Salivaomics - A Review

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Abstract:The term Salivaomics was coined in 2008, which is used for the development of knowledge about the salivary constituents. Saliva contains salivary markers which are used in the diagnosis of cardiovascular diseases, cancer, etc. Diagnosis from saliva is a developing field with appreciation as it contains hormonal status, neurological effects, nutritional and metabolic influence and immunological status, the diagnostic markers are very useful in various assays. There are 5 different branches in Salivaomics which are the diagnostic alphabets which include proteins, mRNA's, miRNA, metabolic compounds, and Microbes. Salivary genomics is the study of whole genomes i.e., DNA of all the organisms. Salivary proteomics is the study of the proteins present in the saliva. Saliva contains about 1,166 proteins. Salivary Metabolomics is the study in which small metabolites present within the system. Salivary transcriptomics is related to the diagnosis of mRNA. Around 185 mRNAs are found common in all healthy humans. Saliva has a proportionately distributed ratio of bacterial colonies like Bacteroidetes, Fusobacterium, Prevotells, Proteobacteria, Veillonella etc. The advantages of Salivaomics include potential diagnostic value, Safer and easier for the health care professionals compared to blood sampling which sometimes causes cross-infection, Multiple samples can also be obtained easily. There are certain disadvantages which make salivaomics less reliable and preference as a diagnostic tool, These include Composition and flow of the saliva is influenced by the method of saliva collection and also stimulation of saliva flow, biomarkers in saliva which are not reliable, Proteins present in the saliva are also affected by the rate of flow of saliva. SALO is a cross-disciplinary interaction creation, which helps in growing semantically enhanced web-enabled will be created within Salivaomics Knowledge Base (SKB).
With this background, the present study aims to review the uses, advantages, disadvantages, techniques and methods used in Salivaomics.

KEYWORDS: Biomarkers, Saliva, Salivaomics, Salivary proteins

1. INTRODUCTION:

Saliva is an oral fluid which contains water, cells, organic and inorganic constituents. The saliva represents the physiological state of an individual. Saliva contains salivary markers which are used in the diagnosis of cardiovascular diseases, cancer, etc. (Farnaud et al. 2010, Brundha 2016). The saliva secretion is 500 - 700 ml/ day of which the major salivary glands

contribute 90 % and minor salivary glands contribute the remaining 10 % of the total saliva (Streckfus and Bigler 2002). The functions of saliva are Cleansing action, Antibacterial activity, Buffering action, Maintenance of pH, Lubrication of the oral cavity, protection from various microbes etc (Figure -1). Diagnosis from saliva is a developing field with appreciation as it contains hormonal status, neurological effects, nutritional and metabolic influence and immunological status, the diagnostic markers are very useful in various assays, patients care (Contreras-Aguilar and Gómez-García 2020, Raskin et al. 2015).

Salivaomics:

The term Salivaomics was coined in 2008, which is used for the development of knowledge about the salivary constituents. There are 5 different branches in Salivaomics which are the diagnostic alphabets which include proteins,mRNA's, miRNA, metabolic compounds, and Microbes (Shah 2018) (Figure - 2).

Salivary Genomics:

It is the study of whole genomes i.e., DNA of all the organisms. There is a recent advancement in genomic technology which accounts for the diagnosis and effective treatment planning of various diseases (Wong 2012). Coeliac disease, a congenital disorder of the small intestine, quantitative measurement of the IgAntigliadin antibodies in the saliva is a specific and effective in the diagnosis of coeliac disease and also, the detection IgAntigliadin antibodies can be used for monitoring patients with a gluten-free diet (Grant 2012)(Brundha 2015).

Salivary Proteomics:

Salivary proteomics is the study of the proteins present in the saliva. It can also be used as a tool for studying oral health and also the pathogen of the disease. Saliva contains about 1,166 proteins, most of the proteins are secreted by the acinar cells of the salivary gland(Helmerhorst and Oppenheim 2007, Timothy, Samyuktha, and Brundha 2019) . The laboratory techniques employed during the diagnosis includes one (or) two-dimensional PolyAcrylamide Gel Electrophoresis (PAGE), Mass spectroscopy, Lectin Probe Analysis and Immunoassays. The protein content in the saliva is only about 30 % present in the circulating blood. Some of the diagnostic salivary proteins include Cystatin, Hystatin, Lactoferrin, α - amylase, albumin, Ig A, Statherin, Proline-rich proteins, Mucin, Lysosomes accounts to about 98% of the total salivary proteins (Wong 2006). In Candidiasis, Candida's Mannan antigen in the oral cavity is a sensitive biomarker along with IgA or IgG antibodies. In Periodontitis condition there is a fall in the salivary proteins which is an indication of Periodontal inflammation, MMP - 8 and MMP - 9 are elevated in chronic periodontitis. Along with MMP's Osteointegrin is also elevated in the presence of Red complex anaerobic pathogens (Mitulović 2019).

