate (IP3R)	Significantly upregulated in OSCC and OLK	The deregulation of IP3R plays an essential role in tumor growth, aggressiveness, and drug resistance via autophagy and energy metabolism.	54
2-phospho-d-glyceric acid	Upregulated in OSCC and OLK	An up-regulation of this compound indicates the elevated utilization of glycolysis during the cell proliferation occurring in OSCC even in the presence of oxygen.	33

the occurrence of OSCC. Additionally, elevated levels of n-eicosanoic acid are associated with the development of OSCC due to the ingestion of saturated fatty acids (40). According to the study by Sridharan et al. (41), significant dysregulation of amino acids, including 1-methylhistidine, 2-oxoarginine, norcocaine, L-isoleucine, and γ-aminobutyryl lysine, has been

observed. Moreover, p-chlorphenylalanine, L-isoleucine, and 4-fumarylacetoacetic acid are significantly down-regulated in OSCC and OLK.

The reason behind the down regulation is that cancer cells utilize more glucose and amino acids for cell proliferation, energy expenditure and tumour growth (42). Especially, lower levels of branched-chain amino acids (BCAAs) are associated with advanced tumours as a result of cancer cachexia and elevated protein production in cancer cells (43-45). Furthermore, the reduction of BCAAs may also be correlated with elevated glycolysis during cell proliferation (38).

As OSCC is a complex disease, it proceeds from an interconnected sequence of biochemical changes, rather than a single disruptive event. Hence, a group of various biomarkers will ameliorate the sensitivity and specificity for OSCC detection. Currently, OSCC is not diagnosed till it reaches an advanced stage, which eventually results in a poor prognosis and survival rate. Thus, early detection of OSCC together with the screening of high-risk populations with OPMD remains to be an unmet need. The amalgamation of several different types of biomarkers together with salivary metabolomic biomarkers and conventional histological examination (i.e., scalpel biopsy) may set off a pertinent approach for early detection of OPMD and OSCC.

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