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Review Article

The Advantages of Saliva Sampling Method for Diagnosis of COVID 19

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Abstract

Several tests for many persons have to be conducted in order to control the pandemic and prevent the possibility of transmission of (Covid -19) to health care workers and other patients. The main aim of this study was to detect the importance of saliva sample for early diagnosis of Corona virus 2019 (COVID-19). Saliva sample considered as an alternative for diagnosis of COVID-19 as salivary gland found to express surface receptor angiotensin converting enzyme II, (ACE2) a receptor that plays a key role within the entry of SARS Corona Virus-2 (SARS-CoV-2) into the cell. The sample can be self-collected from the entrance of the oral cavity easily, in a way the risk of virus transmission to healthcare personnel can be minimized avoiding the use of protective equipment. There are several ways for collection of saliva, such as spitting out, directly from salivary duct gland and collection with sponge- like device. The analysis of Saliva can be used to detect oral cancer, dental caries, periodontal diseases, diabetes, breast cancer, and lung cancer. The saliva polymerase chain reaction (PCR) test demonstrated high sensitivity comparable performance to the current standard naso-pharyngeal and throat swabs. In addition, saliva could be used for monitoring viral clearance, as it contains the highest live COVID-19 viruses and viral load during the first week.

Keywords: COVID19; Diagnosis; PCR; Serological Test; Saliva Sample; Advantages

Introduction

An outbreak of unexplained pneumonia in Wuhan/China at late December was caused by a new strain of coronavirus which has not been previously identified in human, named later as COVID 19 [1]. It spread across the world and considered as pandemic by WHO [2]. This quick spread is due to its highly infectious in nature, and suggested that the transmition through nasal discharge and saliva droplets [3].

Coronaviruses are enveloped RNA viruses, with two strains acute respiratory syndrome coronavirus (SARS-CoV-2) and Middle East respiratory syndrome coronavirus (MERS-CoV). It is highly contagious, human-to-human transmission of SARS-CoV majorly relies on respiratory droplets, which are naturally produced by talking, and coughing which contain saliva [4]. It could cause very large epidemic in the absence of control measures [5]. It was suggested that old people with medical compromised disease might be at greater risk for severe illness from this virus [5].

The target for COVID-19 are all cells that express surface receptor angiotensin converting enzyme II (ACE2), a receptor that plays a key role within the entry of SARS-CoV-2 into the cell. As salivary gland express ACE2 receptor, therefore; ACE2-positive cells in salivary glands also considered target cells of SARS-CoV-2 where it can duplicate, and thereby making saliva perfect specimen for viral detection. Oral epithelial cells express ACE2 with highest expression within the tongue in comparison with buccal and gingival tissues, T cells, B cells and oral fibroblasts [6].

The incubation period of COVID-19 is based on that of SARS and MERS, in addition to observational data from reports of travel-related COVID-19.The symptoms of COVID-19 appear within 2–14 days after exposure to the virus as reported by CDC and they are similar to those of influenza such as fever, cough, and shortness of breath, however; the symptoms varies from patient to patient and sometime symptomless [7]. It was reported that about 80% of the disease transmission was related to asymptomatic cases [3]. Controlling the spread of the viral was very difficult as it was impossible to identify asymptomatic cases.

Because COVID-19 shows a range of clinical features, starting from mild same as flu to a severe life threatening, therefore we must identify COVID-19 patients from those with other diseases by an efficient test in the early stages of infection. This prevent affected individuals from spreading the infection and unnecessary quarantines of negative one [8].

Now there are two commercially available COVID-19 tests, molecular assays, which detect SARS-CoV-2 viral RNA by polymerase chain reaction (PCR), based techniques or nucleic acid hybridization-related strategies. The second one are serological and immunological assays for detecting antibodies against the viruses produced by the patients after exposure to it or depend on detection of antigenic proteins in infected persons [8].

The selection of right site for sample collection for each test is very important to have reliable results and the most commonly used test site are nasopharyngeal and oropharyngeal area [9]. However; there are limitations regarding collection of the sample and healthcare person safety although both are relatively collected easily and the results of the test are highly sensitive, therefore; easily accessible and less invasive site is importantly needed.

