Original Article

Prevalence of dental caries among coronavirus disease 2019-recovered patients and correlation with salivary total antioxidant capacity in Kalaburagi region of Indian subpopulation

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Abstract

Background: Coronavirus disease 2019 (COVID-19) has vastly affected mankind worldwide, and its long-term effect on oral health needs to be studied.

Aims: The aim of the study was to evaluate the prevalence of dental caries and its correlation with salivary total antioxidant capacity (TAC) in Kalaburagi region of the Indian subpopulation.

Methods: About 80 subjects (16–55 years) were included in the study based on selection criteria and were divided into two groups ($n = 40$). Group 1: no history of COVID-19 infection and Group 2: COVID-19-recovered subjects. Each group was subdivided into two subgroups ($n = 20$) A and B with and without dental caries. Dental caries was assessed using the decay, missing, filled, treatment (DMFT) index by the World Health Organization. About 5 ml of unstimulated saliva samples was collected and centrifuged at 2000 rpm for 10 min, and the supernatants were collected. Salivary TAC was estimated using 2,2′-azino-bis-[3-ethylbenzothiazoline-6-sulfonic] acid assay and observed under spectrophotometer (734 nm).

Statistical Analysis: Data were analyzed using the Shapiro–Wilk test for normality distribution followed by one-way analysis of variance and *post hoc* Tukey test ($P < 0.05$).

Results: COVID-19-recovered subjects showed a higher prevalence of dental caries and lower TAC levels than non-COVID-19 subjects.

Conclusion: Higher prevalence of dental caries with lower levels of salivary TAC was found in COVID-19-recovered individuals.

Keywords: Coronavirus disease 2019; dental caries; prevalence; salivary antioxidant capacity

INTRODUCTION

The global pandemic coronavirus disease 2019 (COVID-19) has vastly affected the mankind. According to the World Health Organization (WHO), as of December 19, 2023, there have been 772,838,745 confirmed cases of COVID-19, including 6,988,679 deaths across the world. As of December 19, 2023, a total of 13,534,474,309 COVID-19 vaccine doses have been administered and in India, since its onset in January 2020–December 19, 2023, there have been 45,010,891 confirmed cases of COVID-19 with 533,346 deaths, a total of 2,206,773,886 vaccine doses have been administered.[1] COVID-19 has affected the health system at large including the oral cavity.[2] It has been reported

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that oral health conditions such as dry mouth, sore throat, periodontal symptoms such as gingival bleeding, gum pain, mobility of teeth, and orofacial pain, occur in adults with COVID-19 infection.[3]

Since dental caries is an alarming oral health condition that affects people of all ages worldwide. It is well recognized that dental caries has a multifaceted etiology and pathophysiology.[4] An imbalance between host, environmental, and microbiological variables leads to the occurrence of dental caries. Caries occurrence has been linked to changes in salivary components of the host factor such as pH, viscosity, flow rate, and changes in its composition.[5]

Recently, the diagnostic approaches are inclined toward the noninvasive methods. Saliva contributes to defence mechanism due to the fact that it is a source of enzymatic and nonenzymatic antioxidants that maintain redox balance in addition, it contains variety of indicators/ biomarkers of biological significance. It is easy to obtain with noninvasive retrieval associated with less complexity. It has a varied composition than serum, antioxidant content of saliva, and blood correlate well. It has less practical and ethical issues; hence, it is the preferred body fluid for diagnosis.[6]

Saliva is the most appropriate body fluid to determine antioxidant capacity since salivary antioxidants are the first-line defense and exhibit a significant role in the anticariogenic effect. Stabilization and deactivation of free radicals (FRs) are controlled by antioxidants. Any imbalance between the reactive oxygen species, reactive nitrogen species, FRs, and antioxidants causes oxidative stress. Several studies have reported the association of total antioxidant capacity (TAC) with caries occurrence.[7]

Since COVID-19 has vastly affected the global population, and several studies were reported earlier regarding its effect on oral conditions.[2,3] However, the majority of studies were the questionnaire-based studies, cross-sectional studies, and observational studies, and most of the studies were conducted during COVID-19-infection outburst, i.e., 2020–2022.[8,9] There is a lack of scientific evidence related to the post-COVID-19 effects or long-term effects on dental caries occurrence among the patients who were affected by the COVID-19 infection. Hence, this study was undertaken to evaluate the prevalence of dental caries and its correlation with salivary TAC levels among COVID-19-recovered subjects of Kalaburagi region in Northern Karnataka of India.

