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Comparison of salivary parameters in COVID-19 positive vs COVID-19 negative individuals during a mass campaign: a case-control study

Comparaison des paramètres salivaires chez des individus positifs au COVID-19 et négatifs au COVID-19 lors d'une campagne de masse : une étude cas-témoins

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Article original

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Mots clés : Ph; Protéines totales; Sodium; Potassium; Salive; COVID 19

ABSTRACT

Background: In the study of the pathophysiology and manifestations, particularly orofacial, associated with COVID-19, oral dysfunctions affecting the salivary glands and saliva, could be associated with this infection. Our objective was to study some salivary biochemical parameters in individuals with COVID-19 compared to healthy individuals.
Method: A case-control study was conducted on salivary samples stored at -20°C at the Biochemistry Laboratory of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé I, of 20 COVID-19 positive and 20 COVID-19 negative individuals. For each sample, information's on the individuals were collected from the database of a study on COVID-19 in the context of voluntary screening in universities. pH, total protein, sodium and potassium ions were measured. Statistical analyses were performed using SPSS version 26.0 software with $p < 0.05$ considered statistically significant.
Results: Of the symptomatic COVID-19 cases, 25% had loss of taste, and 5% had loss of smell. COVID-19+ had lower salivary pH (6.6±0.5 vs 7±0; p=0.003). There were no significant differences between the values of total protein, sodium and potassium in Covid-19+ and controls ($p \geq 0.05$). We did not find any factors associated with the biochemical parameters evaluated.
Conclusion: COVID-19 is associated with a loss of taste and acid salivary pH.
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RESUME

Contexte : Dans l'étude de la physiopathologie et des manifestations (orofaciales) associées au COVID-19, des dysfonctionnements bucco-dentaires affectant les glandes salivaires et la saliveiyf pourraient être associés à cette infection. Notre objectif était d'étudier certains paramètres biochimiques salivaires chez les individus atteints du COVID-19 par rapport aux individus en bonne santé. **Méthodologie** : Une étude cas-témoins a été menée sur des échantillons salivaires conservés à -20°C au Laboratoire de Biochimie de la Faculté de Médecine et des Sciences Biomédicales de l'Université de Yaoundé I, auprès de 20 individus positifs au COVID-19 et 20 individus négatifs au COVID-19. Pour chaque échantillon, des informations sur les individus ont été collectées à partir de la base de données d'une étude sur le COVID-19 dans le cadre d'un dépistage volontaire dans les universités. Le pH, les protéines totales, les ions sodium et potassium ont été dosés. Les analyses statistiques ont été effectuées à l'aide du logiciel SPSS version 26.0 avec une valeur p <0,05 considérée comme statistiquement significative. **Résultats** : Parmi les cas symptomatiques de COVID-19, 25 % présentaient une perte du goût et 5 % une perte de l'odorat. Les participants COVID-19+ avaient un pH salivaire plus faible (6,6 ± 0,5 contre 7 ± 0 ; p = 0,003). Il n'y avait pas de différence significative entre les valeurs de protéines totales, de sodium et de potassium chez les participants COVID-19+ et chez les témoins ($p \geq 0,05$). Nous n'avons trouvé aucun facteur associé aux paramètres biochimiques évalués. **Conclusion**: Le COVID-19 est associé à une perte du goût et un pH salivaire acide.

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Introduction

Coronavirus Disease 2019 (COVID-19) is the infectious disease caused by the new coronavirus unknown to science before its appearance in Wuhan, China in 2019 [1]. In May 2022, according to the update of outbreaks by the World Health Organization (WHO), the world had: 224 affected countries and territories, 518,801,767 confirmed cases, with 6,255,868 deaths, 458,941,429 cured and 11,374,822,677 doses of vaccine administered [2]. In Africa, 53 countries are affected with 11,859,004 confirmed cases, 253,169 deaths and 10,848,258 cured [2]. In Cameroon, these data reported 119,947 confirmed cases and 1,930 deaths [2]. COVID-19 has a wide and varied symptomatology, affecting all body systems, particularly the orofacial sphere [3, 4]. According to a study conducted in 2021 by Natto *and al* on the oral manifestations of symptomatic COVID-19 patients, loss of taste is the most common symptom (43.4%) [5]. As saliva is a biological fluid involved in maintaining oral health, people with viral infections are at risk of developing oral diseases associated with salivary gland dysfunction [6,7]. An association between Xerostomia and decreased salivary flow has been reported in several studies [8]. According to a study conducted in 2021 by Gherlone *and al*, oral manifestations of COVID-19 were detected in 83.9% of the study population, of which 43% had salivary gland ectasia, reflecting the hyperinflammatory response to SARS-CoV-2 [89]. Several publications testify to the presence of the SARS-CoV-2 in saliva and the role of the latter in infectivity, transmission of the virus from individual to individual by direct and indirect contact, its use as a screening test for Covid-19, a potential alternative to the current nasopharyngeal tests [98]. In view of the link between SARS-CoV-2 and salivary gland dysfunction on one hand, and salivary gland dysfunction and impaired oral health on other hand, we proposed to conduct a study to investigate the oral status and changes in some salivary parameters in people with COVID-19 compared to healthy people.