Saliva can be collected from the entrance of the oral cavity easily and patients can collect it themselves, in this way the risk of virus transmission to healthcare personnel can be minimized avoiding the use of protective equipment [10]. Many studies considered that saliva as an alternative for diagnosis of COVID-19, although there are some reports concerned about the accuracy of the screening properties of saliva for COVID-19 [11,12]. Therefore, rapid in-home devices must be designed to decrease the infectious rate before vaccines develop or an antiviral drug become available. Saliva can be used as an excellent diagnostic fluid for controlling the spread of COVID-19 because high SARS-CoV-2 RNA is found in the oropharyngeal cavity [11].

Aim of the Study

The main aim of this study was to detect the importance of saliva sample for early diagnosis of COVID-19.

Diagnosis of COVID-19

Still there is no effective and safe vaccine for immunization against COVID-19 [13]. Therefore to control the pandemic relay on testing as many persons as possible to avoid the possibility of transmission to other patients and health care workers, especially transmission from asymptomatic people, who account for nearly 79% of the contagion [3].

Laboratory testing

Molecular assays for detection of viral nucleic acids

Reverse Transcription-Polymerase Chain Reaction (RT-PCR): It is the gold standard for identification of SARS-CoV-2 virus, based on amplifying a tiny amount of viral genetic material in a sample, which are collected from the upper respiratory system using swabs [14]. This test is used to detect SARS-CoV-2 viral RNA during the acute phase of infected patient [15]. RT-PCR was used widely in diagnosis of COVID-19, but it cannot be used to monitor the progress of the disease stages.

Serological test

Serological testing is an analysis of blood serum or plasma including saliva, sputum, and other biological fluids to search for the immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies. Supplementary IgG/IgM antibodies generated against the virus are analyzed via ELISA, but this test has mainly been performed on plasma [16], and antibody tests are not always COVID-19 specific [11]. It recognizes individuals who have developed antibodies to the virus and could be potential convalescent plasma donors. It could also monitor the immune status of individuals and groups over time [15]. This test plays an essential role in epidemiology and vaccine development, and assists both short and long term antibody response. IgM is first detected in the serum after a few days (5 days post-symptom onset) and lasts for 2 weeks followed by a switch to IgG, which is more tolerated and emerge after 10 days post-symptom onset.

This illustrate that, IgG could be use as indicator for current or prior infection. While IgM is an indicator of early stage infection. During the infection increases of IgA antibodies was found in saliva. However; the presence of antibodies does not indicate that an individual is protected from reinfection. Many Researchers have found that IgG appears to persist for weeks to months than IgM, but both decline after 4 weeks post-symptom onset.

The level of IgG play a role in neutralizing antibody levels. Researches is needed to identify the levels of antibodies necessary for protection from reinfection. Recently, the sensitivity of immunological assays have increased for the application of antibodies (primarily monoclonal antibodies) to detect pathogen derived antigens [17].

Sampling sources

Nasopharyngeal and oropharyngeal swab

Currently, RT-PCR tests for COVID-19 generally use samples collected from the upper respiratory system using nasopharyngeal and oropharyngeal swabs. In addition, few studies used serum, stool, or ocular secretions [14].

Nasopharyngeal swabs are commonly used in respiratory virus diagnosis, but unfortunately it shows relatively poor sensitivity for SARS-CoV-2 detection in early infection. Moreover, it is not suitable for monitoring the viral loads, causes discomfort to the patients due to the procedure's invasiveness and requires a trained professional to collect compared with saliva samples [18]. In addition, it has a high risk of transmission of the virus due to close contact between health professionals and patients, and could induce sneezing, coughing and even bleeding [11,12].

The procedure cannot be used for a large-scale testing, because there are very high shortages of swabs and healthcare workers protective equipment's [19] with difficulty in self-collection of nasopharyngeal swabs and less sensitive for detection of virus [20]. In low income countries, where COVID-19 pandemic intensifies these challenges will be further complicated which limits a reliable and less resource-intensive collection method for the sample, therefore; self-collection in the home, is needed urgently.

Recently in several studies, reported that saliva can be used as an alternative sample to nasopharyngeal and oropharyngeal swabs with more sensitive and noninvasive collection methods [21]. Moreover saliva SARS-CoV-2 detection assay approved by the U.S. Food and Drug Administration [22].