Salivary Metabolomics:

In recent times, metabolomics gained popularity and importance in the life sciences. Metabolomics is the study in which small metabolites present within the system. The metabolites may be exogenous or endogenous, including lipid, amino acid, Peptide, Nucleic acid, organic acid, carbohydrates etc., are some of the tools used in diagnosis. Taurine and Piperidine act as diagnostic biomarkers in Oral cancer (V, Jha, and B 2016, Mittal et al. 2011).

Salivary Transcriptomics:

It is related to the diagnosis of mRNA. The salivary mRNA's are detected by high-density oligonucleotide microarray and there are more than 3000 mRNAs are detected supernatantly. Around 185 mRNAs are found common in all healthy humans, any alteration in the ratio is an indication of diseased state and also helpful in their detection. The miRNA's detected in Oral Squamous Cell Carcinoma is regulated epigenetically by Methylation of DNA (Miller et al. 2010).

Salivary Microbiome:

Saliva has a proportionately distributed ratio of bacterial colonies like Bacteroidetes, Fusobacterium, Prevotells, Proteobacteria, Veillonella etc., Each individual will harbour around 75 - 100 species of bacteria (Belibasakis et al. 2019). The alterations in the microbiome ratio are used in the diagnosis of diseases like Sjögren's syndrome, Diabetes, Oral Squamous Cell Carcinoma, Periodontal diseases. This can be detected by using Laboratory techniques like Gene chip array and PCR (Polymerase Chain Reaction) (Thankappan and Sherin 2017).

The knowledge about the various biomarkers, enzymes present in the saliva is of utmost importance because of its potential diagnostic value and will also be a promising diagnostic tool in future. With this background, the present study aims to review the uses, advantages, disadvantages, techniques and methods used in Salivaomics.

This study included around 35 - 40 articles from various search engines like Google Scholar, PubMed, MeSH core, Cochrane, bioRxiv, MedRxiv for data collection. The articles included in the study were between the period of 2000 - 2020, however, few articles were collected before the period because of the unavailability of articles in the specified period. The articles were collected using keywords like saliva, Salivaomics, Salivary protein, Biomarkers etc.

After the collection of relevant articles quality analysis was performed using Health Evidence - Quality assessment tool (Table 2) (*Health Evidence - Quality Assessment Tool*2016) .

Saliva and it's Sample Collection

Saliva samples are used in the diagnosis of various systemic and orodental diseases. The samples which can be used for diagnosis in the oral cavity include Gingival Crevicular Fluid, Saliva, oral cavity swabbing. The saliva can be of two types as Stimulated or Whole saliva and Unstimulated or Resting Saliva. Unstimulated salivary is usually preferred in the diagnostic point of view since it is present in the oral cavity throughout and also contains the biomarkers, protein etc, also has more diagnostic value. In contrast, it is very hard to identify and isolate the biomarkers from the stimulated saliva and it is too dilute (Sindhu and Jagannathan 2014).

Different Methods Of Saliva Collection:

The different methods of saliva collection includes (1) Draining Method - In this method, the subject is asked to sit with his/her head in a bent position, which makes the saliva drool gradually and it is collected using tubes. It is more reliable since it is not stimulated (Bellagambi et al. 2020) . (2)Spitting Method - The subject is asked to collect the saliva within the oral cavity first and is asked to spit it out forcefully in the sample collecting tube.

It has a stimulatory effect on the saliva. It is also used for estimating the flow of the saliva (Topkas et al. 2012). (3)Suction Method - The saliva from the oral cavity is aspirated using a micropipette. Aspirator, saliva ejectors or syringe form the subject (Donzella et al. 2008). (4)Swabbing Method - In this method saliva is swabbed from the subject by using a cotton swab or absorbent pad, is rolled in the saliva present in the oral cavity and it is preserved and analyzed. When the absorbent pad is used the subject is asked to chew the absorbent pad and spit the pad in the tube. It can be used in patients from whom the saliva cannot be collected by using the passive drooling technique. It is less reliable but it can be used for monitoring the drug, hormone, steroid levels in the saliva (Durdiaková et al. 2012).

