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SALIVARY DIAGNOSTICS AND ITS IMPLEMENTATION IN DIAGNOSIS OF COVID19

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ABSTRACT

The conventional treatment for vertical maxillary excess due to Skeletal class II base, is surgical reduction and rigid fixation which has decreased patient acceptance. However, intrusion of some selected MVE cases can be achieved with minimal intervention using mini plate anchorage, thus expediting and simplifying their management. A 15 year old female patient came with a complaint of forwardly placed teeth in the upper front tooth region of the jaw. Intraoral examination showed class II molar occlusion on both sides with increased overjet and overbite. The cephalometric analysis showed class II skeletal relationship with vertical maxillary excess. The treatment plan included Fixed appliance with extraction of 14,24,35, then miniplates and mini-implant assisted anterior maxillary retraction and intrusion. At the end of the treatment anterior maxillary retraction and intrusion was achieved. Thus the hypothesis was accepted as retraction and intrusion using mini plates is an easy, efficient, safe and cost- effective option in the management of VME. It is less invasive with acceptable clinical and radiographic outcome, while avoiding surgery under GA with its risk and complication. Careful case selection is needed for achieving satisfactory result and smile.

KEYWORDS: Maxillary vertical excess (MVE), Maxillary retraction, Skeletal Anchorage, Miniplate anchorage, Minimally invasive intrusion.

INTRODUCTION

The epidemic of coronavirus disease 2019 (COVID-19), instigated by a novel coronavirus (SARS-CoV-2), has become a major public health challenge around the world. In many cases, the rapid spread of infection transmission through person- to-person, either direct contact by sneeze, cough, or droplet inhalation, or contact transmission such as ocular contact or through mucous membranes of the eyes and nose and saliva, and the spread through droplets and aerosol particles [1].

Diagnostic testing for COVID-19 is essential to control the global pandemic. Inspite of increase in diagnostic testing capacity for SARS-CoV-2, in many countries, testing is still inadequate to slow the COVID-19 pandemic. Many people still do not have access to COVID-19 tests, and some that do still experience long delays in receiving results due to imbalance between supply and demand at large testing centres. The use of saliva has various advantages compared to collection of Nasopharyngeal swab.
The close contacts involved in swab collection have a risk to healthcare workers, and collection of saliva may reduce this risk. Presently, rapid testing is taking place with the help of nasopharyngeal, oropharyngeal swab, bronchoalveolar lavage, sputum, urine, and blood [2]. All these approaches are invasive or uncomfortable to the infected person. It is observed that salivary glands are hosting SARS-COV-2 because of angiotensin-converting enzyme and the detection of high viral loads in the saliva and is playing a major role in virus spread, especially from individuals showing absolutely no symptoms. Further, saliva collection does not require specialised equipment, causes less patient discomfort, and may be a useful sample for self-collection. Saliva is proving to be a promising non-invasive sample specimen for

the diagnosis of COVID-19, thus helping to detect the infection and prevent it from further spreading by prompt isolation.

SALIVARY FLUID AS POTENTIAL DIAGNOSTIC TOOL FOR SARS-COV-2

Saliva is a distinctive body fluid secreted by the salivary glands. It has the purposes of lubricating oral mucosa, digesting food, cleaning and protecting the oral cavity, and is one of the most significant factors affecting homeostasis of the oral cavity. The major salivary glands parotid, submandibular and sublingual glands are the major sources of saliva secretion.[3]

The development of the COVID-19 pandemic has emphasized the need for multiple diagnostic strategies to professionally evaluate potential cases in order to deliver information on people exposure and immunity. These outfits presently include virus molecular testing and rapid host immune response assays. Saliva is a biological fluid in which SARS-CoV-2 can be found and for this purpose saliva has been taken into attention in the diagnosis of COVID-19 [4]. The investigative prospective of saliva was recognised by studies that shown that, like serum, saliva contains hormones, antibodies, growth factors, enzymes, microbes and their yields that can go into saliva through blood via passive diffusion, active transport or extracellular ultra- filtration. Therefore, saliva can be a consistent fluid for observing the physiological function of the body [2]. Whereas the low concentration of certain analytes in saliva linked with the blood previously proved challenging, the creation of highly sensitive molecular methods and nanotechnology have to a large opportunity avoided this limitation.

