ORIGINAL RESEARCH

Assessment of Salivary pH amongst the Population during Severe Acute Respiratory Syndrome Corona Virus (COVID 19) Pandemic Era – An Original Research

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Abstract

Introduction: The outbreak of Covid 19 disease is a major public health concern worldwide. Since oral findings and their occurrence may differ greatly in Covid-19 patients, salivary studies are crucial in understanding the role of saliva in maintaining oral health. The purpose of this study was to evaluate and compare the salivary pH level in Covid, post Covid and normal subjects.

Materials and Methods: The study population consisted of 90 subjects which were divided into three groups of 30 subjects each. RT- PCR Positive Covid patients during our study period were taken as Group I and patients who were Covid positive two months prior to our study period were taken as Group II. Group III consisted of normal subjects who were RT-PCR negative for Covid and was never diagnosed with the Covid 19 virus. pH of the saliva was immediately evaluated using Saliva check pH strips.

Results: Our study results reveal that there is significant difference in mean pH between Covid, post Covid and normal subjects.

Conclusion: This study suggests that while comparing salivary pH with normal population, people affected with Covid19 have an acidic salivary pH which may affect their oral health.

Keywords: Covid 19, Saliva sample, Oral health, Saliva pH, Aquaporin, ACE-2 receptors.

Introduction

The world is at risk of the novel corona virus sickness 2019 (COVID-19), which is caused by the corona virus. The virus is primarily transmitted by direct or indirect personal contact through air borne respiratory droplets from an infected person. Despite repeated attempts to halt the spread of the disease, it remains a major public health concern. Presently a number of researchers have investigated the oral cavity as a potential source of infection, as well as the consequence in dental practice and the use of saliva in COVID-19 diagnosis.

COVID-19 has similar clinical features to several other viral diseases. Common oral manifestations associated with COVID-19 reported were dysgeusia, hyposalivation, burning mouth and oral ulcers. However, the majority of Covid patients' main complaints were loss of taste and smell[1,2]. Saliva and its properties or constituents like pH, buffering capacity, enzymes, electrolytes etc play a critical role in the oral chemosensory perception and maintaining oral homeostasis. Oral findings and their occurrence may vary significantly among COVID-19 patients, so salivary studies are important in understanding the oral diseases in such patients.

Maintenance of normal pH (6.7-7.4) in oral cavity would sustain the integrity of teeth by controlling demineralization and promoting re-mineralization of the enamel surface. It also helps in maintaining normal oral microbial flora, electrolyte

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balance and enzymatic function. Salivary pH changes have been linked to xerostomia, caries, periodontal problems, halitosis, and taste sensorial complaints[3,4]. Salivary pH plays an important role in maintaining the integrity of the periodontium through its buffering action, as well as controlling demineralization and promoting re-mineralization, which occurs continuously at the enamel surface. The low salivary pH below 5.5 creates an acidogenic environment for the growth of aciduric bacteria, resulting in dental caries, which lowers the salivary pH even further, creating a vicious cycle[5].

In Covid infection, there is an increase in inflammatory cytokines such as interleukin-6, interleukin-7, and transforming growth factor-beta, which are associated with a periodontal disease.[6] It's worth noting that saliva not only provides an ecological niche for the colonization and development of oral microbes, but it also stops harmful pathogens from overgrowing. The periodontal diseases in humans and other mammals are known to be associated with Gram-negative anaerobic organisms.[7,8]

In humans, ACE2 receptor expression was observed in the cell membrane of duct components including interlobular excretory ducts of salivary glands, but it was not detectable in the squamous epithelium of oral mucosa. SARS-CoV-2 virus has affinity for these receptors and therefore the Covid infection can alter the properties of saliva.[9]

To the best of our knowledge, the scientific literature contains only a few reports of oral findings associated with previous corona viruses. This study aims to evaluate the pH level in Covid, post Covid and normal subjects in order to identify patients at risk for periodontal disease, caries susceptibility, as well as to assess the prognosis of any existing periodontal disease in them and, consequently, to assess the overall oral health status. The objective of the study was to evaluate and compare the salivary pH of Covid, post Covid and normal subjects.

Materials and Methods

The study population consisted of 90 subjects of age group 18-30 years of age. They were divided into three groups of 30 subjects each. RT- PCR Positive Covid patients during our study period were taken as Group I and subjects who were Covid positive two months prior to our study period were taken as Group II. Group III consisted of normal controls who were RT-PCR negative for Covid and had never been infected with the Covid 19 virus. Subjects with any systemic disease or taking any drugs were excluded from the study design. The randomized unstimulated saliva from each subject was collected and the pH was tested. The data was analyzed statistically using IBM SPSS 20 software. (SPSS Inc. Chicago USA)

The study was conducted in Malabar Dental College and Research Centre, Kerala over a period of 11 months. After obtaining the Ethical clearance from the institutional review board, subjects were informed about the objective of the study and consent was taken before taking part. The WHO-recommended safeguards for health-care personnel were followed.

pH of the saliva was immediately evaluated using Saliva check pH strips. A pH test strip was taken and placed into the sample of resting saliva for 10 seconds and then the color of the strip was checked and recorded. pH was determined based on the color change in comparison to the gold standard chart provided by the manufacturer.