Commercially Available Saliva Collectors:

Oragene Is a commonly used technique for saliva collection wherein it also contains the buffering solutions that can be used for protection of saliva samples until the diagnosis of the sample. Sialgene is an alternative method where the saliva is collected by spit in cup technique (Lenander-Lumikari et al. 2008). After the sample is collected in the container it is covered using a plunger, because of this action buffer solutions are released which protects the samples. Oracol is an absorbent pad saliva collection method, which helps in effective diagnosis of HIV, Measles, Hepatitis. Verofy is a method where immunochromatographic sticks are used for producing immediate results (Wiwanitkit 2013).

Processing Of The Sample:

The sample is placed over Ice soon after sample collection which prevents the degradation of the proteins present in the saliva. There are different equipment and devices available for processing and storage of saliva Laboratory Vortex mixer, Refrigerated centrifuged, cryotubes can be used for withstanding a temperature of about -80 degree C(Anthonappa, King, and Rabie 2013) .

Saliva samples are centrifuged for removing the cellular debris which is present in the supernatant layer and is stored for about an hour in ambient room temperature. Quantitative diagnosis is by Assessing the proteins and DNA in the saliva in PCR and electrophoresis methods (Speicher et al. 2015). The saliva samples can be stored at - 20 degrees C for the prevention of bacterial growth. Saliva BioSwab is a commercially available method where the saliva is stored in a cryovial at - 80 degree C for a period up to 6 months in the freezer (Chiappin et al. 2007).

Analytics From Saliva:

The biomarkers, proteins and various other diagnostic tools are estimated by various methods like Shotgun proteomics, Surface immobilised optical protein sensor, Matrix-Assisted Laser Desorption - Mass Spectroscopy (MALDI - MS), Reverse Transcriptase Quantitative-Polymerase Chain Reaction (RT- qPCR), Invitro translation, Construction of Salivary cDNA library etc. The biomarkers present in the saliva are elevated in various conditions of systemic diseases and Orodental diseases (Kaczor-Urbanowicz 2019). The different biomarkers in saliva are illustrated in figure 3 (Figure - 3).

BiomarkersIn Cardiovascular Disease:

C- reactive protein (CRP) Is non-specific biomarker which is increased in various conditions like periodontal disease, Myocardial infarction etc. in myocardial infarction the biomarkers like CRP, myeloperoxidase, myoglobin are increased when compared to healthy individuals.

Increase in lysozyme Level in the saliva is indicative of orodental diseases and hyperglycemia which is associated with hypertension and early stages of cardiovascular diseases (Floriano et al. 2009).

Salivary Biomarkers In Renal Disease:

The biomarkers associated with renal diseases include cortisol, pH, amylase, uric acid, Sodium, Chloride and lactoferrin which are present in saliva. Salivary phosphate level is a biomarker for the diagnosis of Hyperphosphatemia(Savica et al. 2007, Kumar, Ashok Kumar, and Brundha 2016). The salivary phosphate acts as a more sensitive biomarker when compared to serum phosphate in the diagnosis of Hyperphosphatemia and chronic renal disease. The level of creatinine in the serum and the Glomerular Filtration when correlated with the level of phosphate in saliva a positive association was seen, new therapeutic strategies like binding excessive phosphate in the saliva to the receptors in hyperphosphatemia conditions help in reducing the levels of phosphate (Nagler 2008, Nandakumar, Nandakumar, and Gheena 2016).

Salivary Biomarkers In Systemic Diseases:

Cystic Fibrosis is an autosomal recessive inherited disease, it affects mainly the lungs but it also affects the pancreas, kidney, liver and also intestine (Shenoy and Brundha 2016). There is an elevation of Prostaglandin E2, Elevation of Lipids, Electrolytes, Total protein from the SubMandibular salivary gland. Cystic fibrosis In children there is also the elevation of Calcium and Phosphate levels (Streckfus et al. 2000, Prashaanthi and Brundha 2018) . In Breast cancer, there is a mutation in p53 and elevation in CA 15 - 3 and oncogene C-erB2 (Balaji, Brundha, and Path 2016, Kalaiselvi and Brundha 2016). In the ovarian tumour, there is a decrease in DMBT 1 biomarker and an increase in a C125 tumour marker in the saliva (Chen, Schwartz, and Li 1990).

Diabetes is the most common metabolic disorder around the world, is of two types as Type 1 and Type 2 of which is type 2 is more common (Preethikaa and Brundha 2018). In a study by Rao et al., compared the saliva of Type 2 diabetic individuals and Healthy subjects, concluded that around 65 proteins were increased by two times in diabetic individuals (Rao et al. 2009). However further studies can be conducted to identify the unique salivary biomarker in Diabetes. But in Type 1 diabetes when the exhaled Methyl Nitrate has estimated it gives an approximation of the blood glucose level, which may be due to increased oxidation reactions because of the interaction between superoxide dismutase and nitric oxide. Strauss et al.,proposed that the Gingival crevicular blood can be used for estimating the blood glucose level and compared with blood glucose level, demonstrated a positive correlation between the two methods (Novak et al. 2007).

