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Saliva: Its Role in Covid-19 Diagnosis and Preventive Measures

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Review Article

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ABSTRACT

The oral cavity is a moist fluid environment due to the presence of saliva. Saliva has a vast variety of roles in the oral cavity, it also contains a variety of biomarkers, micro-organisms, and enzymes which can be used as diagnostic tools for the detection of diseases in the body. Most of the dental procedures are directly or indirectly related to saliva and aerosol generation. There have been many researches which proves that saliva has a vast role in the virulence and transmission of SARS-CoV-2. Thus, the aim of this review was to evaluate the role of saliva as a diagnostic tool and the measures for the prevention of transmission of SARS-CoV-2.

Keywords: Saliva, SARS-CoV-2, COVID-19, Diagnosis, Prevention.

Introduction

Human saliva is a secretory body fluid that is produced by the salivary glands. Saliva consists mainly of water (94–99%) with organic molecules accounting for nearly 0.5% and inorganic molecules accounting for 0.2%.[1] It plays an important role in digesting food, lubricating oral mucosa, cleaning and preserving the oral cavity, and influencing the homeostasis of the oral cavity. A normal adult usually generates about 600 ml of saliva every day.[2] Saliva offers an ecological niche for the colonization and development of oral microorganisms but it also prevents the overgrowth of specific pathogens in order to preserve the homeostasis of the oral cavity.

It may also acts as a medium for the transmission of various pathogenic microorganisms.[3] Viruses responsible for diseases such as hepatitis viruses, herpes virus infections (e.g., with Herpes simplex types 1 and 2, Epstein-Barr virus, Cytomegalovirus, and Kaposi syndrome herpes virus), and papillomaviruses can be conveyed by salivary transmission—as can potentially other viruses present in saliva such as Ebola and Zika viruses.[4]

An emergent pneumonia outbreak originated in Wuhan City, in late December 2019.[5] The infectious agent of this viral pneumonia was finally identified as a novel coronavirus (2019-nCoV), the seventh member of the family of coronavirus that infect humans.[6] The novel coronavirus belongs to a family of single-stranded RNA viruses known as Coronaviridae.[7] This family of viruses is known to be zoonotic or transmitted from animals to humans. These include Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV), first identified in 2002, and the Middle East respiratory syndrome coronavirus (MERS-CoV), first identified in 2012.[8] On 11th February 2020, WHO named the novel viral pneumonia as "Corona Virus Disease (COVID-19)", while the International Committee on Taxonomy of Viruses (ICTV) suggested naming this novel coronavirus as "SARS-CoV-2" counting on the phylogenetic and taxonomic analysis of this novel coronavirus.[10] This review explores the potential role of saliva in the COVID-19 pandemic, as both a mechanism for the spread of the disease and a readily accessible diagnostic tool for detecting the presence of the virus. Also, it emphasizes the precautionary measures to be taken in the dental practice specifically related to aerosol and direct salivary contamination.

Saliva as a Diagnostic and Informative Fluid

The role of saliva is at par with that of blood. Like blood, saliva also plays a vital role diagnosis of health and disease. Over the past decade, considerable progress has been made in identifying useful biomarkers present in saliva, as well as identifying oral buccal epithelial cells from which DNA can be extracted for human genomic studies and diagnostics. Saliva is also informative for biomarkers which indicate for viral infections such as measles, mumps, rubella, hepatitis A and B. Direct antigen detection is also available for influenza A and B, streptococcus group A (N-acetylglucosamine), salivary estradiol, and several breast cancer biomarkers. In addition to hundreds of specific species of oral bacteria as well as yeast cells, several hormones and narcotics including aldosterone, cortisol, estrogens, insulin, melatonin, progesterone, testosterone, carbamazepine, lithium, methadone, phenytoin, antipyrine, caffeine, cocaine, methamphetamine, marijuana, and various opiates are identified and measured in saliva. And the list continues with the addition of informative messenger RNAs. Further miniature devices are being developed for salivary diagnostics using microcapillary electrophoresis and other nanobiotechnology advances.[10]

