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Review Article

Dentistry Section

Salivary Metabolic Profiling of Systemic Disorders and Oral Neoplastic and Preneoplastic Conditions- A Narrative Review

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ABSTRACT

Salivary metabolic profiling has emerged as an important mode of analysing the metabolic markers that aids in early disease detection in various systemic diseases. The metabolomics study states that, the transitional and the end products of interactions that take place between genes, proteins, and the environment are found to be involved in various disease processes. Salivary metabolomics stands as a highly specific and sensitive method in diagnosis of various conditions making it a better alternative to the conventional serum and tissue-based methods well. These metabolomics studies incorporate various analytical technologies for identifying each component that could be used as a biomarker. Hence, we reviewed the current state of salivary metabolomics, diagnostic efficiency and its associated technologies and its future role in identification and monitoring the disease prognosis. The study selection was done by locating those research papers that provided information on salivary diagnostics using metabolic markers for early diagnosis in systemic disorders, neoplastic and preneoplastic conditions with help of search engines like Pubmed, Google Scholar, Web of Science and Cochrane library. The results of each study were critically evaluated to accentuate the principal role played by these biomarkers in the field of salivary diagnostics.

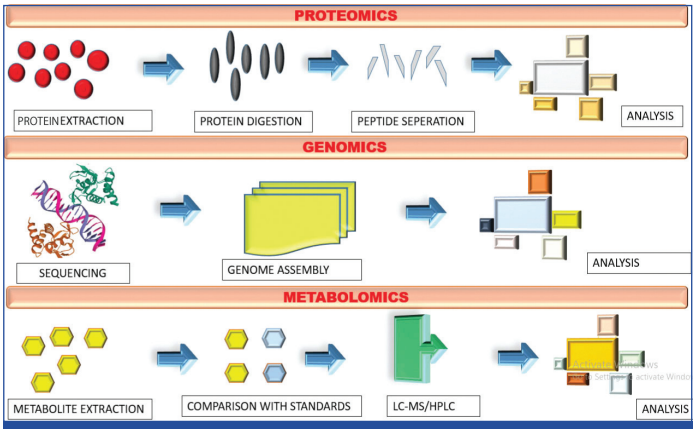
Keywords: Biomarkers, Genomics, Proteomics, Salivary metabolomics, Transcriptomes

INTRODUCTION

Saliva is a biological fluid with an array of metabolites utilised as a prognostic and diagnostic marker. The components present in blood pass into saliva by transcellular, intracellular, paracellular or extracellular routes either by active transport or passive diffusion. Salivary metabolomics is an emerging diagnostic tool that aids in rapid testing, and helps in accomplishing various research laboratory operations [1]. Genomics describes the genes, their functions, and their inter-relationships to identify their combined influence on the growth and development of the individual [2]. Transcriptomes are the RNA molecules from protein coding genes, which direct the synthesis of the proteome. The rich oral microbiota contributes to the high proportion of the salivary RNA. The comprehensive RNA level in the saliva which is devoid of cells ranges from 0.108 µg/mL to 6.6 µg/mL which plays an integral role in diagnosis [3]. Proteomics provides the complete profile of the proteins, proteoforms, and multiple complexes of proteoforms, including their diagnosis, quantification and understanding of various protein characteristics [4]. Salivary proteomics discriminates between physiological and pathological conditions as per the state of the individual [5].

Metabolomics is the fingerprint of the metabolite profile in a biological sample. Metabolomics includes the full repertoire of small molecules and explains the pathway of disease progression to help in the early diagnosis [6,7]. Apart from diagnosing the very progress of the disease, the procedure also distinguish between the disease and the normal metabolic state of the organisms that are being subjected to the procedure of metabolomic studies. The metabolome refers to the final product of the interactions that is found to take place among the proteins, genes and the environment where the process takes place in various disease processes [8]. The various steps involved proteomics, genomics and metabolomics has been depicted in [Table/Fig-1].

Various systemic conditions can be diagnosed with the salivary profile which can be studied by spectroscopic studies using NMR spectroscopy, studies done using gas chromatography along with spectroscopic studies like mass spectrometry (GC-MS).