Salivary Biomarkers In Oral And Squamous Cell Carcinoma (OSCC):

OSCC is a common malignant tumour of the oral cavity and it is also ranked in the top 10 cancers around the globe (Padmaharish 2016). The biomarkers in OSCC include oncogenes (C-myc, C-Fos, C-Jun), Cytokines (TGF - Beta, IL - 8, IL - 1 Beta), Extracellular Matrix Degrading Protease (MMP - 1, MMP - 2, MMP - 9), Hypoxia Markers (HIF - Alpha, CA - 9), Epithelial Tumour Factors (CYFRA 21 - 1), Cytokeratin (CK 13, 14, 16), Micro RNA Molecules, Hypomethylation of cancer-related genes (p16, DAP - K) (Mp, Brundha, and Nallaswamy 2019, Nandakumar and Savitha 2015). In a study conducted by Hu S et al., it was reported that there is a decrease in miR - 125 a - miR - 200 a biomarker. These

biomarkers have to be defined more clearly in further studies which are conducted (Hu et al. 2008, Brundha, Pathmashri, and Sundari 2019).

Salivary Biomarkers In Dental Diseases:

Oral Lichen Planus (OLP) is a chronic inflammatory disease of the oral mucosa. By the Proteomic approach of Salivaomics, three biomarkers are identified by two-dimensional Gel Electrophoresis followed by Mass Spectroscopy (Shreya and Brundha 2017). The biomarkers include Cystatin SA, Chain C of the complement system and Chain B found in D - fragment of fibrinogen. Here, Cystatin SA is decreased whereas Chain C of the complement system and Chain B found in D -a fragment of fibrinogen are increased. Many studies also suggest that cortisol level in saliva also acts as a biomarker (Rudney, Staikov, and Johnson 2009). Candida albicans is a normal microflora of the human oral cavity when there is an imbalance or disturbance like poor oral hygiene, usage of antibiotics for a longer period of time, usage of prostheses, nutritional deficiency etc will lead to alterations in the oral microbiome ratio and it leads to Candidiasis. The detection of Candida's Mannan antigen for the saliva using ELISA kit helps in the detection of candidiasis. However, saliva is used for detecting the disease and is not employed directly but clinical reports suggest the usage of saliva for the detection of the disease (Kurita et al. 2009, Hannah et al. 2019).

Human Papilloma Virus (HPV) is associated with Oral Warts and Oral Cancer. HPV antibodies are detected in the saliva by PCR amplification when compared with the levels in the serum was the same (Harsha and Brundha 2017). And it is proved to yield positive results in the diagnosis however further to prove the efficacy as an alternative method for diagnosis. Salivary based diagnosis yielded positive consistent results to other viruses like Epstein–Barr Virus, Herpes Simplex Virus 1 and also Human Herpes Virus -8, where the viral load in blood and saliva were proved to be the same (Cameron et al. 2003, Sareen et al. 2018).

Salivary Macrophage Inflammatory Protein - 1α, MMP - 8, IL - 1β, IL -6, Prostaglandin E2 (PG E2), TNF - α are increased in Periodontitis and Gingivitis. When Gingival Crevicular fluid is used as a specimen and tested by ELISA confirmed the presence of specific biomarkers of the host like RANKL and Cathepsin, both the biomarkers are present during resorption by the osteoclasts (Agarwal and Lakshmi 2014, Thirumalaisamy and Gajendran 2018). The best biomarkers in dental caries are Statherin and Cystatin especially increased during Occlusal caries (Denny 2009).

Advantages OfSalivaomics:

Saliva based diagnosis has potential advantages when compared to other diagnostic methods and tools which includes. It has potential diagnostic value, Safer and easier for the health care professionals compared to blood sampling which sometimes causes cross-infection (Ravichandran and Brundha 2016), Multiple samples can also be obtained easily, Noninvasive procedure, Inexpensive, More economical in sample collection (Devi 2014), transport and storage, Minimal risk of cross-infection, Manipulation is less during diagnostics, Saliva does not clot unlike blood (Koneru and Tanikonda 2014).

Disadvantages OfSalivaomics:

Despite this number of advantages of salivaomics, there are certain disadvantages which makessalivaomics less reliable and preference as a diagnostic tool. These disadvantages include Certain biomarkers in saliva which are not reliable, Proteins present in the saliva are also affected by the rate of flow of saliva, Changes in the flow of saliva can be seen between

individuals and also in different conditions, certain serum biomarkers cannot reach its way to whole saliva, Composition and flow of the saliva are influenced by the method of saliva collection and also stimulation of saliva flow, the proteolytic enzymes present saliva are derived from the host as well the microbes in the host which also affects the stability of certain biomarkers (Madalli 2013, Malathi et al. 2016).

