SALIVARY ELECTROLYTES COMPOSITION AND IT'S CHANGES IN DIFFERENT TEMPERATURE ATMOSPHERE

THESIS

Submitted to complete one of the conditions

to achieve the degree of Bachelor of Dentistry

Dissection by

ABDAL AZIZ AHMAD ABBAS

J011191121

DEPARTMENT OF ORAL DISEASE

FACULTY OF DENTISTRY

HASANUDDIN UNIVERSITY

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ENDORSEMENT PAGE

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By:

ABDAL AZIZ AHAD

J011191121

Checked and Approved

On Makassar, 26 January 2023

By:

Advisor

<u>Erni Marlina, drg., Ph.D., Sp.PM (K)</u>

NIP. 19750601 200912 2 001

By the knowledge of,
Dean of the Faculty of Dentistry
Hasanuddin University

<u>Prof. Dr. Edy Machmud, drg., Sp.Pros (K)</u>
NIP. 19631104 199401 1 001

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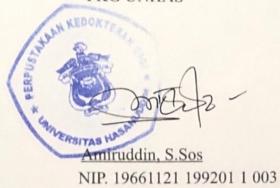
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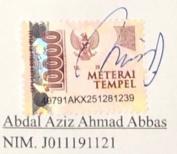
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NIM   : J011191121

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NIM. J011191121

ABSTRACT

Saliva is composed of a variety of electrolytes, including sodium, potassium, calcium, magnesium, bicarbonate, and phosphates. Also found in saliva are immunoglobulins, proteins, enzymes, mucins, and nitrogenous products, such as urea and ammonia. These components interact in related function in the following general areas: (1) bicarbonates, phosphates, and urea act to modulate pH and the buffering capacity of saliva; (2) macromolecule proteins and mucins serve to cleanse, aggregate, and/or attach oral microorganisms and contribute to dental plaque metabolism; (3) calcium, phosphate, and proteins work together as an anti solubility factor and modulate demineralization and remineralization; and (4) immunoglobulins, proteins, and enzymes provide antibacterial action., The normal pH of saliva is 6 to 7, meaning that it is slightly acidic. The pH in salivary flow can range from 5.3 (low flow) to 7.8 (peak flow).

Saliva is a unique fluid that contributed to the development of a new diagnostic tool in the past few years. The research has shown that a wide spectrum of hormones, nucleic acids, electrolytes, and proteins/peptides can be related to multiple local and systemic diseases. It is said that saliva reflects the "body's health" and wellbeing, but until recently its use as a diagnostic tool has been hindered because the examination of the biomolecules that exist in saliva and their relevance and association with different etiologies has been not enough explored. Used for the diagnosis of systemic diseases, saliva is an important advantage, primarily because saliva contains a small amount of plasma. Plasma derived bio- markers in saliva facilitate the continuous monitoring of the oral and general health status.

CONTENT

CHAPTER I
INTRODUCTION

1.1 Background

Saliva is one of the most valuable fluids in our bodies that without many tasks and functions will fail to be executed. This oral fluid will not only assist food digestion but also important in oral lubrication and protection to maintain hard and soft tissues integrity as well as taste buds rehydratation. Saliva is a clear, slightly acidic mucoserous exocrine secretion. Whole saliva composed a complex mix of fluids from major and minor salivary glands and from gingival crevicular fluid, and highly contain bacteria and food debris.[1,2]

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The salivary fluid is an exocrine secretion that consists of approximately 99% water, with a variety of electrolytes (sodium, potassium, calcium, magnesium, and phosphate), proteins such as enzymes, immunoglobulins, antimicrobial factors, albumin, polypeptides and oligopeptides, traces of albumin, and mucosal glycoproteins of great importance in maintaining a balance of the oral health. Saliva also contains glucose, urea, and ammonia in various quantities that can interact and be responsible for several general diseases.[6]

Composition of this body liquid varies and depends on the type of the gland, mucous or serous ones. Its composition differs by the contribution of each gland in order to obtain the total of unstimulated saliva secretion, and the variations are from

65%, 23%, and 8% to 4% for the submandibular, parotid, Von Ebner, and sublingual glands. Components of saliva can have also a non-glandular origin; basically, the oral fluid is considered to be a mixture of the production of salivary glands and other fluids that originate from the oropharyngeal mucosa (oral mucosal transudate, fungi bacteria, viruses, and gastrointestinal reflux liquid)[7]. To the total composition, there is also a contribution from the crevicular fluid that is produced at approximately 2-3 µl/h per tooth and it can be considered as a plasma transudate. The oral fluid also can contain food debris and blooderivate compounds such as plasmatic proteins, erythrocytes, and leucocytes in case there is inflammation present. The composition of saliva based on its constituents is inorganic, organic nonprotein, protein/polypeptide, hormone, and lipid molecules.[7]