METHODS

The institutional ethical clearance for this study was obtained (Reference no. IEC/2020-21/28) and performed accordingly.

The 80 subjects in the age group of 16–55 years, visiting the outward patient's section of our department were selected based on the inclusion criteria as follows. Subjects with no history of COVID-19 infection were chosen as the controls and the study groups consisted of COVID-19-recovered patients with a postrecovery phase between 1 and 3 years, along with a history of polymerase chain reaction positive, treatment with hospitalization, or at-home isolation and subjects who satisfy WHO criteria for caries. Subjects who did not give written informed consent, subjects with a history of medical ailments, pregnancy, medication intake, conditions effecting salivary flow, and patients with a history of smoking, tobacco, and alcohol consumption were excluded from the study. The study subjects were explained in detail about the study procedure, and an informed written consent was obtained before including in the study.

The study participants were divided into two groups ($n = 40$): Group 1: subjects with no history of COVID-19 infection and Group 2: subjects with a previous history of COVID-19 infection. Each group was further subdivided into two subgroups ($n = 20$) A and B as without dental caries and with dental caries [Table 1].

The study subjects were made to sit comfortably in the dental chair and were instructed to rinse their mouth with chlorhexidine mouthrinse (Wellona Pharma, Surat, India) for 1 min. The smooth and occlusal surfaces of the teeth were cleaned with sterile cotton and dried. All the teeth were examined, and the dental caries of the study subjects was evaluated using Decayed, Missing and Filled Teeth (DMFT) scoring criteria as proposed by WHO.[10]

On the day of collection of samples, participating subjects were instructed not to eat or drink anything (water exempted) for at least 1 h before the test session. The unstimulated saliva samples were collected between 9 and 11 am. Each subject was asked to rinse their mouth with distilled water several times for about 5 min and was seated comfortably with eyes open, head tilted slightly forward with restricted orofacial movements, and was instructed to rest for 5 min. Saliva was allowed to accumulate in the floor of the mouth, and the subjects were instructed to

Table 1: Study design

Total sample size $n=80$			
Group 1 ($n=40$) Subjects with no history of covid-19 infection		Group 2 ($n=40$) Subjects with previous history of Covid -19 infection	
Subgroup A without dental caries ($n=20$)	Subgroup B with dental caries. ($n=20$)	Subgroup A without dental caries ($n=20$)	Subgroup B with dental caries. ($n=20$)
DMFT scoring (WHO criteria)			
Saliva sample collection (Spitting method)			
Centrifugation for supernant collection			

Salivary total antioxidant capacity (TAC) by 2,2′-azino-bis-[3-ethylbenzothiazoline-6-sulfonic] acid (ABTS) assay assay

spit into a sterile saliva collecting containers every 60 s for 5 min and about 5 ml of unstimulated saliva sample was collected from each participant. The collected saliva samples were immediately centrifuged at 2000 rpm in a microcentrifuge (Remi, Model RM-12C, Haryana, India) for 10 min, and supernatant was collected for further analysis.

Estimation of total antioxidant capacity of saliva

2,2′-azino-bis-[3-ethylbenzothiazoline-6-sulfonic] acid (ABTS) assay was used for salivary TAC analysis. About 5 mL of 7 mM ABTS solution (S D Fine Chemicals, Maharashtra, India) was added to 88 µl of 140 mM ammonium persulfate solution (S D Fine Chemicals, Maharashtra, India) and incubated for 12–16 h in dark at 37°C after which it is diluted using phosphate buffer of pH 7.2 (Molychem, Thane, India) until the absorbance at 734 nm was adjusted to 0.7 ± 0.05. About 3 ml of the above solution was mixed with 1 µL of saliva samples, and the absorbance was read at 734 nm in a visible spectrophotometer (Systronics model-165, Ahmedabad, India), and the values were recorded.[11]

$$\text{ABTS scavenging activity} = \frac{A_{blank} - A_{sample} \times 100}{A_{blank}}$$