Saliva Ontology (SALO):

SALO is a cross-disciplinary interaction creation, which helps in growing semantically enhanced web-enabled will be created within Salivaomics Knowledge Base (SKB). SALO is a web-based public domain source which helps in gaining better knowledge. Each term provided in ontology has its definition, Source from PubMed, reference annotations in SKB and also databases. SALO associated software tools include Open Biomedical Ontologies (OPB), Foundary, OBO Ontologies includes GO, PRO, CHEBI (Ai, Smith, and David 2010, Humphreys 2019).

Future Perspective:

Despite all disadvantages, Salivaomics will be a future diagnostic tool for its advantages. The disadvantages in Saliva based diagnosis can be rectified in future studies and further biomarkers in various other diseases can also be identified. It can also be used for the early diagnosis of diseases.

2. CONCLUSION:

Saliva is one of the better diagnostic tool options, for its advantageous options like easy to collect, store, contains different biomarkers etc, And the best alternative for blood. The utility of saliva-based diagnosis is successfully demonstrated in various studies for the diagnosis of various systemic and non-systemic diseases. Despite the propitious approach in the tool of Salivaomics is to be explored further and identify and isolate the different biomarkers in various diseases, help in early diagnosis and effective treatment planning. However, as with every new technology or invention, there will be flaws in the processing which has to be considered before they are widely used in clinical settings. Further Salivaomics is a promising future diagnostic tool.

AUTHOR CONTRIBUTION:
V.T.ThamaraiSelvi contributed to data acquisition and drafting of manuscripts.
Dr.M.P.Brundha contributed to the design, editing, critical revision and proofreading of the manuscript.

CONFLICT OF INTEREST:
The authors declare no Conflict of interest.

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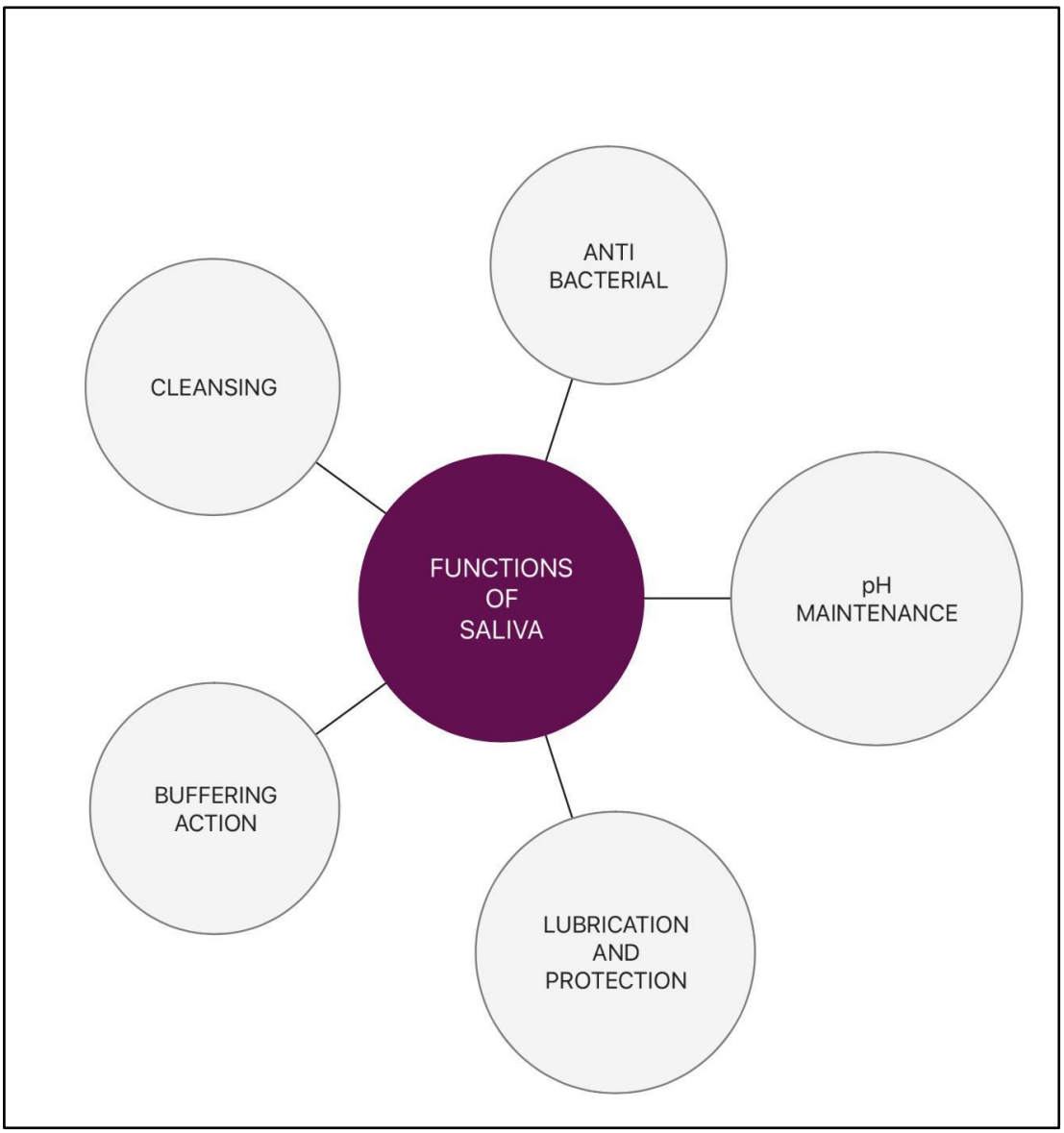