SALIVA AS A POSSIBLE SOURCE FOR SPREAD OF VIRUS

Human saliva is abundant of biologically active components, such as proline-rich proteins, mucins MG1 and MG2, and gp340 [6]. These components intermingle with pathogens and cause multiple influences on their biological performance. The interface between viruses and saliva is a multifaceted biological process. Coronavirus is a group of enclosed single-stranded RNA viruses belonging to the order Nido virales, the coronavirus family, and the coronavirus subfamily [5]. It has 26 known species and can be distributed into four genera (α, β, γ, and δ). Merely the α and β genus are human pathogenic strains. SARS-CoV, SARS-CoV-2 and the Middle East respiratory syndrome coronavirus (MERS-CoV) all belong to the β subdivision. Studies have revealed that early target cells for SARS-CoV infection comprise ACE2-positive cells/keratin epithelial cells in the salivary gland duct and other cells in the lungs, such as ACE2-positive cells/keratin alveolar epithelial cells, which recommended the salivary gland epithelial cells may be infected in vivo after entry of the virus (Liu et al., 2011).[6] Hence, the saliva produced by the infected salivary glands could be a significant source of virus, predominantly in early infection (Liu et al., 2011) [6]. At present, RT-PCR detection results of throat wash and saliva indicated that the content of SARS-CoV RNA in saliva was comparatively higher than that in throat wash, which maintained the possibility of oral droplet transmission of SARS-CoV (Wang et al., 2004).[7] The quantity, distance, and size of saliva droplets vary between individuals, signifying that the infectious intensity and transmission route of saliva droplets differ when the same pathogen is reduced. Each cough can produce about 3000 saliva droplets nuclei, which is almost equivalent to the quantity generated during a 5-min conversation. Each sneeze can create roughly 40,000 droplets of saliva covering several meters in the air. A regular exhalation can create saliva droplets that go beyond one meter in the air. Enormous saliva droplets with increased naturally fall to the ground and small saliva droplets flutter by airflow like a cloud over longer distances. Henceforth, the virus has the likely to initiate disease through both short-distance and long-distance aerosol spread. There is increased risk of infection in people who have direct and unprotected contact with SARS patients (Tuan et al., 2007).[8] Therefore, dental clinicians in near contact with patients, salivary aerosols and plasma need to be highly endangered to condense the risk of infection, mostly during the epidemic period of COVID-19.

ROLE OF SALIVA IN MOLECULAR DETECTION

The zoonotic nature and clinical features associated with this virus are quite typical including fever, non-productive cough mostly, malaise, dyspnoea and pneumonia. While sputum production, haemoptysis, headache and gastrointestinal symptoms such as diarrhoea, nausea and vomiting are less frequently

presented symptoms.
[9] Patients infected with COVID-19 show increased number of leukocytes, greater levels of plasma pro-inflammatory cytokines and abnormal respiratory findings. Swabs from the nasopharynx and oropharynx are the recommended upper respiratory tract specimen types for making diagnosis of COVID-19 the collection of swab requires close contact between healthcare workers and patients that not only increase the risk of transmission of the virus among healthcare workers but also causes discomfort resulting in bleeding, especially in condition like thrombocytopenia[9]. To overcome this problem, saliva has been found as an alternate source for making the diagnosis. It can be used for detecting respiratory viruses, including Coronaviruses because of high consistency > 90%with nasopharyngeal specimens.

SALIVARY FLUID AS BIOMARKERS FOR COVID-19 DIAGNOSIS AND DETECTION

Coronaviruses, such as SARS-CoV and Middle East respiratory syndrome (MERS)-CoV, have developed approaches to reduce or delay the production of interferon (IFN), triggering exuberant inflammatory responses leading to severe pulmonary conditions[10]. Salivary biomarkers and their role in point-of-care application have underlined the progress of the practice of more advanced technologies such as micro/ Nano electro-mechanical systems, paper-based skill, fluorescent biosensors, photometricandelectrochemical approaches, RNA-sequencing ,liquid biopsy, electric field-induced release and measurement technique.
[11] Markers of the inflammatory progression, such as cytokines and chemokine, can be measured in saliva. Such statistics has been recommended to be useful for the identification and prognosis of both oral cavity and systemic diseases. Hence, it is possible to create an inflammatory outline of COVID-19 by studying inflammation-related biomarkers in saliva. Interestingly, some of the known biomarkers in these studies such as C reactive protein, malic acid, guanosine monophosphate, lactate dehydrogenase, and proteins related with macrophage, platelet degranulation and supplement system pathways are shown to be present in saliva.[12] These results support the possible use of saliva-based metabolic/protein/lipid biomarkers as a non-invasive approach for patient stratification in COVID-19 disease. Metabolomics is a method used in the study of small molecules from the metabolic profile of cells, tissues or fluids, which help in the classification of a phenotype. These molecules termed biomarkers, are essential in clinical practice for defining the state of a disease[13] Thus, metabolomics has helped to recognise biomarkers with investigative potential and explanation of metabolic pathways in the most diverse clinical situations, including those containing viral and bacterial pathogens, and more exactly viruses that cause respiratory diseases such as influenza and SARS.