Statistical analysis

Statistical analysis was done with the Statistical Package for the Social Sciences (SPSS) software of version 22 (IBM Statistics, Chicago, IL, USA) using the Shapiro–Wilk test for normality distribution of data, followed by one-way analysis of variance (ANOVA) and *post hoc* Tukey tests. $P = 0.05$ or less was considered a level of significance ($P \leq 0.05$).

Table 2: *P* value by Shapiro–Wilk test for normality distribution of study groups

Group	DMFT score		salivary TAC	
	Subgroup A	Subgroup B	Subgroup A	Subgroup B
Group 1	-	0.1910	0.4240	0.4380
Group 2	-	0.6967	0.0593	0.2111

RESULTS

Shapiro–Wilk test for normality distribution showed that *P* values in all groups were > 0.05; hence, data of all parameters of the study groups were normally distributed. Therefore, the parametric tests one-way ANOVA followed by *post hoc* Tukey tests were used [Table 2].

One-way ANOVA analysis showed that DMFT score was found to be highest in subgroup 2B followed by subgroup 1B. Salivary TAC levels were found to be highest in subgroup 1B followed by 2B. Among subgroups A, the highest salivary TAC levels were found in subgroup 1A [Table 3]. Multiple comparisons between groups by *post hoc* Tukey test showed a significant difference between all the groups except between subgroups 1A and 2A for DMFT score, whereas for salivary TAC values, a significant difference was found among all the groups [Table 4].

DISCUSSION

Since the COVID-19 outbreak has occurred in 2020, the impact of COVID-19 across the globe has been significant with more than 7,023,271 deaths reported to the WHO to date.[1] The COVID-19 infection has vastly affected mankind across the globe, which has a drastic impact on the human body including the oral cavity.[2,3]

Among the oral diseases, dental caries is the most common chronic disease of humankind; therefore, it is a matter of concern that calls for research in this area. Caries management by risk assessment is an evidence-based approach to prevent

Table 3: One-way ANOVA analysis of decay, missing, filled, treatment score, salivary total antioxidant capacity values of study groups

Group	DMFT score (mean±SD)		Salivary TAC (U/mL/min), mean±SD	
	Subgroup A	Subgroup B	Subgroup A	Subgroup B
Group 1	0.0000	3.4000±1.3917	49.1150±0.4768	58.6590±0.4601
Group 2	0.0000	7.0000±1.4868	41.4150±0.8497	31.8980±1.0266
P	<2e-16 (significant difference)		<2e-16 (significant difference)	

SD: Standard deviation, TAC: Total antioxidant capacity

Table 4: Multiple comparisons between groups by *post hoc* Tukey test

Comparison groups	DMFT score		Salivary TAC	
	Difference	*P*	Difference	*P*
Subgroup 1A and subgroup 1B	3.4	0.0000 (significant difference)	9.544	0.0000 (significant difference)
Subgroup 1A and subgroup 2A	0.0	1.0000 (NS)	7.700	0.0000 (significant difference)
Subgroup 1A and subgroup 2B	7.0	0.0000 (significant difference)	17.217	0.0000 (significant difference)
Subgroup 1B and subgroup 2A	3.4	0.0000 (significant difference)	17.244	0.0000 (significant difference)
Subgroup 1B and subgroup 2B	3.6	0.0000 (significant difference)	26.761	0.0000 (significant difference)
Subgroup 2A and subgroup 2B	7.0	0.0000 (significant difference)	9.517	0.0000 (significant difference)

NS: No significant difference, TAC: Total antioxidant capacity, DMFT: Decay, missing, filled, treatment

or treat dental caries at the earliest stages rather than waiting for irreversible damage to the teeth. The risk factors are oral bacteria, diet, and saliva.[12] In the present study, the levels of salivary TAC were incorporated with caries risk assessment, thus enabling to estimate the probability of caries incidence and identify patients at high caries risk among COVID-19-recovered persons.

