Dentika Dental Journal
Journal homepage: https://talenta.usu.ac.id/dentika

Saliva-Based Test as an Alternative Diagnosis for SARS-CoV-2 Patient

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ARTICLE INFO

Article history:
Received 8 October 2022
Revised 14 July 2023
Accepted 14 July 2023
Available online 31 July 2023

E-ISSN: 2615-854X

P-ISSN: 1693-671X

How to cite:
Istiati, Esa Ilham Mei Sasmita. Effect Saliva-Based Test as an Alternative Diagnosis for SARS-CoV-2 Patient. Dentika Dental Journal 2023; 26(1): 60-66

ABSTRACT

Respiratory system is affected by a novel type of virus known as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). The gold standard for its identification is the real-time reverse transcription polymerase chain reaction (rRT-PCR) test, despite being invasive and dangerous to medical workers during the sample-collecting process. However, major salivary glands including the parotid, submandibular, and sublingual, as well as smaller glands dispersed throughout the mouth cavity, release saliva, a hypotonic fluid, containing biomarkers that can be used to identify both local and systemic disorders, including SARS-CoV-2. This fluid can be utilized as a non-invasive, low-risk diagnostic test by medical professionals since it provides insights into patient's overall health. This study aims to determine the potential of saliva as a substitute for other SARS-CoV-2 diagnostic methods. The results from analysis of 5 scientific publications, showed that saliva contained SARS-CoV-2 virus RNA, making it suitable for patient diagnostic testing. Considering the results, the fluid was discovered to be an alternative diagnostic test for patient with the virus.

Keywords: Alternative Diagnostic, Saliva, SARS-CoV-2, Saliva Based Test

ABSTRAK

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) atau biasa dikenal dengan COVID-19 merupakan virus jenis baru yang menginfeksi saluran pernapasan. Saat ini, metode standar untuk mendeteksi SARS-CoV-2 adalah tes reverse transcription polymerase chain reaction (rRT-PCR) secara real-time, namun tes rRT-PCR adalah metode invasif dan prosedur pengumpulan sampelnya berbahaya bagi tenaga kesehatan. Saliva adalah cairan hipotonik yang disekresikan oleh kelenjar saliva mayor seperti parotid, submandibular, dan sublingual serta beberapa kelenjar saliva minor yang tersebar di seluruh rongga mulut. Biomarker yang dimiliki oleh saliva dapat dianalisis untuk mendeteksi penyakit lokal dan sistemik, salah satunya adalah SARS-CoV-2. Penulis ingin mengetahui lebih dalam mengenai potensi saliva sebagai alternatif pemeriksaan diagnosis SARS-CoV-2. Mengingat potensi saliva yang dapat mewakili kondisi sistemik penderita maka saliva dapat digunakan sebagai tes diagnosis non-invasif dan rendah risiko bagi tenaga kesehatan. Untuk mengetahui potensi saliva sebagai alternatif diagnostik SARS-CoV-2. Berdasarkan analisis yang telah dilakukan pada 5 jurnal penelitian membuktikan bahwa saliva memiliki kandungan RNA virus SARS-CoV-2 yang dapat digunakan untuk tes diagnosis pada pasien. Saliva based test dapat digunakan sebagai alternatif tes diagnosis pada pasien SARS-CoV-2.

Kata kunci: Diagnosa Alternatif, *Saliva* SARS-CoV-2, *Saliva-Based Test*

http://doi.org/10.32734/dentika.v26i1.9843

1. Introduction

COVID-19 is a severe acute respiratory disease caused by the coronavirus 2, and a novel type of virus that harms the respiratory system. Furthermore, it has infected more than 2 million people worldwide in less than 5 months. The most common mode of transmission for SARS-CoV-2 infection is through close contact with potentially contagious droplets.[1]