Mechanism of Disease Transmission through Salivary Droplets

The size of droplets, along with the speed of airflow, can determine how far and long they can reach. Huge droplets travelling within a short distance or touching infected surfaces spread the majority of transmissible respiratory infections. Huge droplets with a diameter of more than 60 μm tend to settle rapidly on the surface, thereby reducing the risk of transmission to individuals in close proximity to the source of the saliva droplet. Small droplets with a diameter of less than or equal to 60 μm can cause short-range transmission for individuals with a distance of less than one meter. In a suitable environment, small droplets are likely to fade into droplet nuclei with a diameter less than 10 μm, and then become capable of transmission of long-range aerosols.[11] Thus, the science behind maintaining social distance is that the larger particles from cough droplets are transmitted for less than 1 meter and do not remain suspended in the air.[12]

When speaking, coughing, sneezing, or even breathing, saliva droplets are produced and shaped as particles in a combination of moisture and droplet nuclei of microorganisms. The quantity, distance, and size of saliva droplets vary among individuals, indicating that the infectious intensity and transmission route of saliva droplets differ when the same pathogen is contracted. For a susceptible host to develop infectious droplets of saliva, they can enter the mouth, eyes, or be inhaled directly into the lungs. Thus, the SARS-CoV-2 contamination is minimized to a degree by wearing surgical masks and protective eyewear or face shield in vulnerable healthcare workers.[13]

SARS-CoV-2 in Salivary Gland

It was confirmed that SARS-CoV-2 reaches the cell in the same route as SARS coronavirus, i.e. via the cell receptor ACE2 (Angiotensin Converting Enzyme 2).[14] SARS-CoV-2 can effectively use ACE2 as a receptor to invade cells, which can facilitate transmission from human to human.[15] ACE2 + cells have been shown to be abundant in the respiratory tract as well as cells that are morphologically compatible with the epithelium of the salivary gland duct in a human mouth. ACE2+ epithelial cells of salivary gland ducts have shown to be an early target of SARS-CoV infection and the same might be the situation with SARS-CoV-2 infection, although no research has been reported so far.[16] Chen et al. analysed saliva directly from the salivary gland opening of an infected person and found SARS-CoV nucleic acid, indicating SARS-CoV contamination of salivary glands.[17]

Diagnostic Potential of Saliva for SARS-CoV-2

The detection of 2019-nCoV nucleic acid from the throat or nasal swab of patients is considered a confirmatory test.[18] Throat swabs are relatively invasive, induce coughing and occasionally cause bleeding, which may increase the risk of infection among healthcare workers. With the characteristic of being a non-invasive and fewer hazard to healthcare workers, collecting saliva specimen has the benefits of being more acceptable to patients and more secure for healthcare workers for coronavirus diagnosis. To date, three approaches for saliva collection have been identified - coughing out, saliva swabs, and collection of saliva directly from the salivary gland duct.[19] Oral swabs are likely to be useful in early detection. By extracting oral swabs and checking RNA among 15 COVID-19 patients, Zhang et al. found that half of them (50%) were positive for 2019-nCoV RNA in oral swabs, four (26.7%) had positive anal swabs, six (40%) had a positive blood test and three (20%) had positive serum tests.[20]

The presence of SARS-CoV-2 in the saliva of infected patients also bears implications for a high potential of transmission in the dental operatory and underscores the need for awareness and use of effective PPE practices.

Precautions for Aerosol and Salivary Contamination in Dentistry

Aerosols are a suspension of particles in the air, liquid, or solid, within size ranging from 0.001 to 100 µm.[21] Splatter is a mixture of air, water, and/or solid substances (50 µm to several millimetres diameter).[22] Occupational Safety and Health Act (OSHA) has categorized dentistry as a very high exposure risk job with a high potential for exposure to COVID- 19 during specific aerosol-generating procedures.[23] Aerosol transmission is identified in the spread of tuberculosis, measles, chickenpox, SARS-CoV, influenza virus, and adenovirus.[24] It is possible that the 2019-nCoV will spread through airborne transmission if aerosol-generating procedures in dental practice are implemented.[24]

Hence, other than Personal Protective types of equipment (PPE), face masks, and eye protection, certain precautionary measures need to be implicated in the dental practice for the prevention of transmission through aerosol and direct salivary contamination.