[Table/Fig-1]: Steps involved in proteomics, genomics and metabolomics [3].

Characterisation of vitamins using liquid chromatography studies paired up with mass spectroscopy studies (LC–MS/MS), High performance liquid chromatography procedures being employed in identification of thiols and nucleotides, the inductively coupled plasma mass spectrometry technique atomises the samples and produce ions in order to analyse the trace elements present in the samples [1]. Thus, this review article aimed to analyse and critically evaluate the literature available to bolster the importance of salivary diagnostics in the early diagnosis of systemic disorders and neoplastic conditions.

NEURODEGENERATIVE DISORDERS

The key to manage any kind of neurological disorder is to assess the early symptoms for prompt diagnosis. Invasive procedure such as lumbar puncture is done to aid in diagnosis. Salivary analysis of various metabolic products proves non invasive.

Parkinson's Disease

Parkinson's disease is a chronic neurodegenerative disorder which is often characterised by a persistent and progressive loss of related nervous systems. Identification of specific biomarkers at early stages of the disease process is important for the diagnosis followed by the

evaluation of disease progression and the development of therapeutic interventions [9].

The proteins alpha-synuclein (α-Syn) and DJ-1 biomarkers are known to play a crucial role in the identification of Parkinson's disease. Decreased levels of these proteins from cerebrospinal fluid were reported in various studies of Parkinson's disease [10]. Saliva from the submandibular salivary gland often is identified to be involved by synucleinopathy in the initial stages of Parkinson's disease. Assessment of salivary levels of these proteins would be non invasive and reduce the complications related with CSF collection. The salivary assessment of biomarkers associated with Parkinson's disease can avert the complications associated with the CSF collection methods. Oral biofluid is yet another ideal biofluid for diagnosis and to assess disease progression of Parkinson's disease [10,11].

Alzheimer's Disease

Alzheimer's Disease (AD) is the most common neurodegenerative disorder manifested as cognitive impairment and dementia due to the destruction of the neurons in the hippocampus, basal forebrain which is followed by the cortical areas of the brain. "Amyloid beta peptide and Tau protein" (Microtubule Associated Protein T-MAPT) have been identified to be one of the factors that attribute to the aetiology of AD. The neuron cell death in AD is caused due the accumulation of amyloid beta peptides extracellularly along with the intracellular neurofibrillary tangles have been reported [12,13]. Apart from the above mentioned markers, increased levels of amyloid beta peptides and phosphorylated tau protein in comparison with total tau concentration could be used as a biomarker which has shown significant results [12]. Salivary lactoferrin levels were significantly reduced in AD and can be used as a potential marker in diagnosis of AD [12]. The accumulation of acetyl cholinesterase has been reported to be higher in amyloid plaques and neurofibrillary tangles of AD brains which also can play a role in the diagnosis of the disease.

Huntington's Disease

Huntington's Disease (HD) is caused by Huntingtin gene (HTT) mutation which is an autosomal dominant neurodegenerative disorder. Htt protein here serves as vital component in the diagnosis of HD making it a promising marker. The salivary levels of total Htt protein from HD patients were significantly increased when compared to controls. In addition to it, there was significant increase in salivary concentration of mutated HTT in HD patients in comparison to the healthy controls.

Amyotrophic Lateral Sclerosis

Amyotrophic Lateral Sclerosis (ALS) also known as Lou Gehrig's disease is a motor neuron degenerative disorder with frontotemporal dementia and muscle weakness followed by paralysis leading to death. Chromogranin A (CgA) is a neuroendocrine secretory protein which is elevated in patients with terminal ALS.

Multiple Sclerosis

Multiple Sclerosis (MS) is a chronic autoimmune, neurodegenerative disorder of the Central Nervous System (CNS) showing demyelination, in which the myelin sheaths of neurons were targeted by T-lymphocytes [14]. In this condition the macrophages, microglia and mitochondrial dysfunction generate free radicals in MS. Reactive oxygen species contribute to the plaque formation and measuring their levels serves as a potential biomarker.

