Original Article

SalivarypH:Apossiblediagnostic marker for Covid-19

Mumtaz Gowhar, Aijaz Ul Noor, Ashaqullah Bhat, Ruqaya Aziz,

Background: COVID-19 is a serious and potentially emerging global health crisis. Early diagnosis of infected individuals will play an important role in the management of COVID-19. Saliva, a bio fluid largely generated from salivary glands in oral cavity were reported to show the expression of angiotensin converting enzyme 2 (ACE2). Limited studies are there to find exact correlationofsalivarybiomarkerswithCOVID19.Theaimofthisstudywasthereforetoanalyzethephofthesalivainasampleof123patientsanddetermineitsrelevanceasapotentialdiagnostic biomarker in COVID-19 disease.

Method: 3 groups consisting of clinically healthy subjects, symptomatic and asymptomatic COVID -19 patients were studied. The randomized unstimulated saliva from each patient was collected and pH was tested.

Results: From April 1st, 2020 to September 30th, 2020 we identified a total of 123 COVID-19 patients. The average age of the patients was 55 years and most of patients were male 80 (65.1%).Of all the patients, 57 (46.3%) were symptomatic and 66(53.7%) were asymptomatic. The results demonstrated that salivary pH was slightly alkaline for COVID -19 patients. The salivary pH showed significant differences between symptomatic(P=0.00001)and asymptomatic(P=0.001)patients as compared to control group.

Conclusion: These results indicate a significant change in the pH depending on the COVID-19 infection. We concluded that salivary pH may probably be used as a quick potential diagnostic biomarker n. close monitoring of salivary pH may be helpful for early diagnosis.

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Introduction

Corona virus disease 2019(COVID-19)caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an emerging global health crisis. The clinical features of COVID-19 are varied, ranging from an asymptomatic state to acute respiratory distress syndrome(ARDS).The ARDS is characterized by multi-organ dysfunction driven by hyper-inflammation and a cytokine storm syndrome (increased cytokine levels via interleukins (IL)6,8,10,and tumor necrosis factor (TNF)α.

The clinical symptoms are fever, dry cough, sore throat, difficulty in breathing, fatigue, and others. With no approved treatment or prophylaxis, the virus gets rapidly transmitted by infected saliva droplets, inhalation or contact, and fomites [1-4]. The incubation period ranges from 2to14 days. The World Health Organization(WHO)has declared this a global pandemic by March2020andclaimedthatsheddingofSARS-CoV-2 into saliva droplets or discharge from the nose droplets plays a critical role in viral transmission [5].

Saliva has the potential to play are leinearly diagnosis and prognosis of infectious diseases. Saliva is a complex bio-mixture of fluids produced and secreted from the major and minor salivary glands within pH range of 6.0 – 7.0 [6-7]. Around 99% of saliva is made up of water and the rest 1% is made up of organic components (phosphorousandlacticacid)

Author Affiliations
Mumtaz Gowhar, Department of Physiology, SKIMS Medical College, Srinagar, **Aijaz Ul Noor,** Department of Biochemistry, SKIMS Medical College, Srinagar,, **Ashaqullah Bhat,** Department of Physiology, SKIMS Medical College, Srinagar, and **Ruqaya Aziz,** Department of Biochemistry, SKIMS Medical College, Srinagar.

Correspondence
Prof. Ruqaya Aziz, Department of BiochemistrySKIMS Medical College, Srinagar, Kashmir, India
Email: azizruqaya@gmail.com

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nitrogenous components (total nitrogen, protein, and non-protein nitrogen) and enzymatic components(amylase, maltase, invertase, beta-glucuronidase, hyaluronidase, lysozyme and phosphatases) [8]. When glandular secretions are released into the oral cavity, the fluid is mixed with exocrine, non-exocrine, cellular, and exogeneous components to form whole saliva (WS). WS is a complex fluid, which consists of salivary secretions from different salivary glands, desquamated oral epithelial cells, nasopharyngeal secretion, microbiota (bacteria and their products, viruses and fungi) and may contain blood and its derivatives and food debris in pathological occasions [9].

The study by Liu et al. found that angiotensin-converting enzyme 2-positive ($ACE2^+$) cells were widely distributed in the epithelial cells lining salivary gland ducts. The data of this group further suggest that $ACE2^+$ cells are early target cells of SARS - CoV infection in the rhesus macaques [10]. Besides evidence from animal study, Chen et al. analyzed data from GTEx, HPA, FANTOM5, and Consensus dataset in the Human Protein Atlas (Human Protein Atlas) and confirms the expression of ACE2+ cells in the salivary glands [11].

With the lower risk and non-invasive nature, the saliva specimen collection has the advantage of being more acceptable for patients and healthcare workers. The recent studies demonstrate the possibility of SARS-CoV-2 infection of salivary glands[11-12]. However, there is scanty limited data regarding the use of salivary pH as a diagnostic marker in COVID-19.This study is therefore aimed at evaluating the pH of saliva as a diagnostic marker of COVID – 19 disease.

Materials and methods

Study design

From April 2020 – September 2020, the subjects were recruited from SKIMS-MCH, Srinagar. The presence of SARS-CoV-2 infected patients was detected by real-time reverse transcription PCR. The study was approved by the institution's Ethics Review Committee. The study was conducted according to the Declaration of Helsinki practice. After written informed consent,123 patients with COVID-19 disease were enrolled in this study COVID-19.