Preprocedural Mouth Rinse

Preoperative antimicrobial mouth rinse could minimise the number of microbes in the oral cavity.[25] Previous studies have shown that SARS-CoV and MERS-CoV were highly susceptible to povidone mouth rinse.[26] Since 2019-nCoV is vulnerable to oxidation, it is recommended to use pre-procedural mouth rinse containing oxidative agents such as 1% hydrogen peroxide or 0.2% povidone-iodine to reduce the salivary load of oral microbes, including potential 2019-nCoV carriage. Enveloped viruses such as herpes simplex virus 1 and 2, human immunodeficiency virus, cytomegalovirus, influenza A, parainfluenza and hepatitis are vulnerable to the virucidal effect of chlorhexidine.[27,28,29,30]

It has been recommended that the patient performs a 1-minute mouth rinse with 0.2% to 1% povidone, 0.05% to 0.1% cetylpyridinium chloride, or 1% hydrogen peroxide before the dental procedure.[31]

Rubber Dam Isolation

It has been stated that the use of a rubber dam could minimize airborne particles by 70% in ~3-foot diameter of the operating field.[32] When a rubber dam is applied during the procedure, extra high-volume suction for aerosol and spatter should be used along with regular suction.[33] It is also important to enforce the implementation of a complete four-hand operation. If rubber dam isolation is not feasible, manual devices, such as Carisolv and hand scaler, are recommended for caries removal and periodontal scaling to minimize the generation of aerosol as much as possible.[16]

Use of Disposable (Single-Use) Devices: such as mouth mirrors, syringes, and blood pressure cuff to prevent cross-contamination is recommended.[34]

Radiographs: The most common radiographic technique in dental imaging is intraoral x-ray examination; however, it can stimulate saliva secretion and coughing.[35] Extraoral dental x-rays, such as panoramic radiography and cone-beam CT, are therefore suitable alternatives during the COVID-19 outbreak. Sensors should be double-barriered when intraoral imaging is required to prevent perforation and cross-contamination.[41]

Anti-Retraction Handpiece

Aspiration and expulsion of debris and fluids generated during the dental procedure might occur with the use of a high-speed dental handpiece without anti-retraction valves. A study found that the anti-retraction high-speed dental handpiece would substantially reduce the backflow of oral bacteria and HBV into the tubes of the handpiece and dental unit as compared with the handpiece without an anti-retraction feature.[37] Therefore, there should be a prohibition of the use of dental handpieces without anti-retraction function during the epidemic period of COVID-19. To reduce the risk of developing toxic aerosols, dentists should eliminate the use of ultrasonic devices, high-speed handpieces, and 3-way syringes.

Additionally, dental teams should be familiar with treatment options that minimise or eliminate AGPs. Micromotor handpieces can be used which can be a little time-consuming but will inhibit the formation of aerosols. Also if possible, caries excavation should be performed with manual excavating instruments.

Negative-Pressure Treatment Rooms/Airborne Infection Isolation Rooms (AIIRs)

It is worth noting that patients with suspected or confirmed COVID-19 infection should ideally be treated in negative-pressure rooms or AIIRs and not in a routine dental practice setting.[38] Alternatively, patients could also be treated in isolated and well-ventilated rooms. Ventilation of the operating room should be adequate, i.e. natural ventilation with airflow of at least 160 L / s per patient or in rooms with a negative pressure with at least 12 air changes per hour.[39] High-Efficiency Particulate Air (HEPA), an extraoral evacuation device can be used effectively to monitor the generated aerosol.[40]

Sterilization and Disinfection of all the Surfaces that Come in Direct Contact with the Saliva of the Patient