According to the WHO, 90,308 persons were infected with SARS-CoV-2 in March 2020. Among them 5,726 were treated, resulting in an overall mortality rate of 3,087, or 2.3%. The majority of deaths occurred among the elderly and people with comorbidities. Furthermore, this pandemic has impacted about 200 countries worldwide, with the most significant outbreaks reported in the United States, Italy, and Spain, as stated by Indonesia's Ministry of Health. In Indonesia, there was a notable surge in positive cases at the end of January 2021, with a staggering rate of 36.18%. This figure exceeded the WHO-recommended safe level of 5% by more than seven times. The Case Fatality Rate (CFR) in the country was 2.8%, which was extremely higher than the global CFR of 2.3%.[2]

To identify viral RNA in individuals suspected to have SARS-CoV-2, samples were typically collected from nasopharynx, oropharynx, or sputum. However, the current method of using the rRT-PCR test for sample analysis is invasive and poses a risk to healthcare workers.[3] Saliva biomarkers provide a potential alternative for detecting both systemic and localized diseases. Saliva samples are recommended as a tool for respiratory virus detection, including Influenza A and B viruses due to their simpler collection process and shorter testing time.[4] Compared to nasopharyngeal swab, it has several advantages, which included a simpler and more patient-friendly collection procedure, making diagnostic test easier. Furthermore, saliva collection can be self-administered without requiring special training or assistance.

Based on the provided information, this study aimed to explore the potential of saliva by conducting a literature review. Furthermore, it was proposed that this fluid could reflect patient's systemic condition, hence, suggesting its utility as a non-invasive and low-risk diagnostic test for health workers.

2. Discussion

Wuhan, China was the origin of the virus outbreak that emerged in late 2019. The rapid spread of this infection prompted the WHO to declare a global health emergency in late January 2020. The sickness was initially attributed to a novel coronavirus, named severe acute respiratory syndrome coronavirus 2 (SAR-CoV-2) (COVID-19), which caused the disease commonly known as COVID-19.[5]

On a single RNA chain, the virus is predominantly composed of nucleotide material, and consists of 4 major proteins, namely surface (S), envelope (E), membrane (M), and nucleocapsid are the four major proteins that make up this virus. Furthermore, the size is approximately 30 kb (N) [5]. SARS-CoV-2 is a member of the SARS-CoV-2 virinae subfamily, which belongs to the SARS-CoV-2 viridae family. This family encompasses various viruses, including AlphaSARS-CoV-2, BetaSARS-CoV-2, GammaSARS-CoV-2, and DeltaSARS-CoV-2. CoVs have a relatively larger single-stranded positive-sense RNA (+ssRNA) genome, typically ranging from 27 to 32 kilobases, which sets them apart from other RNA viruses. The genome is surrounded by nucleocapsid proteins (N), which form a capsid and are then encased by a sheath connected to known as M, S, and E proteins. The recently discovered SARS-CoV-2 is a member of the coronavirus family and it has a genome size of about 29.9 kb.[6]

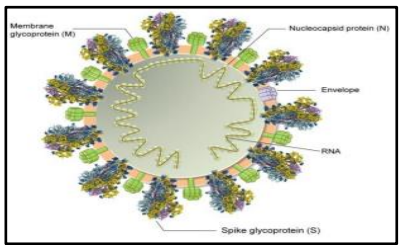

Figure 1. Structure of SARS-CoV-2.[21]

During this time, the levels of leukocytes and lymphocytes remain within the normal range or exhibit a slight decrease, while patient is still asymptomatic. The virus primarily spreads through the bloodstream in the early symptomatic phase, targeting tissues such as the heart, lungs, and gastrointestinal tract that produce Angiotensin Converting Enzyme 2 (ACE-2). Symptoms during this stage are typically mild, and following the initial assault, a second phase begins around 3 to 7 days later. Patient now experiences fever, breathing difficulties, worsening lung lethargy, and a decline in lymphocytes. Hypercoagulation ensues, and inflammation markers begin to rise. In the following stage, when left untreated, the inflammation becomes uncontrollable, leading to cytokine storms, Acute Respiratory Distress Syndrome (ARDS), sepsis, and other complications. The virus became attached to the respiratory tract and initiated a protein production process. This mechanism involved the activation of the spike protein, which utilized ACE-2 and the spike protein fragments as an entry point for the virus. The activity of TMPRSS2 enhances the cellular uptake of SARS-CoV-2 virus. This entire mechanism is referred to as the viral infection process, where the virus enters the target cell and releases RNA. The viral protein enables replication in the cell, resulting in an increased number of virus replicas, which are then moved to the outer edge.[7]