Figure -1 Elucidating the Different Functions Of Saliva

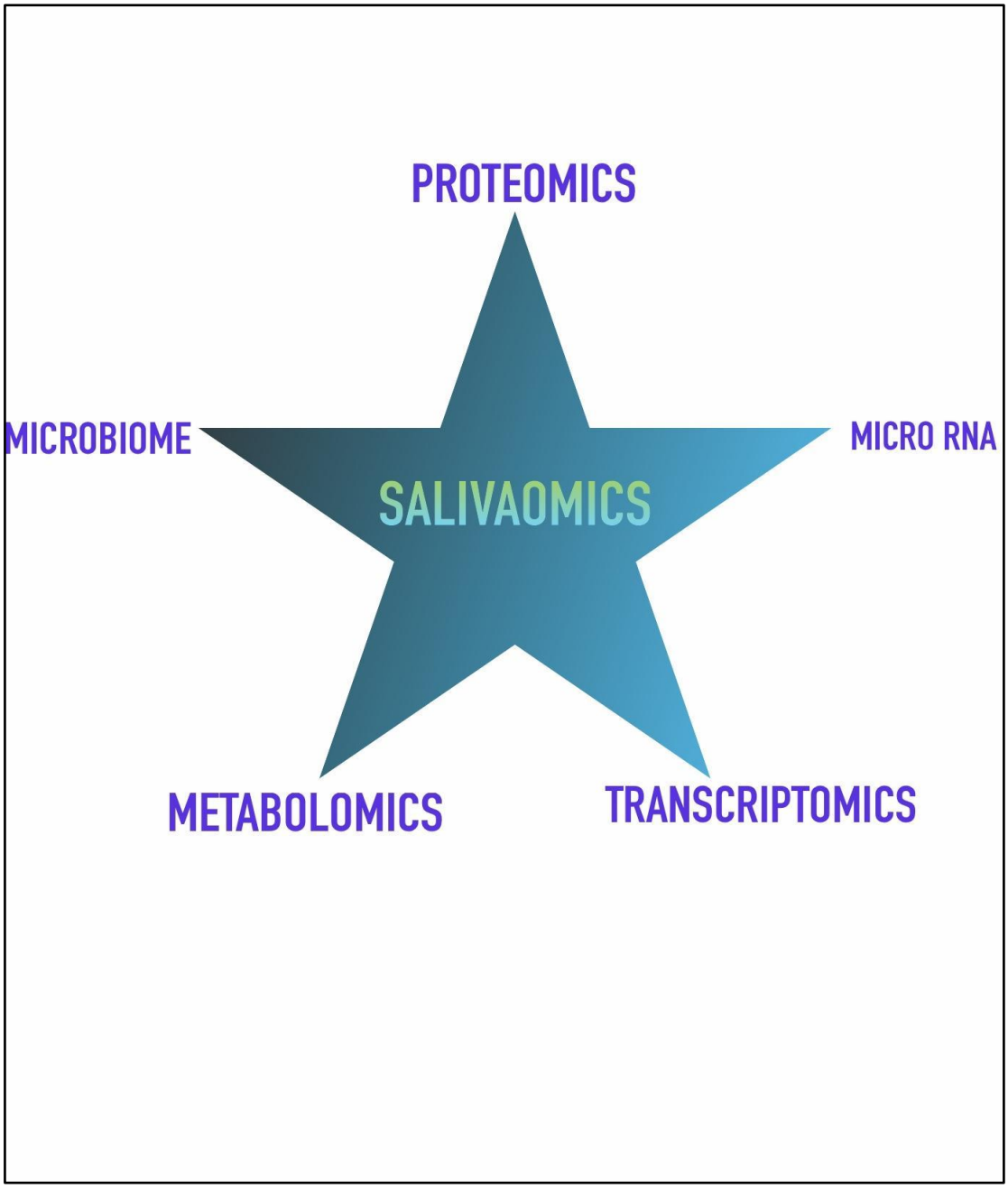

Figure- 2 Representing The Different Branches Of Salivaomics

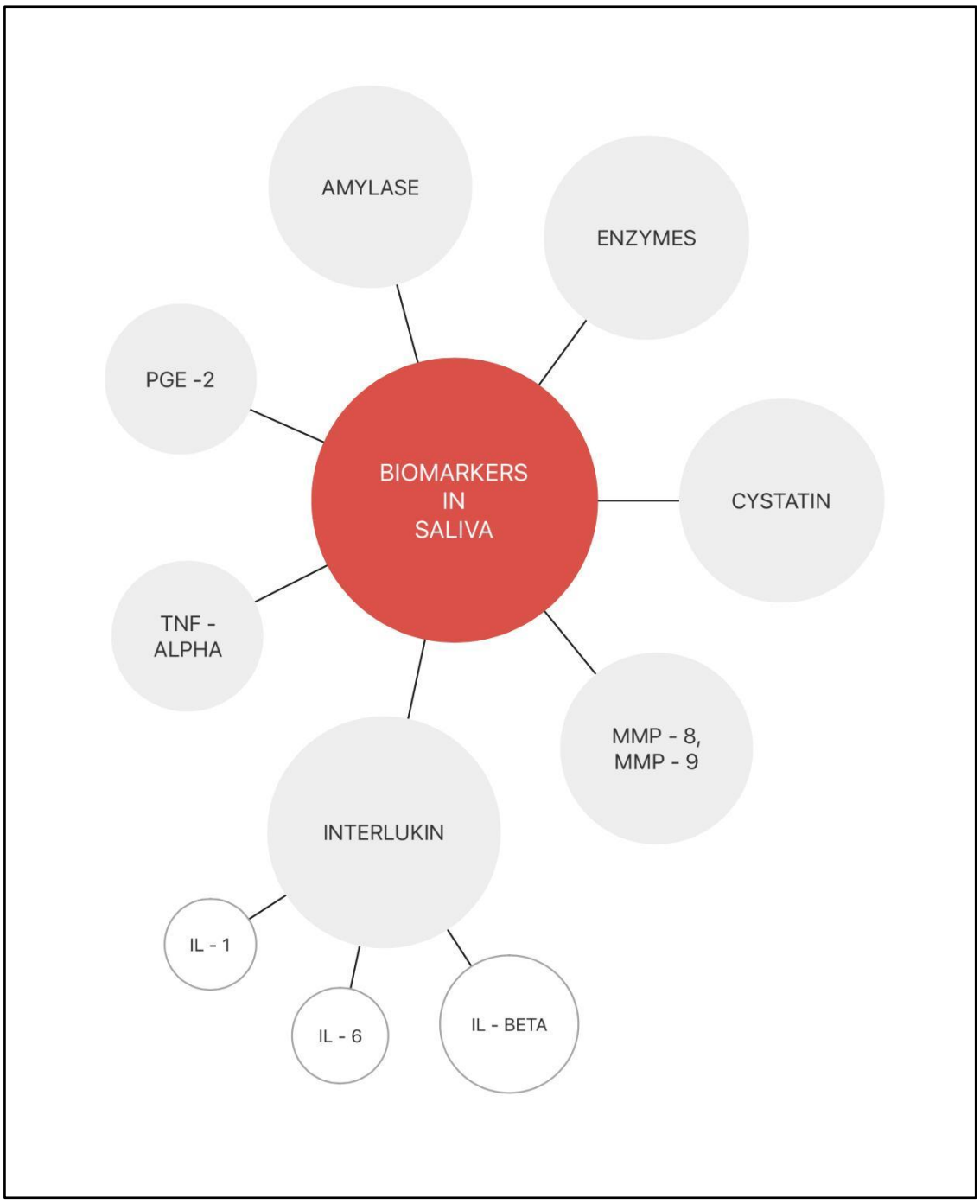

Figure - 3 Portraying the Various Biomarkers Present In The Saliva

S. No	AUTHOR	YEAR	LEVEL	QUALITY OF RESEARCH
1.	*Agarwal R et al.,*	2014	Level 3	Weak
2.	*Ai J et al.,*	2010	Level 2	Moderate