Saliva sample

Saliva is a biological watery liquid secreted into the mouth, 90% from major salivary glands (parotid, submandibular and sublingual glands) and 10% from minor salivary glands within pH from 6 to 7 [23]. It contain 99% water and the rest 1% crevicular fluid, desquamated oral epithelial cells, microorganisms, respiratory secretions, gastric acid from reflux, food debris in pathological occasions, and sometimes blood [24]. Usually saliva contains growth factors, cytokines and secretory IgA. In addition about 600–1000 mL of the saliva flows each day from the human salivary glands [25]. which keep the oral cavity wet and has the ability washout in order to maintain the normal physiological activities of the oral cavity [26]. Moreover, it acts as a lubricant for chewing and swallowing, buffering, balance of remineralization, and anti-microorganisms [27].

Nowaday saliva is used to diagnose several diseases such as malignancies,hereditary diseases, autoimmune diseases, infections, periodontal diseases, dental caries [27], systemic disease with relevance to oral diseases and for monitoring the levels of hormones, drugs and bone turnover markers [28]. The presence of viral RNA, microRNA, antigens and DNA, or host antibodies in saliva are the bases for the diagnosis of saliva- based viral infections. Saliva is a non-invasive diagnostic tool. It can detect some viruses up to 29 days after infection. This novel can be used for early diagnosis and for monitoring the disease with the treatment [29].

Collection of saliva

There are several ways for collection of saliva without compromising the quality and quantity of saliva [30] such as spitting out, directly from salivary duct gland and collection with sponge- like device [25]. The cheapest one is spitting out technique, which includes nasopharyngeal/or oropharyngeal/airway secretions. A relatively more pure saliva can be obtained by using Sponge- like devices, however; this technique needs special equipment that is some time not available. Pure saliva can also be collected from the ducts of major salivary gland, but the process requires special equipment and time consuming.

The importance of saliva in diagnosis of diseases

The composition of saliva is full of information to analyze and compare the physiology or pathology of the human body. Saliva as potential diagnostic tool has been studied and it could be become as alternative to other biological fluids such as urine or serum in the diagnosis of diseases [27]. It contains biomolecules such as DNA, RNA, microRNA, protein, and metabolites [31]. This property give it the chance to be recognized biological matrix to detect diseases in general similar to serum and other body fluids [32], Saliva is secreted in the oral cavity which is the entrance and an outlet of the body, supposed to play a role in early diagnosis and close contact transmission in infectious diseases. Saliva was used to isolate proteins, peptides, and sheds of viruses via many molecular assays [32]. Antibodies against viral infections have been detected in saliva and the status of immunization for rubella, mumps, measles and hepatitis can be confirmed by analyzing IgG, IgM and IgA in saliva [33]. Saliva tests can be used against other viruses (HIV, Zika, etc.) in laboratory and at-home settings [34].

The importance of saliva in diagnosis of Corona viruses

An experimental study found a production of SARS-CoV-2 specific secretory IgA in the saliva of mice intranasal immunized with SARS-CoV-2 virus- like particles [35]. This could prove that anti-SARS-CoV-2 antibodies might also be present in human saliva.

To *et al* have demonstrated a high sensitivity and specificity of saliva- based tests, with >90% agreement between saliva and nasopharyngeal swabs [12]. In Hong Kong hospital To et al reported the accuracy of a human saliva sample from eleven of twelve (91.7%) COVID-19 patients.

Pasomsub., *et al*. in a Cross-Sectional Study showed the value of testing saliva sample as a non-invasive method for detection of SARS-CoV-2 [21].

The Saliva PCR test demonstrated high sensitivity with comparable performance to the current standard nasopharyngeal and throat swabs [21], in their study on drooled saliva, two cases of COVID-19 showed positive salivary samples but negative respiratory swabs.

It was reported that self-collection of saliva sample for SARS-CoV-2 testing is feasible and can produce reliable test results [36].

Jamal., *et al.* [37] studied 91 Self-collected saliva, there results detected that saliva is more sensitive and consistent than nasopharyngeal swabs specimens by spitting into the tube, RT-qPCR sensitivity was 89% for NPS and 72% for saliva. NPS was more sensitive than saliva for SARS-CoV-2 detection especially if the patient was later in illness.