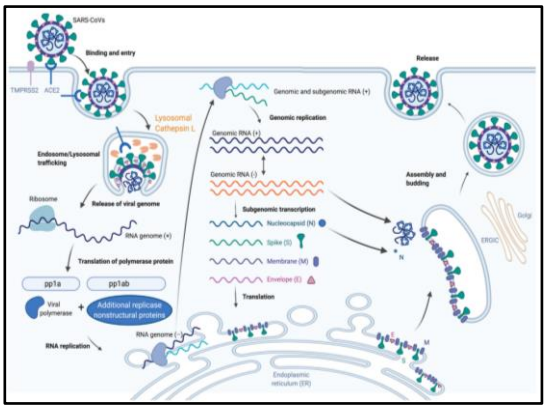

Figure 2. SARS-CoV-2 entry pathway.[8]

The common cold CoV tends to cause mild URT symptoms and gastrointestinal disorders. Symptoms such as fever, coughing, and dyspnea are the most typical. However, as the pandemic progresses, it became evident that SARS-CoV-2 was solely not limited to causing rapid respiratory and gastrointestinal disease but has also been associated with long-term consequences such as myocardial inflammation. Severe cases of SARS-CoV-2 are not restricted to the elderly population, as previously reported, but have been observed in children and young adults.[8]

Saliva consists of 99% water, while the remaining components include glycoproteins (mucin), enzymes (amylase), electrolytes (sodium, potassium, and bicarbonate), antimicrobial substances (lactoperoxidase enzymes) and bacterial cells.[9] Mucin is also produced by major salivary glands and minor mucous glands located in the buccal mucosa, labial and palatal. The predominant mucins in saliva are MUC5B and MUC7.[10]

In addition to immunoglobulins, mucin, enzymes, metabolites, hormones, and electrolytes, the formulation also includes several proteins. This comprehensive composition allows for the quantification of biomarkers that can indicate an individual's immunological, inflammatory, endocrine, and metabolic health, as well as the identification of infections in saliva.[10]

Saliva offers new avenues for the direct detection of viral infections, enabling the use of cytokines and other bioanalytes to expedite diagnostic methods. Real-time evaluation of the biochemical components of this fluid has shown the presence of secretory IgA, protein, glucose, and urea.[11] When an infection occurs, T cells & macrophages secrete cytokine IL-6, which promotes inflammation and enhances the immune response. Meanwhile, IL-10 acts as an anti-inflammatory cytokine, limiting and terminating the immune response.[12] T cells, classified as T CD4 and T CD8, played a significant role in the immune response. CD8 cytotoxic or T

cells contribute to the immune response against viral antigens on infected cells, aiming to halt the spread of viral infection. Helper T cells (CD4), a subpopulation of T cells, aids B cells in producing antibodies.[13] Macrophages are mononuclear phagocytic cells that play a role in both innate and adaptive immune systems.[14] Alanine aminotransferase (ALT) is an enzyme that catalyzes the transfer of amino acids from alanine to a-ketoglutarate.[15] C-reactive protein (CRP) is a blood protein that serves as an indicator of inflammation, with increased production by the liver in response to infection, injury, or inflammation. Neutrophils, a type of white blood cell, are markers of infection or inflammation. [16] Neutrophils, a type of white blood cell, are markers of infection or inflammation.[16] The presence of cytokine hormones at the site of inflammation stimulates an increase in circulating neutrophils, resulting in heightened activity.[17] Laktat dehydrogenase (LDH) is an enzyme that is required to catalyze the conversion from pyruvic to lactic acid under conditions of anaerobic glycolysis. Urea is a nitrogen product that is excreted by the kidneys from dietary protein. Several of the bioanalytes stated above can be discovered in saliva and utilized for early and accurate diagnosis of SARS-CoV-2, as well as to evaluate exposure levels, disease prevalence, and the progression of severe cases.