Saliva sampling

Blood samples were collected in the morning between 9:00 am and 11:00 am. after an overnight (8–10 hours) fast, during which subjects were requested not to drink any beverages aside from water. The subject is made to sit comfortably, in a standard dental chair. For the collection of saliva, the subjects were requested to rinse the mouth thoroughly with distilled water to remove any food debris. The saliva is allowed to accumulate naturally in the floor of the mouth. The subject spits out it into the graduated test tubes about once a minute for up to 10 min to collect about 5 ml of saliva. The pH of the saliva was immediately measured in order to prevent any deterioration of the sample.

LaboratoryAnalysis

The laboratory analysis was carried out in the Department of Biochemistry, Sheri-Kashmir Institute of Medical Sciences Medical College and Hospital (SKIMS-MCH). The pH of saliva was measured as soon as possible and not later than3 0minutes after the collection of the sample.The pH measurement was done directly, using a hand-held digital pH meter (Hanna, Model pHel 1, Z379395-1EA,Sigma-Aldrich Chemicals Pvt.Ltd, Bengaluru, India) place over a stand to prevent variations in the readings due to handling movements. The pH has along, slim stem with a double junction gel-filled electrode, making it possible to measure little quantity of samples in small vials. The pH meter's range of measurement is 0 to14pH. The electrode was immersed in the sample in a closed container, the digital reading was allowed to stabilize for few seconds, and the final stable reading was used to determine the salivary pH value. The electrode was washed with distilled water in between readings and placed in a standard solution of pH 7. This ensured consistent readings and allowed for continuous monitoring of any drift.

StatisticalAnalysis

The collected data was entered in the Microsoft Excel Sheet and statistical analysis was undertaken with the version 26.0 of SPSS software (SPSS Inc., Chicago, IL, USA).

Results

Demographic distribution of study participants are summarized in Table 1. Among 123 subjects involved in the study, 80 (65.1%) were male and 43 (34.9%) were female. The mean age of the study participants were 55. Out of 123, 57 (46.3%) were symptomatic and 66 (53.7%) were asymptomatic for COVID -19 infection.

It was found that the pH of saliva from COVID-19 patients was slightly alkaline as compared with that of the clinically healthy group (control population). As shown in Table 2, the average pH of the control group was 6.7 ± 0.12. The average pH of the group having symptomatic COVID -19 patients was7.34±0.57(P=0.00001), where as the average pH of the group having asymptomatic COVID -19 patients was 7.2 ± 0.60 (P = 0.001). (Table 2 and 3)

Discussion

Saliva has been used as a diagnostic method for a variety of systemic diseases. Since saliva is non-invasive and easy to obtain, considerable research effort have been made to validate biomarker profile in saliva for clinical diagnostics. As a result, emerging evidence suggests that whole saliva can be used to diagnose a variety of systemic and oral diseases [13-15].The use of saliva for the diagnostic of emerging viral diseases is now well established [16-17], however routine applications are still rare because of the lack of well-standardized protocols The recent studies reported the detection of RNA viruses in the human saliva. The Niedrig M et

al.reporteda number of salivary-based detection methods for Ebola virus [17]. Another study by Khurshid Z et al.suggest that the human saliva can be a diagnostic tool for zika virus detection[18].

The findings by Wang WK et al. reported large amounts of SARS-CoV RNA in the saliva from 17 SARS infected-patients and suggests that saliva should be included in sample collection guidelines for SARS diagnosis [19]. Zelus BD et al. have reported that the murine coronavirus S protein can be triggered to a membrane-binding conformation at 37°C either by soluble receptor at neutral pH or by alkaline pH (pH 8) alone,without

Characteristics		N=123
AverageAge		55
Gender	Male	80(65.1%)
	Female	43(34.9%)
SymptomaticPatients		57(46.3%)
AsymptomaticPatients		66(53.7%)

Table1:Demographicaldataofthestudypopulation(n=123)

Group	Characteristics	AveragepH
GroupA	SalivarypHofControlGroup	6.7±0.12
GroupB	SalivarypHofSymptomatic Patients	7.34±0.57
GroupC	SalivarypHofAsymptomatic Patients	7.2±0.60

Table2:AveragepHofvaluesofCOVID-19symptomaticandasymptomaticpatients

Comparisonbetweengroups	*P*values
Avs.B	0.00001
Avs.C	0.001
Bvs.C	0.12

Table3:Pvalueforsymptomaticandasymptomaticgroupcomparison

.requiring previous activation by cleavage between S1 and S2 [20]. In our study, the salivary pH from SARS-CoV 2 infected patients was slightly alkaline as compared with that of the control group. This finding supports the possibility that salivary pH can be used as an indicator of prognosis during SARS-CoV-2 infection. It is conceivable that alkaline pH exposes a hydrophobic domain that presumably initiates fusion with host cell membranes in SARS-CoV-2 infected patients. To our knowledge, this report is the first that demonstrates the possibility of salivary pH for SARS-CoV-2 detection.

A limitation of the present work is the small sample size, therefore future study with large sample size is required to draw definite conclusions. Also, this is a single institute study and needs to be replicated in further multicentric settings.

Conclusion

In our study, the pH of saliva from COVID-19 patients was slightly alkaline and found that there is a relationship between SARSCoV-2 infected patients and alteration of pH.The findings of our study suggest that salivary pH can detect possible SARSCoV-2 infected patients with altered oral homeostasis.

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Competing Interests:The authors declare that they have no competing interests.

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