Autism Spectrum Disorders

Autism Spectrum Disorder (ASD) is a group of neurodevelopmental disorders with impairments in communication, interaction and display of restricted repetitive patterns of behaviour. Autistic disorder, Rett syndrome, Childhood disintegrative disorder, Asperger's disorder, Pervasive developmental disorder-not otherwise specified are the group of disorders of ASD. Salivary microRNAs (miRNAs/miR) profiling of ASD patients in comparison to control group expressed 14 different types of miRNAs in saliva samples [12].

AUTOIMMUNE DISORDERS

Systemic Lupus Erythematosus

Systemic Lupus Erythematosus (SLE) is a chronic inflammatory and also an autoimmune disease. IL-6 levels were found to be increased in SLE. Salivary Plasminogen xActivator Inhibitor (PAI-1) and Monocyte Chemoattractant Protein (MCP-1) levels were elevated in SLE patients [14].

Rheumatoid Arthritis

Rheumatoid Arthritis (RA) is an autoimmune disease of the bone. Salivary TNF-α level are higher in RA and have been employed in the early diagnosis of the condition [15].

Sjogrens's Syndrome with Increased Risk Of Developing Lymphoma

Sjogren's syndrome is an autoimmune disease of salivary glands. There is an increased lymphocytic infiltration resulting in oral and ocular findings. This condition presents with abnormal B-cell hyperactivity [16]. Siglecs are a group of transmembrane receptors and are expressed in myeloid restricted manner on the surface of various immune cells. These siglecs are found to be in higher levels in the saliva of primary Sjogren's syndrome patients [16].

All the important markers associated with the neurological and autoimmune disorders are mentioned in [Table/Fig-2] [10,12,14,17,18].

Systemic disorder	Salivary biomarker
Neurological disorder	Synuclein (-Syn), DJ-1, Amyloid beta peptide and tau protein, Acetylcholinesterase, Htt protein, CgA, ROS and miRNA [10,12]
Autoimmune disorder	IL- 6, PAI-1, MCP-1, TNF-α [14]
Inflammatory disorder	pANCA, α-amylase, globulin, anti-OmpC, ALCAs, ACCAs, AMCAs, anti-L, and anti-C and PAB, CRP, Hs-CRP and β2-microglublin, miR-21, M2PK, CHI3L1 [17,18].

[Table/Fig-2]: Salivary biomarkers in systemic disorders [10,12,14,17,18].
CgA: Chromogranin A; ROS: Reactive oxygen species; pANCA: Atypical perinuclear Antineutrophil cytoplasmic antibodies; IL: Interleukin; PAI: Plasminogen xActivator Inhibitor; MCP-1: Monocyte chemo-attractant Protein; ASCA: Anti-saccharomyces cerevisiae antibodies; PAB: Pancreatic autoantibodies; CRP: C-reactive protein; highly sensitive CRP:Hs-CRP

INFLAMMATORY DISORDERS

Diabetes Mellitus

Salivary glucose possess promising values that are been explored for a long time now and it can be potentially correlated with glucose levels obtained from the blood results. Those proteins that are present in blood are present in saliva as well. Therefore, saliva can be functionally compared to that of blood in reflecting the exact physiological status of the body and even the pathology at times [13]. Salivary α-amylase level of whole saliva is found to be higher in diabetics, while salivary globulin level is lower in diabetics [17].

Inflammatory Bowel Disease

Inflammatory Bowel Disease (IBD), the term includes two conditions the Crohn's Disease (CD) and Ulcerative Colitis (UC), the aetiology of which is unknown [18]. IBD is diagnosed with the help of the following antibodies atypical perinuclear Anti-neutrophil Cytoplasmic Antibodies (p-ANCA), Anti-Saccharomyces Cerevisiae Antibodies (ASCA), anti-OmpC, ALCAs, ACCAs, AMCAs, anti-L, and anti-C and Pancreatic Autoantibodies (PAB) and are considered as potential biomarkers [2]. C-Reactive Protein (CRP) and highly sensitive CRP (Hs-CRP) and β_2-microglublin are certain inflammatory markers [17].