Han., *et al.* [38] found that the positivity of saliva samples RT-qPCR in 11 patients was 80% in week 1, 33% in week 2, and 11% in week 3.

Mucous plugging in the lungs who have of the patients who have COVID-19 was reported in a case reports study. This suggested that oral epithelial cells could be a possible route of entry for the SARS-CoV-2. The ability of the virus particles to penetrate the mucous layer and infect the underlying epithelial cells need to be studied [39].

Saliva in the form of liquid biopsy is used for the safety of healthcare providers, from the transmission of disease. This sampling method has advantages in comparison with the use of nasopharyngeal swabs, nasopharyngeal aspirates and oropharyngeal swabs. In addition, it was suggested as an alternative to NOS for detection of COVID-19 [12]. One study protocol of SARS-CoV-2 has been published, aimed to analyze IgG, IgM and IgA in different biological fluids with self- collected saliva for rapid SARS-CoV-2 diagnosis [39].

Further studies and experiments are needed to explore the benefit of using the saliva or oral fluid in the prevention and early detection of COVID-19 [10].

Clinical advantages of saliva sampling

Saliva samples has many clinical advantages as it is less invasive approach for disease diagnosis and monitoring of general health as compared to NOS or blood samples [10]. In addition, it is painless, and relatively cheap technology with no need for expensive equipment/instruments (swabs, suction tubes or special collection devices) for collection. It needs a sterile container, and clear instructions to the patients to collect the saliva sample themselves. In order to minimize the risk of virus transmission to healthcare personnel and avoid the use of personal protective equipment. It was reported that self- collection of saliva sample is feasible and produce reliable results [36]. The content of salivary COVID-19 (viral load) has been shown to be highest during the first week after symptom onset [12]. This confirms the role of saliva as a probable source for transmission of viral, and it could be detected in the saliva as long as 25 days following the onset of symptoms, suggesting its potential use for monitoring viral clearance [12]. Anew kit for RT-PCR assay (TaqPath COVID-19 Combo kit) was developed by Rutgers Clinical Genomics Laboratory, using self-collected saliva samples, which is quicker, painless, and reduce the risks to healthcare providers than other sample collection methods [41], Using saliva as a diagnostic tool opens the possibility of using it to detect antibodies, chemokines, cytokines beside detection of the viral pathogen, which allow the application of rapid diagnostic devices [42]. There are several studies involving quantitative analysis of the biochemical components of saliva in real time, some of those studies revealed the presence of proteins, glucose, urea, secretory IgA, cortisol, phosphates, among others [43]. Featuring saliva as a fluid with diagnostic potential for COVID-19 biomarkers discovery.

Since saliva sampling is a non- invasive specimen, type, it well-suited for serial viral load monitoring. It is reported that the highest SARS-CoV-2 load in saliva is after the first week of symptom onset, followed by a gradual decline, this underlines that saliva is a good candidate for SARS-CoV-2 detection in earlier disease phase [12,44].

The temporal profile of SARS-CoV-2 load in saliva has reported to be more accurate [44] as compared with that of nasopharyngeal swabs, suggesting its suitability for disease monitoring and detection of SARS-CoV-2 for cases with moderate to severe symptoms [44] and for asymptomatic or mild cases. This is very important for the screening of the suspicious/asymptomatic cases and for the surveillance of the healthcare workers in large scale population based point prevalence studies [36]. Although saliva can be a potential source of viral transmission it can be used for monitoring the response to antivirals in clinical trials [45]. Serologic studies need to be performed on saliva sampling to measure the immune response for SARS-CoV-2 infection. There are so far no results describing the presence of antibodies against SARS-CoV-2 in human saliva, this definitely clarify the need of future studies on the potential use of salivary immunoglobulins for COVID-19 in diagnostics, disease progression and immunization monitoring.

Conclusion

Saliva samples can be used for early diagnosis and monitoring COVID-19 as a less invasive approach as compared to NOS or blood samples. viruses and viral load shown to be highest during the first week after symptom onset and can be detected in the saliva as long as 25 days following the onset of symptoms, suggesting its potential use for monitoring viral clearance.

Declaration of Competing Interest

The authors have no conflicts of interest related to this article.

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