3.	*Anthonappa RJ et al.,*	2013	Level 1	Weak
4.	*Balaji S et al.,*	2016	Level 2	Moderate
5.	*Belibasakis GN et al.,*	2019	Level 1	Moderate
6.	*Bellagambi FG et al.,*	2020	Level 3	Weak
7.	*Brundha MP et al.,*	2015	Level 2	Strong
8.	*Brundha MP et al.,*	2016	Level 3	Weak
9.	*Brundha MP et al.,*	2019	Level 1	Weak
10.	*Cameron JE et al.,*	2003	Level 3	Moderate
11.	*Chen DX et al.,*	1990	Level 3	Weak
12.	*Chiappin S et al.,*	2007	Level 2	Strong
13.	*Contreas Aguilar et al.,*	2020	Level 1	Moderate
14.	*Denny PC et al.,*	2009	Level 1	Moderate
15.	*Devi TJ et al.,*	2014	Level 2	Weak
16.	*Donzella B et al.,*	2008	Level 3	Weak
17.	*Durdiakova et al.,*	2012	Level 3	Weak
18.	*Farnaud et al.,*	2010	Level 2	Moderate
19.	*Floriano et al.,*	2009	Level 1	Moderate
20.	*Grant MM et al.,*	2012	Level 3	Weak

21.	*Hannah R et al.,*	2019	Level 2	Strong
22.	*Harsha L et al.,*	2017	Level 3	Weak
23.	*Helmerhorst EJ et al.,*	2007	Level 1	Weak
24.	*Humphreys P et al.,*	2019	Level 3	Moderate
25.	*Hu S et al.,*	2008	Level 1	Moderate
26.	*Kaczor - Urbanowicz et al.,*	2019	Level 2	Strong
27.	*Kalaiselvi R et al.,*	2016	Level 3	Weak
28.	*Koneru S et al.,*	2014	Level 2	Moderate
29.	*Kumar MDA et al.,*	2016	Level 1	Weak
30.	*Kurita H et al.,*	2009	Level 2	Moderate
31.	*Lenander-Lumikari, M.*	2008	Level 1	Moderate
32.	*Madalli VB et al.,*	2013	Level 3	Weak
33.	*Malathi M et al.,*	2016	Level 2	Strong
34.	*Miller CS et al.,*	2010	Level 3	Weak
35.	*Mittal S et al.,*	2011	Level 1	Weak
36.	*Mitulovic et al.,*	2019	Level 3	Moderate
37.	*Brundha MP et al.,*	2019	Level 1	Moderate
38.	*Nagler RM et al.,*	2008	Level 2	Moderate

39.	*Nandhakumar E et al.,*	2015	Level 3	Moderate
40.	*Nandhakumar et al.,*	2016	Level 1	Strong
41.	*Novak BJ et al.,*	2007	Level 2	Weak
42.	*Padmaharish V et al.,*	2016	Level 2	Moderate
43.	*Prashanthi N et al.,*	2018	Level 3	Weak
44.	*Preethika S et al.,*	2018	Level 1	Moderate
45.	*Rao PV et al.,*	2009	Level 2	Strong
46.	*Raskin et al.,*	2015	Level 2	Strong
47.	*Ravchandran H et al.,*	2016	Level 3	Strong
48.	*Rudney JD et al.,*	2009	Level 2	Strong
49.	*Sareen A et al.,*	2018	Level 3	Weak
50.	*Savica V et al.,*	2007	Level 2	Strong
51.	*Shah S et al.,*	2018	Level 1	Moderate
52.	*Shenoy PB et al.,*	2016	Level 1	Moderate
53.	*Shreya S et al.,*	2017	Level 2	Weak
54.	*Sindhu S*	2014	Level 3	Weak
55.	*Speicher DJ et al.,*	2015	Level 2	Strong
56.	*Streckfus C et al.,*	2000	Level 2	Strong

57.	*Streckfus et al.,*	2002	Level 1	Weak
58.	*Thankappan S et al.,*	2017	Level 1	Moderate
59.	*Thirumalaisamy V et al.,*	2018	Level 1	Moderate
60.	*Timothy CN et al.,*	2019	Level 2	Strong
61.	*Topkas E et al.,*	2012	Level 3	Weak
62.	*V DS et al.,*	2016	Level 2	Moderate
63.	*Wiwanitkit et al.,*	2013	Level 1	Weak
64.	*Wong DT et al.,*	2006	Level 2	Moderate
65.	*Wong DTW et al.,*	2012	Level 2	Weak

Table 1 represents the Quality Analysis performed using Health Evidence - Quality assessment tool for gathering the Articles from Previous Literature