MiR-21 levels are found to be increased significantly in mucosa of the individual with IBD. Significant rise in M2-Pyruvate Kinase (M2PK) in UC, CD, and Colorectal Cancer (CRC) thus, serves as a potential marker. This also rises in the CRC arising in IBD patients. Intraepithelial neoplasia in the mucosa of UC cryptoepithelium shows rise in the mucosa CHI3L1 levels. This marker also used to monitor the malignant degeneration of UC into CRC as there is significant rise in the mucosa CHI3L1 levels during their progression. Most commonly studied markers in the inflammatory disorders are mentioned in the [Table/Fig-2] [10,12,14,17,18].

NEOPLASTIC AND PRENEOPLASTIC CONDITIONS

Different Carcinomas

All the commonly associated salivary markers different carcinomas in are mentioned in [Table/Fig-3] [19-23].

Systemic disorders	Salivary biomarkers	Suspected neoplastic and preneoplastic
Parkinson's disease	Alpha Synuclein (α-Syn) and DJ-1	Skin cancer, prostate cancer, breast cancer, melanoma [19]
Systemic Lupus Erythematosus (SLE)	Plasminogen xActivator Inhibitor (PAI-1) and Monocyte Chemo-attractant Protein (MCP-1)	Non Hodgkin's lymphoma, Hodgkin's lymphoma, leukaemia, multiple myeloma, cervix, vagina/vulva, renal, bladder, oesophagus, gastric, hepatobiliary, lung, oropharynx, larynx, non melanoma skin, and thyroid cancers [20]
Rheumatoid Arthritis (RA)	Salivary TNF-α	Haematologic & renal cancer [21]
Diabetes mellitus	Salivary α-amylase, salivary globulin	Colorectal, postmenopausal breast, pancreatic, bladder, and endometrial cancers [22]
Inflammatory Bowel Disease (IBD)	Atypical perinuclear Antineutrophil cytoplasmic antibodies (pANCA), Anti-Saccharomyces cerevisiae Antibodies (ASCA), anti-OmpC, ALCAs, ACCAs, AMCAs, anti-L, and anti-C and pancreatic autoantibodies (PAB),	CRC [23]

[Table/Fig-3]: Salivary biomarkers in systemic disorders and those disorders transforming into neoplastic and preneoplastic conditions [19-23].
p-ANCA: Atypical perinuclear Antineutrophil cytoplasmic antibodies pANCA; ASCA: Anti-saccharomyces cerevisiae antibodies; PAB: Pancreatic autoantibodies

Oral Squamous Cell Carcinoma

Oral Squamous Cell Carcinoma (OSCC) is reported to be the most common malignant neoplasm involving the oral cavity worldwide. The diagnosis of OSCC is done using a panel of markers from the interleukins family mainly the IL-6, IL-8, IL-1, and TNF-α [24]. These proteins were found in high levels in saliva in patients with OSCC. Increased levels of IgG have also been detected in OSCC which ascertains their role in angiogenesis [24]. There is an altered levels in salivary levels of Ki-67 and Cyclin D1 in OSCC. Cell-surface glycoprotein such as CD44, CD59, or Carcinoembryonic Antigen (CEA) were found to be over expressed in studies conducted using western blot, or Magnetic Resonance Spectroscopy (MRS) based studies. The zinc finger protein family (ZNF) such as ZNF510, Cyfra 21-1, and CK19 have also been used as a tool in the diagnosis. The salivary levels of ZNF510 have been incorporated to discriminate between the early and stages (T1+T2) and the advanced stages (T3+T4) in OSCC. Many unique proteins or panels obtained from non targeted proteomic techniques are used as oncological markers Liquid Chromatography with tandem mass spectrometry (LC-MS/MS) studies have demonstrated that a panel of proteins namely the Mac-2 Binding Protein (M2BP), Myeloid-related protein 14 (MRP14), CD59, catalase, and profiling have shown 90% sensitivity in the diagnosis of OSCC. A panel using Matrix metalloproteinase-1 (MMP1), Kininogen-1 (KNG1), ANXA2, Heat Shock 70 kDa Protein 5 (HSPA5) were able to predict Oral Potentially Malignant Disorders (OPMDs) which showed malignant transformations [25]. Along with these biomarkers that are present in higher amounts in OSCC determine the primary and pathological state of individuals. The biomarkers like Resistin (RETN) are studied best using Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) coupled to LC-MS/MS which identifies the biomarkers and are considered to be the best tool in biomarker analysis [5]. Using nano-LC-MS/MS and validation by Western blot and Enzyme-Linked Immunosorbent Assay (ELISA), S100A8 was identified as a potential biomarker of OSCC [26].