Results

The mean pH of saliva in RT PCR positive Covid infected group, post Covid subjects and normal controls were found to be 6.54, 6.967, 7.353 respectively. Among Covid subjects the maximum level of pH was 7.2 while it was only 7.6 among post Covid subjects and in normal controls. Similarly, the minimum pH level in all 3 groups was found to be 6.4.

Statistical analysis for all the continuous variables, the results were either given in Mean±SD, and for categorical variables as percentage (Table 1). To compare salivary pH of Covid, post Covid and normal subjects using Dental Saliva Check-pH strips, one-way ANOVA (Table 2, Graph 1) with Games-Howell Post Hoc analysis (Table-3) was applied. The mean difference is significant at the 0.05 level.

The mean pH with standard deviation in Covid group was 6.540±0.224, post Covid group was 6.967±0.217 and in normal controls was 7.353±0.343. The comparison of mean pH among the group was found to be statistically significant at the 0.05 level. The mean pH was higher in normal group compared to Covid and post Covid subjects.

Study also showed a significant difference in mean pH between Covid patients and post Covid subjects. This suggests that there is a considerable mean pH difference between the groups, confirming that the Covid 19 infection has a substantial impact on salivary pH.

Table 1: Comparison of mean pH among the group

pH	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Covid pH	30	6.5400	.22376	.04085	6.4564	6.6236	6.40	7.20
post Covid pH	30	6.9667	.21709	.03963	6.8856	7.0477	6.40	7.60
Control	30	7.3533	.34314	.06265	7.2252	7.4815	6.40	7.60
Total	90	6.9533	.42617	.04492	6.8641	7.0426	6.40	7.60

Discussion

The oral cavity is a potential reservoir for pathogens which can predispose to various infections. The literature review suggests that Covid-19 could have a potential impact on the oral health status[3]. The primary focus of this study was to assess the variation of salivary pH in Covid, post Covid and normal subjects. Watanabe et al discovered that the method using the pH indicator strip is highly reproducible both within samples and between the examiners. They also confirmed that by using linear regression analysis, the results obtained using a pH strip and those acquired using a pH meter were found to be in accord[10]. In our study, pH value was ascertained in the resting saliva sample using the pH strips.

In normal subjects the pH of saliva is maintained near neutral. In our study we found that the control group has a mean pH of 7.353±0.343 which is in normal range. Multiple comparison test using James Howell with Post Hoc analysis revealed a statistically significant difference between all the 3 groups. In contrast to the pH of control group the Covid positive group (6.540±0.224) had a statistically significant difference of -.81333. Comparison between the salivary pH of control and post Covid group (6.967±0.217) also showed a significant difference in pH (-.38667). Equating the mean pH values of Covid positive and post Covid patients, there also existed a significant difference (-.42667). These values clearly indicate in Covid patients the pH of saliva reduces considerably. Comparison of mean pH among groups were done using ANOVA test and were statistically significant at the 0.05 level.

In a similar study conducted by Ahmadi-Motamayel F et al also showed that salivary pH in the HIV-positive group was lower (6.99±0.46) than the normal controls, but in their study the difference obtained was not statistically significant.[11] Research conducted by Alessia et al indicated that miRNA of Epstein Barr Virus can be transferred from infected B lymphocytes to salivary gland epithelial cells via exosomes, where it decreases STIM1 (Stromal Interaction Molecule1) and Aquaporin-5 (AQP5) protein levels in patients with infectious mononucleosis. Aquaporin-5 is critical for saliva production. It is a specific channel protein found in the acinar cells that allows for rapid transcellular migration of water in response to an hydrostatic/osmotic pressure gradient. The decrease in STIM1 adversely affects the regulation of calcium signaling and NFAT activation there by altering the biochemical properties such as electrolyte balance and buffering capacity of saliva.[12,13,14] The same mechanism could be one of the reasons for change in salivary pH of Covid patients also.

The most prevalent oral fungus infection, candidiasis can also trigger host immune responses by activating T cells. These T cells stimulate the production of inflammatory cytokines like TNFα, IL-6, and IL1β[15]. TNF-α suppresses the transcription of Aquaporin-5 and destroys human salivary gland acinar cells. These inflammatory responses may impair salivary glands, changing the biochemical characteristics of saliva, most notably its buffering ability.[16,17] Normal functioning of salivary gland is necessary to maintain adequate levels of bicarbonate/carbonate ions, phosphate ions, proteins so as to maintain the pH within normal range[18].

