Saliva NMR-Based Metabolomics in the War Against COVID-19
Gilson Costa dos Santos Junior,* Claudia Maria Pereira, Tatiana Kelly da Silva Fidalgo, and Ana Paula Valente

ABSTRACT: COVID-19 is an emergent, worldwide public health concern. Joint efforts have been made by scientific communities of various fields to better understand the mechanisms of action of SARS-CoV-2. The need to understand the pathophysiological fingerprint and pathways of this disease make metabolomics-related approaches an indispensable tool for properly answering concerns relating to disease course. Determination of the metabolomic profile may help to explain the heterogeneous spectra of COVID-19 clinical phenotypes and be useful in monitoring disease progression as well as therapeutic treatments. In this sense, saliva has proven to be a strategic biofluid, owing not only to its appeal as a noninvasive sampling method but also due to the capacity of the virus to invade epithelial cells of the oral mucosa and salivary gland ducts via ACE2 receptors. Accordingly, important changes in metabolism have been described relating to COVID-19, indicating that metabolomics may open new avenues for understanding the pathophysiology of this disease, especially via longitudinal study designs. Thus, we discuss the importance of comprehending the SARS-CoV-2 salivary metabolomic fingerprint and also highlight the situation of Brazil on the frontlines of the war against COVID-19.

INTRODUCTION

A severe respiratory disease that was first reported in Wuhan, China,1 in December 2019 has been officially named COVID-19 by the World Health Organization.2 The causal agent of this disease was identified as the novel coronavirus, 2019-nCoV,3 that was renamed by the Coronavirus Study Group (CSG) of the International Committee on Virus Taxonomy as severe acute respiratory syndrome, coronavirus 2, i.e., SARS-CoV-2.4 COVID-19 is a worldwide public health concern. The ongoing COVID-19 pandemic has resulted in nearly 26,763,217 confirmed cases and 876,616 deaths as of September 6, 2020.5 Nasopharyngeal and oropharyngeal swabs are the recommended specimen types for detection of SARS-CoV-2; however, the importance of saliva in human transmission and detection of this virus has also been discussed.6 A proper understanding of the pathophysiology of this disease will provide more targeted treatment strategies.

Metabolomics is a very useful tool that has the unique feature of connecting all “omics” techniques and best represents the phenotype.7,8 Nuclear magnetic resonance (NMR) and mass spectrometry (MS) are the most used techniques in metabolomics. NMR has the advantage of being able to analyze most biofluids with only minor preparation. Additionally, NMR is non-destructive and more reproducible than MS.9,10 NMR approaches can identify the differences in low molecular weight molecules that exist in states of health or disease. NMR metabolomics has elucidated metabolic pathways related to different local and systemic diseases, such as osteoarthritis,11–13 Type 1 diabetes,14 melanoma,15 oral cancer,16 and dental caries.17,18

NMR METABOLOMICS OF VIRAL INFECTIONS

Most of the infections promoted by different types of viruses, including SARS-CoV,19 Zika Virus,20–22 Dengue Virus,23–24 Chikungunya Virus,25,26 Influenza Virus,27–29 HIV,30 and Hepatitis C,31 have been shown to modulate the metabolic profile of their target cells.

For instance, even 12 years after recovery, patients affected by SARS-CoV infection continue to present serum alterations in lipid metabolism as well as enhanced levels of lactate, cysteine, alanine, aspartic acid, and succinic acid.19 Additionally, it has been demonstrated that Zika virus infection leads to modulation of phospholipids and carboxylic acids in the microglia.20 Meanwhile, infections caused by Dengue Viruses...
enhance the levels of fatty acids, acylcarnitines, glycerolipids, fructose, and hydroxyl ketones, while infection by West Nile Virus enhances the levels of sphingolipids and glycerophospholipids while reducing the levels of cholesterol esters. Recent studies have been conducted to determine the metabolomic profile of the serum and plasma of COVID-19 patients. Shen et al. performed a cross-sectional study of serum metabolomics in healthy subjects and patients diagnosed with different severity levels of COVID-19 and found differences in amino acids, carbohydrates, fatty acids, and glycerophospholipids among the groups. An additional cross-sectional study also identified plasma profiles relating to various COVID-19 outcomes, demonstrating changes in the plasma at different stages of the disease.

It is worthy to note that the aforementioned studies were all conducted using either in vitro models or invasive methods. Therefore, noninvasive methods and longitudinal studies to better understand the disease evolution are still lacking. Saliva has been extensively used in NMR metabolomics studies; however, saliva NMR metabolomics are still under explored in the study of viral infections.