Other important group of biomarkers that play an important role in the salivary diagnostics of OSCC are the group of "MicroRNAs (miRNA/miR)". These are useful both in the diagnosis and treatment of OSCC. "microRNA", also explains the process oncogenesis in the head and neck cancers. MiR-125a and miR-200a levels are found to be down regulated in salivary samples of OSCC patients [27]. Association of miR-195-, miR-26b, miR-483-5p, miR-375, miR-143, miR-155-5p with oral cancer has been studied by many authors. The levels of these biomarkers in the saliva play a major role in preoperative diagnosis and follow-up postoperatively. In contrast to others miR-195-5p levels was down regulated in OSCC tissues in comparison to the non tumour samples [28].

Defensins that are present within the granules of polymorphonuclear neutrophils possess increased cytotoxic activity. These peptides are found in increased numbers in OSCC which could be used as a prognostic marker [29]. Cathepsin V, ADAM9, kallikrein and MMP-1, levels are found to be comparatively higher in patients with OSCC where the healthy individuals and those with other oral diseases showed reduced levels of these biomarkers. Proteases are considered to be of higher importance when the prognosis of the disease has to be assessed [30,31].

Different biomarkers in various preneoplastic and neoplastic oral conditions has been summarised in [Table/Fig-4].

Neoplastic and preneoplastic oral conditions	Salivary biomarkers
Oral Squamous Cell Carcinoma (OSCC)	IL-6, IL-8, IL-1, TNF-α, IgG, Ki-67, Cyclin D1, CD44, CD59, ZNF510, Cyfra 21-1, CK19, M2BP, MRP14, CD59, catalase, profilin, MMP1, KNG1, ANXA2, and HSPA5, MiR-125a and miR-200a, miR-195-, miR-26b, miR-483-5p, miR-375, miR-143, miR-155-5p, defensin, Cathepsin V, ADAM9, kallikrein and MMP-1 [4-7]
Oral Lichen Planus (OLP)	Cortisol, Oxidative stress related molecules, Igs, and cytokines, glucocorticoids and adiponectins [24]
Oral Leukoplakia (OL)	C4d, MDA, endothelin-1, CK10 and lactate dehydrogenase [24,32]
Oral Submucous Fibrosis (OSMF)	S100A7, zinc, copper, iron, superoxide dismutase, albumin, uric acid, miR-21, miR-31 [33-35]
Mucoepidermoid carcinoma	miRNA-127-3p, mmu-miR-140-5p, hsa-miR-374, hsa-miR-222, hsa-miR-15b, hsa-let-7g, hsa-miR-132, hsa-miR-519b-3p, hsa-miR-223, and hsa-miR-30a-3p [36]
Kaposis sarcoma	miR-375 [37]

[Table/Fig-4]: Salivary biomarkers in oral neoplastic and preneoplastic conditions [4-7,24,32-37].
ZNF: Zinc finger protein family; M2BP: Mac-2-binding protein; MRP-14: Myeloid-related protein 14; Kininogen-1; Annexin A2

Mucoepidermoid Carcinoma

Mucoepidermoid Carcinoma (MEC) is a malignant salivary gland neoplasm that may occur in the oral cavity. They are commonly reported in the palatal region. These tumours present with highly variable biologic behaviour. The miRNA-127-3p in MEC is found to be an anti-proliferative that alters the cell cycle. This could be used as a marker in assessing the prognosis of the neoplasm [28]. Salivary microRNA namely mmu-miR-140-5p, hsa-miR-374, hsa-miR-222, hsa-miR-15b, hsa-let-7g, hsa-miR-132, hsa-miR-519b-3p,

hsa-miR-223, and hsa-miR-30a-3p were increased in malignant parotid gland neoplasms [36].