For many years now, researchers investigated the importance of the changes that occurred in the saliva, changes that affect the flow rate and composition. The changes in the fluid are valuable regarding the diagnosis of oral and systemic diseases. At first, the examination of saliva was used in order to identify the local gland diseases, such as inflammatory and autoimmune diseases, but later on the researchers expanded their work, highlighting the potential for diagnosing multiple general diseases.[7] Further researches conclude that salivabased biomarkers are not only preferred but also are accurate in discerning healthy subjects from those afflicted with local diseases such as periodontal disease, burning mouth syndrome, and used as an indicator of stress and chronic pain. Several studies report substance P, a neuro-peptide associated with inflammation status and pain, as well as the stress

hormone cortisol, and markers of oxidative stress can be repeatedly detected within salivary secretions[7].

Based on that important knowledge, it is highly possible to utilize saliva component, specifically for the electrolyte composition as a sensor for prevention and infectious diseases, including the ongoing pandemic virus COVID-19.[14] However, to allow safe investigation of saliva sample, precaution measurement the study involving saliva composition needs to perform cautiously to avoid infection to people working with the saliva. One simplest method for inactivation of targeted viruses is by heat-killing. Although some studies revealed heatinactivation effectivity in eliminating COVID-19 virus, however most of this study focus on utilization of the samples for PCR downstream application to

detect the RNA viruses.[15,16,17,18] It is the aim of this proposed pilot study to observe whether the temperature method might also effective to generate safe saliva sample material for assay involving electrolyte composition.

1.2 Hypothesis

This research aiming on discovery of the temperature effect on the electrolyte composition of saliva to provide safe and reliable saliva samples for bioassay downstream. Two outcomes could be expected from this research, whether the temperature would not affect the composition of saliva, or it would be affected by the temperature changing.

1.3 Research Aims

Obtain statistically supporting data as part of the main research regarding Inteligencia   Artificial application for infectious diseases saliva chemical composititon which is an ongoing collaboration research between Dentistry faculty, Medical faculty, and Mathematics and Science Faculty of Hasanuddin University to detriment the effect of temperature on the composition of saliva in reducing occupational hazard for the health worker.

1.3.1 Academic benefits

1.3.1.1 Beneficial to the academic community as reference for the effects of environmental temperature on saliva flow rate and composition.

1.3.1.2 As a baseline to further research on downstream of any saliva assay to detect COVID-19

1.3.1.3 As a pilot project for the AI saliva chemical composition for COVID-19 detection.

1.3.2 Public benefits

The public are beneficial in case of stable saliva composition might be a based to create a new method of covid-19 detection that is much less uncomfortable and easy to carry without potential of sample-origin infection.

CHAPTER II

LITERATUR REVIEW

2.1 Saliva Content and Composition

Saliva is one of the most valuable fluids in our bodies that without many tasks and functions will fail to be executed. This oral fluid will not only assist food digestion but also important in oral lubrication and protection to maintain hard and soft tissues integrity and the taste buds rehydrated. Saliva is a clear, slightly acidic mucoserous exocrine secretion. Whole saliva composed a complex mix of fluids from major and minor salivary glands and from gingival crevicular fluid, and highly contain bacteria and food debris.[1,2]

The terms major and minor refer to the anatomic size of the glands, but that does not mean that the bigger the better and more useful to produce saliva, paradoxically, it could be argued that the minor salivary glands are the most important because of their protective components. Major glands do produce more saliva than minor glands, but the quality of content and thus the type of protection varies.[3]

The average daily flow of whole saliva varies in health between 1 to 1.5 litre. Percentage contributions of the different salivary glands during unstimulated flow are as follows: 20% from parotid, 65% from sub-mandibular, 7% to 8% from sublingual, and less than 10% from numerous minor glands. Stimulated high flow rates drastically change percentage

contributions from each gland, with the parotid contributing more than 50% of total salivary secretions.[3]

2.2 Saliva Associated with Local and Systemic Conditions

Some studies conducted to explore the effect of general health or factors such as alcohol on the function of salivary glands. In some chronic diseases for example, chronic pancreatitis, insulin-dependent diabetes mellitus, renal failure, anorexia and bulimia nervosa, and celiac disease, the salivary amylase level is elevated. Amylase is also strongly affected by changes in flow rate and total protein concentration.[11] The studies suggested that chronic alcoholics affects oral health and causes changes in their salivary glands.[11]Moreover, consumption of ethanol affects salivary function in the short term by reducing salivary secretion and secretory protein synthesis. The mechanism by which acute ethanol exerts an effect is not known, but suggested that acute alcohol consumption causes a decrease in the stimulated whole saliva flow rate.