Perspectives of SARS-CoV-2 Salivary Metabolomics

The COVID-19 pandemic has driven the world’s attention towards fast and easy diagnostics. Interestingly, most of the current tests require buccal swab collection, since the virus can be transmitted through the saliva or nasal fluids. Moreover, the virus enters cells through ACE2 receptors, which are highly expressed by epithelial cells of the oral mucosa and salivary gland ducts, suggesting a probable route of SARS-CoV-2 entry. Despite great efforts to maximize the availability of diagnostic tools as well as the intense search for therapies to provide efficient control of the pandemic caused by the SARS-CoV-2 outbreak, many questions related to the metabolic pathway of this disease remain to be addressed while the literature remains scarce. Metabolomics points out intermediate metabolites that may be targets of SARS-CoV-2 infection. An understanding of the disease course will require investigations at the level of the metabolic phenotype, which results from the local microenvironment and the affected cell metabolism. In this sense, more than 200 endogenous and exogenous biomolecules from whole saliva can be identified by NMR, including molecules relating to glandular metabolism, such as salivary secretion, as well as gingival fluid, which carries systemic metabolites, dietary compounds, and products derived from oral health and pharmaceutical products.

Whole saliva is composed of a variety of electrolytes, lipids, and proteins, including components of the immune response with antiviral activity, such as α- and β-defensins, secretory immunoglobulin A, agglutinin gp340 (DMBT-1), lactoferrin, lysozyme, and cathelicidin (LL-37) as well as low molecular weight metabolites, such as carbohydrates, amino acids, organic acids, polyphenols, and fatty acids, among others; these various metabolites are responsible for maintaining the balance of oral tissues and can reflect both local and systemic conditions.

The infection resulting from SARS-CoV-2 is not well understood at the cellular metabolite level; in this sense, the SARS-CoV-2 metabolic fingerprint would benefit from the comprehension of both the disease course as well as the heterogeneous spectra of clinical phenotypes seen from COVID-19, which vary from mild flu symptoms to acute respiratory distress syndrome (ARDS) and, in some cases, multiple organ failure and death. Moreover, this sort of analysis would aid in the comprehension of vulnerable populations, such as the elderly people and people with specific systemic comorbidities. Recently, the Australian National Phenome Center (ANPC) started a collaboration with Bruker (the largest NMR manufacturer in the world) in an attempt to deliver diagnostic and prognostic solutions for COVID-19 utilizing NMR and MS. However, at this point, the researchers are collecting only plasma and urine. In this context, more international cooperation, longitudinal studies, and observational and clinical trials are essential to determine the COVID-19 metabolic profile, establish the evolution of the disease, and evaluate potential treatments.

In March 2020, the Council for State and Territorial Epidemiologists (CSTE) added a temporary loss of taste (ageusia) and smell (anosmia) to the symptoms compatible with SARS-CoV-2 infection. The exact mechanisms underlying this olfactory and gustatory dysfunction remain uncertain. It is possible that SARS-CoV-2 targets the olfactory epithelial support cells that express molecules involved in coronavirus entry and dissemination (ACE2 receptor and Transmembrane Serine Protease 2-TMPRSS2), thus leading to anosmia and alterations in taste perception. Some drugs used in the treatment of cardiovascular diseases (e.g., ACE inhibitors, angiotensin II receptor blockers, and calcium channel blockers) are known to affect taste perception. It is Probable that SARS-CoV-2 alters taste sensitivity by a mechanism analogous to the ACE inhibitors. In this scenario, saliva metabolomics emerges as a potential strategy for identifying biomarkers with diagnostic potential as well as providing a description of the metabolic pathways involved in the olfactory and taste disorders observed in COVID-19 patients. Although it seems that the taste changes in patients with SARS-CoV-2 infection are mostly related to neurological events, metabolomics will open new perspectives of analysis, as mentioned above. The concentration of salivary components directly affects taste perception. The taste receptors are bathed in saliva and adapt to the concentrations of salivary components, such as salt, glucose, and urea. In order to perceive a taste, the concentration of a food must exceed the basal salivary levels resulting in sweet, sour, salty, bitter, and/or umami taste. For example, renal failure subjects presenting with higher levels of urea in their saliva also have a higher threshold for bitter taste perception. There is evidence that subjects insensitive to the perception of oleic acid present higher levels of acetate and butyrate in their saliva, along with a reduced concentration of lysine and fatty acids. An additional study demonstrated that sensitive oleic acid perceivers present increased levels of acetate, propionate, formate, lysine, valine, and GABA in their saliva, while insensitive oleic acid perceivers demonstrated higher concentrations of galactose, glucose, lactate, threonine, phosphocholine, and ethanolamine. Additionally, L-Arg seems to be capable of modulating bitter taste function and suppressing the bitterness of quinine by specifically blocking the T2R4 receptor. Therefore, these findings reinforce the role of additional factors related to metabolomics and taste perception in SARS-CoV-2 infected patients.