The major outbreak of COVID-19 infection was during 2020 and 2021. However, till today, COVID-19 cases are reported to occur all over the world causing health problems and psychological issues. It has been reported that even after taking the COVID-19 vaccines, individuals are at risk to be affected by COVID-19 infection, hence post COVID-19 health issues including oral diseases such as dental caries have to be evaluated; hence, this study was undertaken. The time lapse between the occurrence of COVID-19 infection and screening for dental caries and saliva collection in this study was chosen between 1 and 3 years, to evaluate the long-term effects of COVID-19 infection. However, the previous studies reported the immediate effects of COVID-19 infection on the occurrence of oral diseases including dental caries.[2,3]

Saliva contains primarily antioxidants such as uric acid, peroxidase, albumin, and glutathione; their most important function is to scavenge FRs and control oral bacteria that form dental plaque, leading to dental caries.[5,6] In the present study, salivary TAC levels were measured using ABTS assay. It is a novel method for assessing antioxidant capacity. It is more sensitive to identifying the antioxidant activity since it has faster reaction kinetics. This photometric assay is based on the reduction by the presence of antioxidant compounds of metastable radical (ABTS+).[11]

In this study, the mean DMFT score was found to be highest in the COVID-19-recovered subjects compared to non-COVID-19 subjects. Most studies reported that there was a decline in the provision and utilization of routine oral health services particularly during lockdown periods.[13,14] According to Sari and Bilmez,[15] COVID-19 pandemic has significantly changed people's eating habits by increasing their consumption of stuff high in sugar content. Dental caries occurs more frequently among individuals who consume substantial quantities of carbohydrates and worsens in response to a high-sugar diet and poor oral hygiene.[16] It was reported that patients only visited dentists in an emergency due to a concern about contracting SARS-CoV-2. Therefore, oral diseases have increased due to all the aforementioned factors during the COVID-19 outbreak.[17]

However, this can be attributed to less salivary flow, lower salivary pH, and lower TAC levels in these individuals compared to controls.[18] In the present study, salivary TAC of COVID-19-recovered subjects (group 2) was less compared to non-COVID-19 subjects (Group 1). This may be due to the presence of higher amounts of FRs that generate oxidative stress causing exhaustion of antioxidants in the body. In the present study, salivary TAC levels were increased in non-COVID-19 subjects with caries (subgroup 1A) compared to without caries (subgroups 1B) which is in accordance with studies by Ahmadi-Motamayel *et al*.,[19] reporting higher TAC values in caries active group and Hegde *et al*.,[20] reported increased salivary TAC in early childhood caries and rampant caries. According to Hegde *et al*.[18] and Shetty *et al*.,[21] the increase in salivary TAC levels is due to an increase in the suspension of proteins and cariogenic activity. Levels of antioxidants alter in response to an infection or disease. Pincemail *et al*.[22] in a recent study reported that oxidative stress was found to be higher during the recovery phase of critical COVID-19 patients and found that salivary TAC levels were decreased, which is in accordance with the present study.

In the present study, COVID-19-recovered patients (Group 2) exhibited a decrease in salivary TAC levels with an increase in DMFT score; hence, it is important to set preventive measures at early stages to prevent dental caries occurrence; however, some of the limitations of the present study include small sample size. Other methods to estimate the TAC levels and confounding factors such as age and gender were not considered. The quality of saliva collected might also influence the study outcome. The study limited measuring the TAC activity at follow-ups to determine how this parameter changes according to the treatment of caries. Further studies with more research variables, more biochemical parameters, fewer confounding factors, and longitudinal studies should be designed to gain more detailed information.

CONCLUSION

Within the limitations of the study, COVID-19-recovered subjects exhibited lower salivary TAC levels with a high prevalence rate of dental caries compared to non-COVID-19 subjects with and without caries.

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Conflicts of interest

There are no conflicts of interest.

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