Both chronic and acute alcohol consumption cause significant changes in the physiology of the human body. Consumption of a high dose of alcohol causes overall stress, elevated blood pressure, hypoglycemia, changes in the lower esophageal sphincter and promotion of the secretion of gastric acid juice. The mechanisms by which ethanol exerts its acute effects are not clear. The effects of ethanol on membrane lipid composition have been extensively studied, but these changes do not explain the changes in saliva composition. Interestingly, Proctor et

al found different responses in protein synthesis in rat parotid, sublingual, and submandibular glands after acute alcohol intake. They concluded that alcohol may affect some aspects of secretory cell function, in addition to its effects on autonomic innervation. The mechanism by which ethanol reduces protein

synthesis in all salivary glands is unclear.[11]

The effects of chronic ethanol exposure on salivary electrolytes have been extensively studied both in human beings and in rats. Data on the effects after acute ethanol ingestion are, however, unclear or known only for ions such as sodium and potassium. Our results showing a decline in sodium and calcium concentrations as BAC increases, are in good agreement with studies on human beings and rats. We also studied changes in calcium and phosphate after acute alcohol consumption. Interestingly, the impaired output of all 4 studied electrolytes was highly significant both immediately after ingestion of ethanol and when BAC levels had reached maximum height. The low sodium and calcium concentrations in saliva might be explained by the decrease in secretion rate. Our results also suggest that acute alcohol consumption may cause temporary dysfunction in cells of the striated ducts observed as changes in electrolyte concentrations in this study and in acinar cells, observed as a reduction in amylase activity in the salivary glands in this study. The electrolyte analyses indicate that salivary ductal resorptive activity might be diminished in the presence of high blood concentrations of alcohol.[11]

Periodontitis can be classified based on the three phases of evolution: inflammation, connective tissue degradation, and bone turnover. There are, associated with each phase of the periodontal disease, different salivary biomarkers

that can stage the evolution and the status of the patient. At the beginning of the inflammatory phase, prostaglandin E2, interleukin-1, interleukin-6, and tumor necrosis factor-alpha are found in a high number, released from a variety of cells. As the stages progress and the disease becomes more advanced with severe bone loss, the levels of tumor necrosis factor, interleukin-1, and RANKL are elevated and directly related to the degree of bone destruction. The specific biomarkers for the bone, such as pyridinoline crosslinked carboxyterminal telopeptide of type I collagen, are being transported in the crevicular fluid into the periodontal pocket and finally become a component of saliva.[7]

A recent study outlined the existence of certain correlations between salivary superoxide dismutase levels and the gingival index, pocket depth, and clinical attachment loss found in patients that were diagnosed with chronic periodontitis. Saliva's potential of diagnosis is seen as a noninvasive and easy way to diagnose patients with premalignant conditions.[8]

Other disease such as Sjögren's Syndrome: Sjögren's syndrome (SS) is an autoimmune chronic systemic disease that has important symptoms: xerostomia and keratoconjunctivitis. Patients diagnosed with SS have a decreased salivary flow rate and a modified composition of the saliva. It was shown the fact that this syndrome is accompanied with significant changes in the proteome and transcriptome, having also important alterations in the levels of IL-4, IL-5, and cytokine clusters.[9]

Additionally Alzheimer's disease (AD) which is one of the most common neurovegetative disorders that occur to the aging population, actually is initiated years before it becomes clinically manifest. Until now, the specific biomarkers for this disease could be found in the cerebrospinal fluid through the amyloid levels or using structural and functional magnetic resonance imaging, procedures that proved to be invasive and time-consuming. Further researches show that the existence of protein Ab and tau or a-Syn and DJ-1 in human saliva can be considered proteins that are related to Alzheimer's disease and Parkinson's disease, suggesting actually the implication of saliva and its potential in the diagnosis of neurodegenerative diseases. Risk factors in the development of Alzheimer's disease are systemic infections, brain infections due to bacteria or virus involvement, but the association of various antimicrobial peptides in this disease is still not completely clear.[7]

The human immunodeficiency virus (HIV) saliva composition is possible through antibody-based screening assays. The diagnostic test is an antibody assay might be perform via Western blot test of the blood or saliva sample or via a polymerase chain reaction via blood. These specific tests have the aim at identifying the p24 antigens and antibodies against HIV-1 and HIV-2.[10]