Saliva has emerged as a valuable biological matrix for early disease detection, sharing similarities with serum and other bodily fluids. Furthermore, it contains various biomolecules such as DNA, RNA, proteins, and metabolites. The advantages of saliva sampling have garnered attention in the field of public health. Recent technological advancements have unveiled several saliva-based biomarkers, linking them to some diseases. This includes the mixture of the microbiome, transcriptome, metabolome, and proteome in the fluid. Due to its diagnostic potential, saliva can serve as an alternative sample for detecting respiratory viral infections, making it particularly useful for large-scale screening efforts. At least, three pathways for detecting SARS-CoV-2 have been identified in saliva.[11]

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1. When the fluid originating from this tissue can reach the oral cavity
2. When the oral cavity can be reached by blood through an exudate containing regional proteins
3. From major and minor salivary gland infections, viral particles mixed with saliva are released. According to Meutia Wardhanie Ganie, salivary glands serve as very early proliferative sites for the virus. Therefore, the increased expression of ACE-2 in the glands, indicates that they are the initial target organ. Saliva can be an important source for early diagnosis of disease before respiratory symptoms appeared.[18]

The ACE -2 receptor present in salivary glands captured target cells, namely SARS-CoV-2. This allows the virus to replicate and establish colonization. As a result, saliva has become an ideal specimen for virus detection.[10]

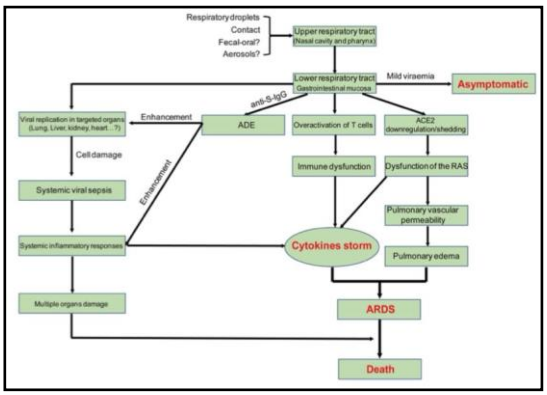

Figure 3. Clinical manifestation of SARS-CoV-2 infection.[22]

The two primary upper respiratory tract specimens recommended for diagnostic testing are naso and oropharyngeal swabs. However, there were various drawbacks associated with their use. These include patient pain and the requirement for medical staff involvement, which increased the risk of nosocomial transmission. This collection system led to sneezing and coughing, potentially realizing infection-carrying aerosols.

Additionally, individuals with thrombocytopenia or blood coagulation disorders may experience bleeding as a result of this technique. Another concern was the molecular testing of the nasopharyngeal swab, which has certain limitations, such as the requirement for sample collection by experts and the time-consuming analysis process conducted in a laboratory for molecular diagnostics. These limitations especially in the pandemic can pose challenges for test providers.[19] Additionally, it was observed that oropharyngeal and nasopharyngeal swab (NPS) sample collection often causes significant discomfort for patient and necessitates the involvement of skilled specialists. This close contact between patient and the healthcare provider further increases the risk of transmission.[11]