Kaposi's Sarcoma

Kaposi's Sarcoma (KS) is an angioproliferative sarcoma affecting multiple sites of the skin and oral cavity, associated commonly with Human Immunodeficiency Virus (HIV) and Human Papilloma virus type-8 infections. The incidence of HIV associated kaposi's sarcoma has drastically come down following the Antiretroviral therapy (ART) treatment. MiR-375, which is considered a potential biomarker is found to be down regulated in patients HIV-KS patients following combined antiretroviral therapy (cART). Whereas, HIV-KS patients who have not taken cART treatment showed upregulated MiR-375 [37].

Oral Lichen Planus

Oral Lichen Planus (OLP) is a chronic inflammatory disease mucocutaneous disorder. OLP is an inflammation triggered by the apoptosis mediated by the cytotoxic T-lymphocytes against the epithelial cells. The malignant transformation rate of OLP is found to be 1% over a 5-year average period [38]. The diagnosis of OLP has been made using cortisol, Oxidative stress related molecules, Igs, and cytokines are mostly protein based. Extensive studies have been conducted to find the relationship between psychological status and levels of cortisol hormone in patients with OLP. Many studies revealed that the elevated levels of this glucocorticoid are common among affected individuals [24]. A study done by Lorenzo-Pouso AI et al., reported that the adiponectin levels were higher in OLP patients [24]. The levels of IgA and IgG are considerably increased in OLP patients when compared to control population [37,38].

Oral Leukoplakia

Oral Leukoplakia (OL) is an Oral Potentially Malignant Disorder (OPMD) characterised by white plaque of questionable risk having excluded (other) known diseases or disorders. The annual average of malignant transformations of this condition is 1%.There are no specific markers to predict the malignant transformation of OL [31]. The salivary proteomic studies focused on to study the cytokines based on ELISA techniques and the studies mainly studied the following IL-6, IL-8, and TNF-α [24].

Few other proteins that can differentiate between OL and OSCC are C4d, Malondialdehyde (MDA), endothelin-1, and lactate dehydrogenase [39]. Camisasca et al., reported that in a 2-DE gel-based proteomic study, 22 spots were more abundant in patients with OL than in controls. One spot corresponded to CK10 [40]. The authors later validated this marker by immunohistochemistry.

Oral Submucous Fibrosis

Oral Submucous Fibrosis (OSMF) is a potentially malignant disorder with a high prevalence rate in India. It account for almost 6-8% of the malignant transformation rate ranging from 4.5% to 7.6%. The role of lactate dehydrogenase has been studied widely, and their levels are found to be increased in OSMF. A study done by Kallalli BN et al., highlighted the elevated levels of lactate dehydrogenase in both OSMF as well as in OSCC [33]. Alternatively trace elements can be used as a marker in the diagnosis of OSMF. Altered levels of trace elements like zinc, copper and iron can be observed in OSMF. An antioxidant enzyme superoxide dismutase activated by zinc is reduced in OSMF [33].

Salivary albumin and uric acid levels in OSMF patients showed mild decrease in their levels. However, Tiwari P et al., in their study highlighted that the free radicals produced by the areca nut in OSMF have least effect on the albumin present. Similarly, the uric acid levels were also reduced [35]. S100A7 levels were assessed in OSCC and OSMF patients. Many researchers have reported the antifibrotic activity and reduction in fibroblast proliferation has been associated with S100A7 [39] Raffat MA et al., reported the increased levels of S100A7 in OSMF patient's salivary samples which could be used as a potential biomarker [41]. The potentially malignant disorders of the oral cavity like leukoplakia and OSMF showed increased levels of salivary miR-21 and miR-31nand is considered to be used as an adjuvant method of screening in OPMD. Hung KF et al., in their study highlighted the increased levels of miR-21 and miR-31 in the saliva in OPMD [42].

CONCLUSION(S)

In summary, the salivary biomarkers are considered to be a valuable tool in diagnosis of the preliminary stages of the disease. These biomarkers in many neurological disorders and premalignant conditions are considered to play a key role in assessing the condition well ahead in order to promptly treat the condition. The salivary extraction being the highly non invasive procedure, the biomarkers can be easily identified by the help of advanced analysing tools. The overall content of the study lead to the identification of potential biomarkers in various systemic disorders. And thus, the application of these non invasive metabolite panel will help in early and precise risk detection and that which can lead to early risk management and medical intervention.

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