2.3 Saliva as A Pathway of Communicable Disease

SARS-CoV-2, a novel emerging coronavirus, has caused severe disease (COVID-19), and rapidly spread worldwide since the beginning of 2020. SARSCoV-2 mainly spreads by coughing, sneezing, droplet inhalation, and direct contact. SARS-CoV-2 has been detected in saliva samples, making saliva a

potential transmission route for COVID-19. The participants in dental practice confront a particular risk of SARS-CoV-2 infection due to close contact with the patients and potential exposure to saliva-contaminated droplets and aerosols generated during dental procedures. In addition, saliva-contaminated surfaces could lead to potential cross-infection. Hence, the control of saliva-related transmission in the dental clinic is critical, particularly in the epidemic period of COVID-19.

The interaction between viruses and saliva is a complex biological process. Human saliva is abundant of biologically active components, such as proline-rich proteins, mucins MG1 and MG2, and gp340. These components interact with pathogens and cause multiple influences on their biological behavior.

Coronavirus is a group of enveloped single-stranded RNA viruses belonging to the order Nidovirales, the coronavirus family, and the coronavirus subfamily. It has 26 known species and can be divided into four genera (α, β, γ, and δ). Only the α and β genus are human pathogenic strains. SARS-CoV, SARSCoV-2 and the Middle East respiratory syndrome (MERS) coronavirus (MERSCoV) all belong to the β subgroup. Studies have shown that early target cells for SARS-CoV infection include 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 suggested the salivary gland epithelial cells may be infected in vivo after entry of the virus.

Therefore, the saliva produced by the infected salivary glands could be an important source of virus, particularly in early infection, Study of the invading

process of SARS-CoV-2 revealed this pathogen invades human cells through ACE2 which functions as a cell receptor. This process is similar to the invasion process of SARS-CoV, suggesting a similar invasion mechanism of these two species of coronavirus. Therefore, salivary glands may also become a potential source of the transmission of SARS-CoV-2, which is not to be neglected.[23]

2.4 Saliva and potential use as a marker for diseases

Collecting saliva in order to identify biomarkers associated with orofacial pain is a painless method and is easy to collect and store. Recently, saliva and synovial fluids have piqued the interest of numerous researchers and clinicians as possible alternatives to serum[7].

In a study suggesting that exposure to different temperatures has an immediate effect on saliva flow rate and composition. With decreasing temperature saliva flow rate and output of total protein and amylase increase. MUC5B output shows a similar trend, although not significant, but this might be due to the limited number of participants. Future studies with a higher number of participants might confirm this trend.[12]

During exposure to cold two effects may play a role; the physiological response of the body to maintain homeostasis in a cold environment and an effect of cold on receptors in the mouth that directly may have an effect on saliva secretion.

It is much useful to determine the chemical changes that will occur during temperature changes to oral fluid to be used in beneficial researches in the future including the help of covid-19 screening method.[13]

2.5 Saliva and COVID-19 Association

Centers for Disease Control and Prevention considers saliva contact the lead transmission mean of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes the coronavirus disease 2019 (COVID-19). Saliva droplets or aerosols expelled by sneezing, coughing, breathing, and talking may carry this virus. People in close distance may be exposed directly to these droplets or indirectly when touching the droplets that fall on surrounding surfaces and ending up contracting COVID-19 after touching the mucosa tissue of their faces. It is of great interest to quickly and effectively detect the presence of SARSCoV-2 in an environment, but the existing methods only work in laboratory settings, to the best of our knowledge. However, it may be possible to detect the presence of saliva in the environment and proceed with prevention measures. However, detecting saliva itself has not been documented in the literature. On the other hand, many sensors that detect different organic components in saliva to monitor a person's health and diagnose different diseases, ranging from diabetes to dental health, have been proposed and they may be used to detect the presence of saliva.[19]

2.5.1 COVID-19 Virus in Saliva

Saliva is a complex mixture of components secreted by three major salivary glands, namely the submandibular, parotid and sublingual glands, and other small parts of the mouth that also produce saliva but in small quantity, such as the gingival fold and oral mucosa. The components of human saliva include water, organic proteins, such as amylase, peroxidase, lysozyme, cortisol, and mucin, glucose, cholesterol, urea, and in- organic components or electrolytes. The concentration of these components has been used as an indicator or an auxiliary means of diagnosis of various diseases. Saliva-producing glands have been shown to host SARS-CoV-2 in individuals with COVID- 19 while being asymptomatic. Saliva tests for COVID-19 have received recent emergency approval as a noninvasive COVID-19 test by the U.S. Food and Drug Administration (FDA).[20]