Patients recovered from severe acute respiratory syndrome caused by SARS-CoV were recruited after 12 years of infection for metabolic evaluation of the consequences of the disease [14].
The comparison of patients' serum with healthy individuals showed differences in organic acids, amino acids, phospholipids, carnitine and inositol derivatives. These results represent the practical application of metabolomics in the estimation of long-term effects. MicroRNAs, non-coding RNAs of 20-nucleotide to 22-nucleotide length, silencing gene appearance by a transcript-specific target-mediate inhibitory action, play a key role in numerous cellular processes including cell development and distinction immunity, cell metabolism, proliferation, apoptosis and cancer [15]. The importance of monitoring microRNA is associated to the fact that a single microRNA can be occupied in several cellular regulatory pathways, which involve dissimilar molecules. There are studies reporting a specific microRNA upregulation and down regulation of nuclear factor- κB pathway and IFN pathway associated with some viruses including respiratory virus infection [16]. Moreover, in this context, since microRNAs related with extracellular vesicles are recognized to be protected from enzymatic degradation, several studies have been focused on the investigation of the expression of microRNAs in extracellular vesicles gained from saliva as potential biomarkers. Therefore, the fact that microRNA existing in biological fluid can replicate the molecular event within the cellular background, make them a potential exhaustive marker to check the cell- infection status; this is particularly important in a low replicative condition in which virus cannot be present in biological fluid[17] provides an opportunity to evaluate virus pathological effect-associated diseases as in COVID-19.

SALIVARY ANTIBIODIES AGAINST COVID-19

Viral antibodies have been spotted in saliva and the immunisation status of measles, rubella, mumps and hepatitis can be tested by analysing IgG, IgM and IgA in oral fluids[18]. In addition to RT-PCR- based RNA detection of SARS-CoV-2, finding of IgM and IgG against SARS-CoV-2 in serum/plasma samples of patients with SARS-CoV-2 have been reported [19]. Regarding SARS-CoV-2, only a study procedure aimed to analyse IgG, IgM and IgA in changed biological fluids including self-collected saliva for rapid SARS-CoV-2 diagnosis has been published [20]. Viral antibodies have been noticed in saliva and the immunisation status of measles, rubella, mumps and hepatitis can be confirmed by analysing IgG, IgM and IgA in oral fluids [18]. Though, there are so far no outcomes describing the presence of antibodies against SARS-CoV-2 in human saliva. This clearly permits future studies on the potential use of salivary immunoglobulins for COVID-19 in diagnostics, disease progression and immunisation monitoring.

METHODS OF SALIVARY TESTING FOR SARS-COV-2

Salivaomics is the study of salivary "omics" methodologies including the genome, the epigenome, the transcriptome, the proteome, the microbiome, and the metabolome [21]. The capability to collect a sample in a non-invasive, safe, and cost effective fashion, with the benefits of higher patient comfort and compliance makes the adoption of saliva for each of these "omics" techniques an attractive proposition for all parties concerned (patients, researchers, and clinicians) here are many properties of human saliva that attract clinicians or researchers to adopt the use of saliva specimens and reinforce the use of this non-invasive fluid in diagnostic algorithms [22]. Some of these are highlighted below: Non-invasive, Simple collection protocols, Non-infectious sample, Easily disposal, Easily transportable, Cost effective, Not subject to cultural and religious "taboos", Safe and effective ,Higher patient compliance.