Disinfection of all surfaces that may be touched by the patients should be performed with sodium hypochlorite 0.1% or 70% isopropyl alcohol.[31]

Sterilization of Handpiece

Studies have shown that viral DNA and viable viruses can possibly be retained inside high-speed dental handpieces and scaler devices. It is challenging to clean the internal area of the handpiece because of limited access. The proposed disinfection approach is to discharge 20-30 seconds of air and water after each patient, to flush out infected material that may have reached the turbine and the air and water tubes. The manufacturer's instructions for cleaning, lubrication, and sterilization should be performed.[41]

Sterilization of Dental Burs

Dental burs are used for various procedures in clinical dentistry, some involving caries excavation, access cavity preparation, and crown preparation.[42] Burs can become heavily contaminated with necrotic tissue, saliva, blood, and potential pathogens during these procedures and can be a possible vehicle for cross-infection.[43-45] Burs are unique because of their complex architecture which makes it difficult to pre-clean and subsequently sterilize. Inadequate sterilization causes cross infection among the patient and transmission of disease between the patient and dental personnel.[43,46] Under suitable conditions, steam under pressure (Autoclave) can kill all microorganisms including bacterial spores, and is considered to be relatively the best method for decontaminating dental burs.[42] In the midst of COVID-19, it is necessary for considering new bur for each patient.

Sterilization of Endodontic Instruments

One of the fundamental phases of the sterilization process is the cleaning of coarse debris consisting of necrotic and protein material, blood residue, and dentinal mud that is deposited on the endodontic instrument.[47] A study conducted by Popovic et al. compared different methods of disinfection and cleansing of endodontic instruments and reported the use of ultrasonic trays as a method giving efficient results.[48] The most common sterilization techniques used in the last thirty years have been autoclaving, glass-bead sterilization for 45 seconds at 240°C, UV light at 240–280 nm, laser sterilization, and exposure to 2% glutaraldehyde. Steam sterilization in an autoclave does not alter the mechanical and physical properties of most nickel-titanium instruments and is considered the most effective method for the sterilization of endodontic instruments.[47]

Disinfection of Impressions and Prosthesis

All dental impressions of only emergency work should be sent to the laboratory after thorough disinfection in the dental office before dispatch.

Proposed disinfection protocol (According to ISOI Guidelines) is:

1. Thoroughly wash the impression in running water after removing it from the mouth. [For all materials including alginates, polyethers and vinyl polysiloxanes.
2. Gently scrub with a camel hair brush and liquid detergent under running water. [For all materials including alginates, polyethers, and vinyl polysiloxanes.
3. Immerse the impression in a solution of 5.25% Sodium Hypochlorite with 1:10 dilution. The solution should be changed daily. [Only for vinyl polysiloxanes.]
4. Alginates and Polyethers are generally sprayed with an intermediary level disinfectant for the required time. Hydrophobic materials should be immersed in disinfectant solution for ten minutes.

There shall be minimal distortion. Hydrophilic silicones and alginates should be sprayed to avoid dimensional changes.

5. A packet containing prosthesis received from the laboratory should be disinfected first with disinfectant spray and the prosthesis should be disinfected by immersing in glutaraldehyde or any other suitable disinfecting solution for an appropriate time.[41] The microbe that felled one child in a distant continent can reach yours today and seed a global pandemic tomorrow. It has once again proved its relevance with the emergence of CoV disease 2019 (COVID-19) as the latest pandemic that is affecting human health and economy across the world. So, it is very necessary to maintain social distancing, mental distancing.[49,50]

Conclusion: Saliva may be a viable alternative to the nasopharyngeal specimen for COVID-19 testing. Dental professionals should be aware of the different protocols to be followed to prevent the transmission of the virus through aerosols and direct salivary contamination in the dental practice. Further studies are needed to investigate the efficacy, feasibility, and scalability of using salivary specimens for SARS-CoV-2 detection and surveillance on a nationwide basis.

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