2.5.2 COVID-19 detection using saliva as a sample

The complexity of SARS-CoV-2 detection and the lack of knowledge about the virus lead us to wonder how to determine whether an environment presents a high risk for contracting COVID-19 for its occupants. In such case, one can detect the presence of human saliva and take preventative measures. The detection of saliva in an environment may not ensure the presence of the virus but it may indicate the need for taking septic measures that may minimize the possibility of infection. Portable and robust sensors that detect SARS-CoV-2 in the field seem to be complex to realize at this time. On the other hand, there has been interest in detecting different saliva components for health monitoring and disease diagnosis

in recent years. Such sensors may identify a saliva droplet by detecting one or a few of its components.

These sensors target the detection of organic or inorganic components of saliva. Furthermore the sensors are classified and presented based on their working principle, construction, and properties, such as sensitivity and the sample size needed for performing the detection. Considering the need to detect specific viruses, including SARS-CoV-2. There are also existing sensors that detect different viruses that cause upper respiratory disease and spread through saliva, such as influenza, Middle East Respiratory Syndrome (MERS), and SARSCoV-2.

While saliva sensors present an opportunity to detect the possibility of unveiling an infectious agent, there has been interest in directly detecting specific viruses. Therefore, we also surveyed some of the sensors that detect influenza and other corona′viruses that spread through saliva droplets, such as SARS-CoV-2. Figure 1 shows a classification of these virus sensors according to their working principle, detection elements, and sensitivity. They are also classified into chemical, electrochemical, capacitive, chemiresistive, optical, and electrical sensors. [21]
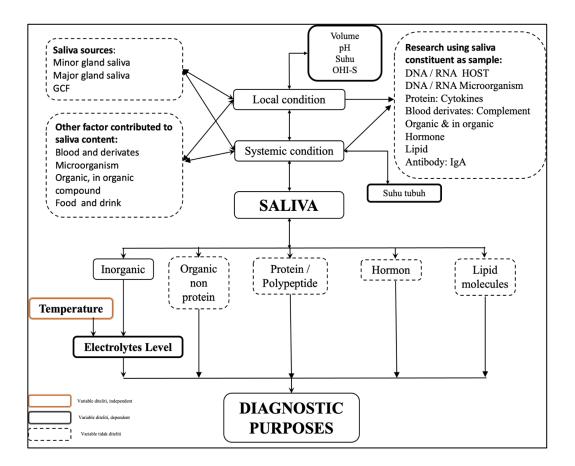
2.5.3 Inactivation of COVID-19 virus in saliva sample

Inactivation of SARS-CoV-2 can be done by different methods; like chemical inactivation using 0.5% of Povidone-Iodine oral antiseptic, and/or 70% alcohol can be rapidly inactivate the virus within 30 s contact time. Lysis buffers which are available in RNA extraction kit are also effective for SARS-CoV-2 inactivation without additional means. However, some detergents using for sample

treatment can inhibit PCR reactions. Some of cannot inactivate viruses properly without RNA degradation. Different strain of the virus could be inactivated with related temperature range. The famous method of SARS-CoV-2 inactivation at 56 °C for 30 min prior to extraction procedures was showed that leads to a clear drop of viral infectivity (>5 Log10 reduction). The other SARSCoV-2 inactivation at 60 °C for 60 min, 92 °C for 15 min, 80 °C for 5 min, and 100 °C for one minute also could be resulted significantly decrease the viral infectivity in a clinical specimen. However, sample treatment that use heat before molecular testing might destroy the viral RNA and can lead to false-negative

results.

After COVID-19 declared as a global pandemic, the numbers of suspected cases were increased day today and that needs to maximize laboratory testing capacity by high throughput automated and point of care testing instruments for SARS-CoV-2 detection. For these types of platforms sample inactivation is an important procedure to protect health care workers from the expo- sure of SARSCoV-2 infection. However, some studies showed that sample inactivation by using heat can leads to RNA degradation and false-negative results, An experimental study was conducted at Ethiopian Public Health Institute (EPHI) from September 25 to October 15, 2020. Concluded that heat inactivation at 56 °C for 30 min does not have statistically significant effect for the qualitative rRT-PCR detection

(positivity or negativity rate of detection) of SARS-CoV-2 infection. However, this study showed that there was statistically significant Ct value increment after heat inactivation at 56 °C for 30 min compared to untreated samples. So, a false negative result in high Ct value (especially greater than 35) might be the challenge

of this protocol. [22]

CONCEPTUAL FRAMEWORK