The very first instance of a method for saliva collection from a patient was in the early 19th century (1934) by Wainwright for the analysis of salivary calcium (Ca2+). In Wainright's method, the patient's head was tipped forward with the mouth pointing vertically downwards and saliva was allowed to drip from the mouth into a filter funnel [23].Normal healthy adults produce around 0.5 to 1.5 liters of saliva per day (or approximately 0.5 mL/min) but in various systemic diseases, and in pathological and physiological conditions, there may be a considerable (negative) impact on the salivary flow rate[24]

Type of Whole Mouth Fluid	Method of Collection and Type of Collection Device
Whole Saliva (WS)	Patients should refrain from eating, drinking, and oral hygiene actions for at minimum 1 h before saliva collection. (Optimal collection time is 8–10 a.m.). Before collection perform a 1 min oral rinse with distilled water and then after 5 min collect ~5 mL of saliva. Collected sample must be processed in the laboratory within 1 h [25]
Un-stimulated Whole Saliva (USWS)	Passive drooling: In this method restrict oral movement and drain saliva from the lower lip into a plastic vial. Spitting method: Instruct subject to spit into a collection vial. In this method 14 times more bacterial contamination is introduced into the sample[26]
Stimulated Whole Saliva (SWS)	For the stimulation of glands, chewing different things like natural gum, a piece of paraffin wax, citric acids, and powdered drink crystals have been used[27]
Parotid Gland	Method introduced by Carlson and Crittenden (1910). In this method a double chambered metallic cup with two outlet tubes is used. One end holds the cup in place using vacuum suction. The second half acts as a collection vehicle for saliva. Specimen collection can be enhanced by smearing citric acid (10%; 1 mL) on the dorsum of tongue every 30 s. Discard the first 1.5 mL of saliva prior to sample collection[28]
Submandibular/Sublingual Gland	Truelove, Bixler, and Merrit (1967) used a "V"-shaped collector. This method is similar to that for parotid gland collection, but in this case the initial 2 mL is discarded[29]
Minor Glands	Kutscher *et al.* (1967) used capillary tubes for collecting saliva from minor glands located at the everted surface of the lower lips[30]

Two companies from the United States, namely Epitope, Inc. (Oregon, now OraSure Technologies, Bethlehem, PA, USA) and Saliva Diagnostic Systems, Inc. (Vancouver, WA, USA), were two of the early pioneers in the area; each developed commercially viable saliva collection devices in the 1990s, and each of these has been used in proteomic analysis and other areas of research and clinical practice[31]. The OraSure Device from Epitope/OraSure was the first saliva collection device to be linked to a clinical test for the human immunodeficiency virus (HIV) and the company was successful in gaining Food and Drug Administration (FDA) approval for the device in conjunction with a laboratory enzyme-
linked immunosorbent assay (ELISA) test for the HIV virus.[10] These early devices spurred new developments in saliva collection device technology resulting in devices that produce "cleaner" specimens, thereby allowing clinicians to analyze saliva more easily than before. Other devices that have been used historically include the Salivette device from Sarstedt, and the Salimetrics Oral Swab (SOS). Newer devices on the market "mimic" whole saliva collection using passive drool. These devices, which includes the (A) Salivette® (Sarstedt); (B) Quantisal® (Immunalysis); (C) SCS® (Greiner- BioOne), Super•SAL™ (Figure D) and Versi•SAL® (Figure E) technologies. These devices (Figure D,E)
have been used successfully to collect hormones [31], proteins, and biomarkers potentially useful in the diagnosis of Parkinson's disease [31] as well as infectious agents including the Ebola virus and Lassa fever. The critical thing is that the latest generation of devices provide a standardized sample of saliva, representative of whole mouth saliva.

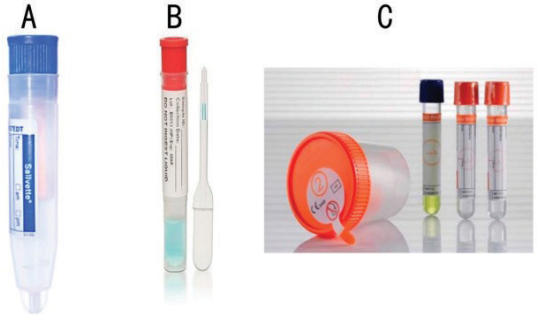

Figure A: (A) Salivette® (Sarstedt); (B) Quantisal® (Immunalysis); (C) SCS® (Greiner-BioOne) [9,10]