Salivary metabolomics is a promising field in terms of better understanding COVID-19 phenotypes and how SARS-CoV-2 impacts human physiology. However, specific challenges relating to this biofluid, as well as other technical limitations,
must be outlined so that strategies to overcome these limitations can be developed. The major concern is the difficulty involved in screening a large number of subjects in order to find valid, representative biomarkers. In this sense, networked collaborations between medical centers will be required to safely obtain the samples, while investments in metabolic centers will be needed to address automatization and biosafety issues. It is also recommended that small groups conduct multicentric studies to increase the sample size.

Another barrier to the saliva metabolomics approach is the low signal in saliva when compared with others biofluids, such as plasma, serum, or urine, due to the lower concentration of metabolites. Whole saliva is composed of ~99% water, while the remaining 1% consists of organic and inorganic content. Although seemingly minor, the 1% composed of metabolites remains as a unique information source from the oral cavity regarding the body’s physiological condition. The reduced levels of salivary metabolites can be overcome by using higher field magnets, pulse sequence improvements, and concentrating samples via the lyophilization process. Another limitation is the fluctuation in metabolites levels that occurs throughout the day; these oscillations are controlled by the circadian cycle, which is independent of sleep or food intake. For this reason, sampling for metabolomic studies that use saliva as the biofluid must occur at the same period of the day, preferably in the early morning (i.e., 8:00 to 10:00 am), in order to avoid these fluctuations.

Furthermore, it has been demonstrated in the literature that intraindividual variability in the metabolic composition of saliva is strongly influenced by dietary habits. Additionally, Walsh et al. demonstrated that inter- and intraindividual variation is not reduced by standardization of diet intake on the day before saliva collection. If food consumption patterns modulate the metabolic fingerprint of saliva, then why are there numerous studies that do not perform dietary interventions on their volunteers and how did this method successfully elucidate the salivary metabolomic fingerprint of specific diseases? To answer these questions, some key reflections must be pointed out. Variability in food consumption increases interindividual variability among subjects in the same study group, and this variation is possibly overlaid by the metabolic changes resulting from the disease process when compared to the control subjects. Another key point that deserves attention is the short period of confounding effects on salivary metabolomics from food and drink intake. Protocol recommendations for NMR-based salivary metabolomics include that the participants should abstain from ingesting substances prior to saliva collection. Saliva collection should be done at least 1 h after eating, as this is an adequate interval of time for the elimination of carbohydrate peaks that blur salivary metabolite results. Additionally, Fidalgo et al. preconize refraining from oral activities including toothbrushing for 2 h prior to sample collection. Finally, a key concern in the use of salivary metabolomics for analyzing systemic conditions is the interpretation of biomarkers without considering the current oral condition. Several studies have demonstrated that salivary metabolomics can reflect oral status and oral disease fingerprint, including dental caries and periodontal disease. Therefore, to control for this issue, it is highly recommended to examine the oral cavity to determine whether the eligible biomarker is actually related to a disturbance in the oral condition.

Identification of the salivary profile of SARS-CoV-2 infected individuals throughout the different stages of the disease could help predict disease progression, recovery, and the therapeutic effects of various treatments. All told, salivary metabolomics is a promising, noninvasive tool for shedding light on how this virus affects host metabolism, and may provide valuable information regarding the pathophysiology of this disease.

**PERSPECTIVE FOR BRAZIL**

It is important to highlight that a recent projection points to Brazil as the next putative global pandemic epicenter for COVID-19, and in September of 2020, Brazil became the country with the second most confirmed cases, equal to 4 123 000. Fortunately, Brazil has the biggest NMR facility in Latin America, the National Center of Nuclear Magnetic Resonance, which houses several field magnets, including 400, 500, 600, 700, and 800 MHz magnets as well as the recently installed 900 MHz magnet (the strongest NMR field magnet in Latin America). These conditions place Brazil on the frontlines of the war against COVID-19, however, also with powerful weapons against this virus.

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**Author Contributions**

G.C.S.J. conceived the presented idea and designed and revised the manuscript. C.P., T.K.S.F., and A.P.V. made critical revisions and designed the final version of the manuscript.

**Notes**

The authors declare no competing financial interest.

Compliance with Ethical Requirements: Gilson Costa dos Santos Junior, Claudia Pereira, Tatiana Kelly da Silva Fidalgo, and Ana Paula Valente comply with ACS ethical policies. The above authors consent that the research did not involve humans or animals. The above authors consent that no ethical approval is required by the National Committee of Ethics and Research Committee.

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