One advantage of using saliva over blood samples is the presence of IgA antibodies. IgA has been detected in the serum of SARS-CoV-2 patient, and it appeared to emerge earlier than IgM or IgG antibodies. This might have happened two days after the first signs and symptoms. Unlike IgM and IgG antibodies, which are frequently seen in saliva at lower levels than in blood, IgA being the primary antibody in the mucosa, can be easily detected.[20] This mucosal immune system does not only work when infected with SARS-CoV-2 but when it is invaded by microorganisms. The system consists of inductive and effector sites. The inductive sites are responsible for antigen uptake and priming of naïve T and B cells which then migrate to other mucosal effector sites, Meanwhile, effector sites are where secretory IgA (sIgA) is produced, initiating mucosal immunity. This biologic fluid can be employed for diagnosis while keeping in mind saliva's potential for infectiousness. It has advantages over the nasopharyngeal swab, such as easier collection procedures. This makes it more convenient for patient undergoing diagnostic test, as the simple procedure allows for self-retrieval without requiring specialized training.[19] However, when using saliva as a sample for testing, similar to the nasopharyngeal swab, there is a potential risk of bias. This is because the oral and upper respiratory tract mucosa have a high expression of ACE-2, which makes it easier to detect SARS-CoV-2.

Using patient's saliva has the advantage of reducing the danger of nosocomial infections for medical staff. Additionally, it minimizes the need for personnel to wait during sample collection, allowing for better time management in a clinical setting and reducing the potential for additional viral transmission. Another benefit is that patient finds saliva collection stress-free and painless, leading to a higher acceptance rate. Therefore, this fluid can be effectively utilized for various purposes, including epidemiological study, large-scale sampling, or benefiting specific populations such as children.[10]

Saliva can serve as an alternative way to diagnose SARS-CoV-2 patient. These were caused by several things, namely:

1. The oral cavity served as the estuary for the propagation of the virus through the upper respiratory tract, gingival crevicular fluid (GCF), and the main and minor salivary glands.
2. The relatively high intensity of ACE-2 expression in the mucosal layer at the tissues of the mouth and nose.

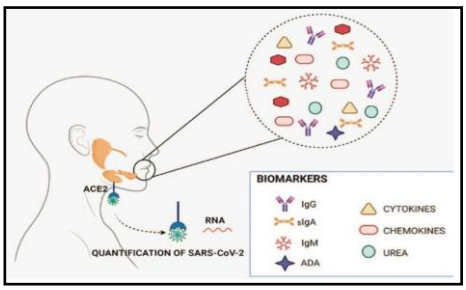

Figure 4. High intensity of ACE-2 expression as a biomarker in the mucosal layer.[11]

In the oral cavity, there were a lot of viruses that can be utilized as a diagnostic sample. The draining method, which involved allowing saliva continuously run into the tube, can be used to collect samples. However, due to the highly transmissible nature of the virus, it was recommended to use straws or other measures to prevent its spread. The collected samples were subjected to rRT-PCR analysis. This examination converted the viral RNA material into DNA, allowing for amplification to reach the detection threshold.

Therefore, saliva-based test/SARS-CoV-2 offered the advantages of ease and more accuracy, and it is a non-invasive method that does not cause discomfort.

References

[1]. Atmojo JT, Akbar PS, Kuntari S. Definition and path of transmission of severe acute respiratory syndrome SARS-CoV-2 virus 2 (SARS-CoV-2) or covid-19. J Health Educ. 2022; 9(1): 57–64.

[2]. Suni NSP. Tingginya kasus aktif dan angka kematian akibat covid-19 di Indonesia. J Info Singkat 2021; 13(3): 13-8

[3]. Dharmayanti NLPI, Hewajuli DA. Development of reverse transcriptase-polymerase chain reaction technology in identifying genomes avian influenza and newcastle diseases. WARTAZOA. 2014; 24(1).

[4]. Rahatina VEF, Adi S. Detection of SARS-CoV-2 using saliva. ODONTO: Dent J. 2021; 8(1): 94-100.

[5]. Rosyda M, Rahani FF. Analisis epitope sel T pada SARS-CoV-2 dengan pendekatan bioinformatika. JNTETI 2020; 9(3): 233-8

[6]. Wang MY, Zhao R, Gao LJ, Gao XF, Wang DP, Cao JM. SARS-CoV-2: Structure, biology, and structure-based therapeutics development. Front Cell Infect Microbiol. 2020; 10: 587269.