SARS-CoV-2 can attach to ACE-2 receptors on the epithelium of salivary glands, fuse with them, replicate, and lyse cells to trigger apparent signs and symptoms, such as discomfort, inflammation, and pain in major salivary glands. Liu et al. studied SARS-CoV and showed that epithelial cells of salivary gland have elevated ACE-2 expression indicating ACE-2 receptor is a critical COVID-19 receptor. Cytokines storm caused by Covid 19 infection can also facilitate the inflammatory reaction that destroys the tissue of the salivary glands (the cytolytic activity of SARS-CoV-2 lyses the acinar cells) as the immunopathological process continues and can alter the normal salivary flow rate, protein and electrolyte concentration altering the biochemical properties. In SARS-CoV infections, xerostomia could be aggravated due to nasal congestion and rhinorrhea, where the oral breathing increase and it can impair salivary gland function and leads to xerostomia.[19,20]

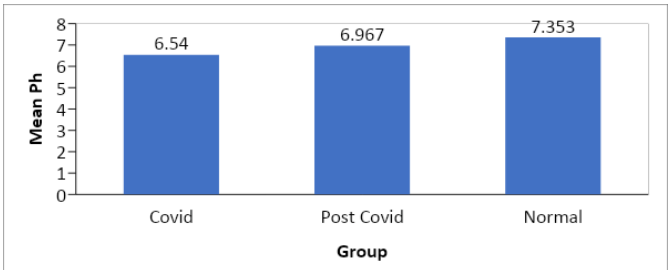
Alsopandemic-induced psychosocial factors have a greater impact on normal salivary gland function and quantitative secretions. Due to widespread Covid 19 pandemic, Patients were increasingly exhausted and emotional deterioration was becoming more frequent due to social isolation such that a new term corona blue has appeared. Mental health issues such as

Table 2

pH	Sum of Squares	Df	Mean Square	F	Significance
Between Groups	9.931	2	4.965	69.302	0.05

Table 3

Category (I)	Category (J)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Covid pH	post Covid pH	-.42667*	.05692	.000	-.5636	-.2898
	control	-.81333*	.07479	.000	-.9940	-.6327
post Covid pH	Covid pH	.42667*	.05692	.000	.2898	.5636
	control	-.38667*	.07413	.000	-.5658	-.2075
control	Covid pH	.81333*	.07479	.000	.6327	.9940
	post Covid pH	.38667*	.07413	.000	.2075	.5658



Graph I

mood swings, headaches, depression, stress often led to poor nutrition intake and poor personal hygiene which contributed to development of oral ulcers, xerostomia, and plaque deposition on teeth. All these factors could have an impact in the pH of saliva.[21,22]

A longitudinal study to follow up the duration of varied pH in Covid patients to be conducted as, long standing acidic pH has serious implications in oral environment.

A case–control study demonstrated an association between periodontitis and the severity of COVID-19 infection. It has been found that there is a clear correlation between these two diseases. Recurrence of COVID-19 infection showed alteration of normal saliva flow rate, pH and salivary constituents. Moreover, the cytokine storm syndrome caused by the Covid 19 virus could also accentuate existing periodontitis in patients[23].

According to Shaila Mukhi et al simple biochemical tests revealed a substantial difference between salivary pH, composition, and flow rate in gingivitis and periodontitis. A significant decrease in pH of saliva and salivary flow rate were noted in periodontitis patient compared to normal controls.[24]

A study by Takahashi et al on the effect of pH on the growth of periodontal pathogens showed that P. gingivalis grows at a pH of 6.5-7.0, P. intermedia grows at a pH of 5.0-7.0 and F. nucleatum grows at a pH of 5.5-7.0. These microorganisms showed an increased rate of growth at low pH. Thus, change in pH could alter the normal oral microbial flora and could lead to periodontal disease[25].

In a study done by Kho HS et al in-end stage renal disease patients, a decreased immune-competence as well as the change in normal constituents of the saliva was noted. This variation reduced the buffering capacity, which decreased salivary pH.[26]

Our study was designed to investigate the change in salivary pH of Covid patients. We had divided our study subjects into 3 groups (Covid, post Covid and normal subjects) with 30 participants in each and it was learnt that the mean salivary pH of Covid and post Covid subjects were significantly lower than that of normal subjects. The change in pH of saliva could be due to the change in its components as well as the flow rate due to Covid infection per se. Accordingly, all the aforementioned alterations in saliva caused by Covid 19 infection can lead to unpredictability of oral health. Long term follows up of the cases to measure the impact of this pH change in Covid and post Covid subjects, also the time taken for this low pH to come back to normal was not taken into consideration and this constitutes the limitation of this study.

Conclusion

Saliva samples offer great potential and advantages over other biological fluids in research because they do not require invasive intervention and can be used to evaluate oral health.

In this study we have observed a statistically significant difference in the pH of saliva between Covid, post Covid and normal subjects. Our study suggests that pH has a great influence on SARS-CoV-2 Infection and Covid-19 severity. Covid infection reduces the salivary pH and this low pH acts on ACE 2 expression in Covid patients which further increases the severity of the infection. A change in the pH of saliva even if it is for a short period of time will have its own negative impact on oral health and a long-standing acidic environment will have more serious adverse effects. A longitudinal study to determine the time taken for the acidic saliva to become normal, must be designed with a larger sample so as to validate the duration of low pH in oral cavity after Covid infection.

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