Versi SAL®, (E) Corporation Super SAL by Oasis Diagnostics®

PCR DETECTION IN SARS-COV-2 WITH SALIVARY FLUID TESTING FOR SARS-COV2

At Present, RT-PCR is the most universally used diagnostic test for the exposure of SARS-CoV-2 RNA in the biological samples. For comprehensive testing as in the case of SARS-CoV-2, accurate collection of the type and the site of biological specimen collection is crucial for obtaining unfailing test results[32]. Biological samples from the upper tract such as nasopharyngeal swabs, oropharyngeal swabs, throat swabs, nasal swabs and lower tract such as broncho alveolar lavage respiratory tract scan and tracheal aspirates can be used for the detection of SARS-CoV-2 with varying degree of test sensitivity[33] Presently, nasopharyngeal/oropharyngeal swabs where virus samples are collected by separately rubbing the nasopharyngeal wall and the posterior pharynx/tonsillar areas through mini tip swabs, are regularly used for SARS-CoV-2 detection. Despite the extensive use, the collection of nasopharyngeal/ oropharyngeal swabs has a number of restrictions. The collection of these swabs is less satisfactory to patients as compared with non-invasive approaches like saliva collection, as it tends to cause patient discomfort and even bleeding. Likewise, the risk for disease transmission to the healthcare workers when collecting these samples is high, as it needs active participation of the test taker. Also collection of these samples stresses the use of personal protective and healthcare resources, both of which tend to be in little source in a pandemic like COVID-19 [34].

ADVANTAGES AND DISADVANTAGES OF SALIVARY SAMPLING

Saliva has been studied comprehensively as a possible diagnostic tool and it is likely to become a auxiliary for other biological fluids such as serum or urine in disease analysis[18]. Compared with other investigative fluids, saliva sampling has both advantages and disadvantage in use for thediagnosis of COVID-19.(table 1)

Advantages	Disadvantages
Safer collection for health professionals than other biological trials such as nasopharyngeal swabs and blood. Non-invasive method for diagnosis of the disease	Not at all times reliable for measurement of certain markers
No patient discomfort and anxiety for sampling.	Substances of saliva can be predisposed by the method of collection, degree of stimulation of salivary flow, inter individual dissimilarity and oral hygiene status.
Easy collection and applicable in isolated areas.	Serum markers can reach whole saliva in an unpredictable way.
Comparatively cheap technology. Economical	Medications may upset salivary gland function and subsequently the quantity and composition of saliva.
Suitable for children, anxious, disabled and elderly patients.	Possibility for degradation of salivary proteins due to occurrence of proteolytic enzymes.
Potential multisampling.	
Easy to handle, No need for expensive equipment or instruments Simply needs a sterile container	

Table 1: table represents the most common advantages and disadvantages of saliva sampling method in diagnosis of covid19.

FUTURE OF SALIVARY SAMPLING IN SARS- COV-2

Saliva collection is quite comfortable for patients as well as being easy, cheap, and non-invasive with minimal equipment required. It should also minimize the nosocomial transmission of COVID-19 to healthcare workers. The use of saliva-based SARS-CoV-2 testing offers several clinical advantages and is scientifically well founded. Saliva-based testing can be an alternative to the more widely used nasopharyngeal/oropharyngeal swabs for COVID-19 diagnosis and disease monitoring. The beneficial role of saliva as a quick, non-invasive diagnostic modality and the various possibilities it presents with, for investigation, during the course of the disease process, prognosis or presence of any antibodies to the novel COVID-19 virus, needs further exploration. Additionally, the involvement of any other receptors or cellular proteases which may throw more light on the pandemic disease pathogenesis may pave way to targeted drug therapies.

CONCLUSION

The search for salivary biomarkers associated with the development and progression of COVID-19 could allow a better distinction between asymptomatic, mild, moderate or advanced disease. Saliva biomarkers have a prospective to be an essential guide in COVID-19 prognosis, making possible the development of sampling procedures. Knowledge of this kind might lead to the development of point-of-care devices, which can be extremely useful for understanding of the evolution of contagions and immunological responses in population studies. Right now, in this uncontrollable pandemic situation, all research centers , health agencies, and health care providers must explore the diagnostics opportunity and rapidly develop automated molecular point-of-care assays.

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