[7]. Mu'afa K, Asih TSN. Numerical completion of the SARS-CoV-2 (Virus Dynamics Model) causes of SARS-CoV-2 with the body's immune system. PRISMA. 2021; 4: 718-26.

[8]. Harrison AG, Lin T, Wang P. Mechanisms of SARS-CoV-2 transmission and pathogenesis. Trends Immunol. 2020; 41(12): 1100–15.

[9]. Saragih JA, Purwoningsih E, Maslina S, Rahman S. Saliva content test in diabetic patients melitus tipe 2. Universitas Muhammadiyah Sumatera Utara; 2017.

[10]. Sarkar A, Xu F, Lee S. Human saliva and model saliva at bulk to adsorbed phases – similarities and differences. Adv Colloid Interface Sci. 2019; 273: 102034.

[11]. Fernandes LL, Pacheco VB, Borges L, Athwal HK, Paula Eduardo F, Bezinelli L. Saliva in the diagnosis of COVID-19: A review and new research directions. J Dent Res. 2020; 99(13): 1435–43.

[12]. Azhari A, Jalaludin D, Irawan A. Proteomis: Database and technology quantum. QUANTUM 2017; 8(1): 1-12.

[13]. Febrianty H, Djati MS. Modulasi Sel T CD4+ dan CD8+ pada spleen ayam arab putih (Gallus turcicus) dengan ransum yang mengandung daun pepaya (Carica papaya L). J Biotropika 2015; 3(3).

[14]. Handajani J, Fatimah S, Asih R, Latif A. Penurunan kadar IL-1β makrofag terpapar agregat bakteri actinomycetemcomitans setelah pemberian minyak atsiri temu putih. Maj Ked Gi Ind. 2015; 1(2): 2442–576.

[15]. Kendran AAS, Arjana AAG. Pradnyantari AASI. Aktivitas enzim alanine-aminotransferase dan aspartate aminotransferase pada tikus putih jantan yang diberi ekstrak buah pinang. Buletin Veteriner Udayana. 2017; 9(2): 132–8

[16]. Setyowatie L, Sukanto H, Murtiastutik D. C-Reactive Protein pada Berbagai Derajat Keparahan Psoriasis Vulgaris. Berkala Ilmu Kesehatan Kulit dan Kelamin. 2016 Aug;28(2)..

[17]. Prihandari R, Muniroh L. Jus Semangka Menurunkan Neutrofil Tikus Jantan Galur Wistar yang Terpapar Asap Rokok. MGO 2018; 11(2): 166.

[18]. Ganie MW. Uji Sensitivitas dan spesifisitas spesimen saliva pasien covid-19 menggunakan pemeriksaan polymerase chain reaction untuk identifikasi infeksi Sars-Cov-2 di kota Medan. Thesis. Medan: Program Pasca Sarjana FK USU, 2021.

[19]. Aita A, Basso D, Cattelan AM, Fioretto P, Navaglia F, Barbaro F. SARS-CoV-2 identification and IgA antibodies in saliva: One sample two tests approach for diagnosis. Clin Chim Acta. 2020; 510: 717–22.

[20]. Azzi L, Maurino V, Baj A, Dani M, d'Aiuto A, Fasano M. Diagnostic salivary tests for SARS-CoV-2. J Dent Res. 2021; 100(2): 115–23.

[21]. Gunardi WD. Pemeriksaan diagnosis laboratorium SARS-CoV-2: Keterbatasan dan tantangannya saat ini. J Ked Meditek. 2021; 27(2): 173-82.

[22]. Jin Y, Yang H, Ji W, Wu W, Chen S, Zhang W. Virology, epidemiology, pathogenesis, and control of COVID-19. Viruses. 2020; 12(4): 372.