Materials and Method

We conducted a case control study. Our work took place at the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé I, precisely at the Biochemistry Laboratory. We carried out our work over a period of 5 months from January 2021 to May 2022. Twenty (20) COVID-19 positive and 20 COVID-19 negative individual's saliva were collected from the database of a study on COVID-19 in the context of voluntary screening campaign at the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé I from March to July 2021. All individuals were confirmed infected with SARS-CoV-2 or not by real-time reverse transcriptase-polymerase chain reaction (RT-PCR) test from a nasopharyngeal swab sample in the previous study.

The sampling method was consecutive and not exhaustive. All the saliva of individuals aged 18 years or more, well stored were used. We had access after request to salivary samples obtained from individual's during the campaign. We then verified the identification of the samples and group the positive and negative cases according to our selection criteria using the initial data base. The data collected were age, sex, ethnicity, marital status, religion, occupation, history of COVID-19 (previous testing, type of testing performed and result), clinical symptoms and signs. We had total unstimulated saliva samples already collected in the screening context according to the following protocol: Participants received at any hour were asked to refrain from eating, drinking, mouth rinsing, and physical activity at least 01 h before saliva collection. In a room, they were asked to rest for 5min, eyes open, without swallowing; sitting comfortably with arms resting on the knees, head down and tilted slightly forward. They had to swallow all the saliva in their mouths, to accumulate the new saliva in the mouth and pour it every minute for 5 minutes into a sterile jar. The total unstimulated saliva collected was aspirated (the liquid, not the foam) from the container with a 5ml disposable sterile syringe, transferred to a cleaner jar, and stored in a secure cooler for transport and cool storage at -20°C. pH measurement with universal indicator pH paper 1-14 made by comparing the paper turn with the colorimetric scales.

Total proteins were measured using the diagnostic kit BIOLABO TOTAL PROTEIN Biuret Method (Reference LP87016, LOT 082009A1, Expiry date 2023/08). Sodium and potassium ions were quantified using of the diagnostic kit, BIOREX

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SODIUM POTASSIUM monoliquid, Reference BXC0146A, Lot 211453, Expiry date 2022/12). The data on the questionnaires were entered, aggregated using CS.pro (Census and Survey Processus system) version 7.5, and then analyzed using SPSS (Statistical Package for the Social Sciences) version 26.0. All tests used were one-tailed (equivalence study) with an α threshold set at 5%. Categorical variables were described in terms of numbers and percentages and were compared using the Chi-square test. Quantitative variables in the form m of mean with their standard deviation, and were associated with qualitative variables using Student's t test. A p value at 0.05 was considered statistically significant for all analyses. Our study was approved by the ethics committee of the faculty of medicine and Biomedical Sciences of the University of Yaoundé I. Administrative authorisations were obtained from the study site. Anonymaty and strict respect of the fundamental principles of research were ensured

Results

The table below shows that the majority of individuals were between 25 and 34 years of age, 40% (8) respectively for COVID-19 positive cases, and between 18-25 years of age, 45% (9) respectively for COVID-19 negative cases. The mean age was 27.65 ± 7.94 years versus 28.45 ± 6.5 years with extreme values of 18 and 48 years for COVID-19 positive cases and 20 and 39 years for COVID-19 negative cases. The gender distribution was equal in the COVID-19 positive group, 50% (20) males and 50% (20) females; in the COVID-19 negative group, females were in the majority, 70% (14) respectively. Single people were in the majority in both groups, i.e. 90% (18) of COVID-19 positive cases vs. 70% (14) of COVID-19 negative cases (p=0.114). Regarding occupation, students were in the majority in both COVID-19 positive and negative individuals, i.e. 60% (12) vs. 50% (10) (p=0.525). A previous test was performed in 95% (19) of the positive cases compared with 75% (15) of the negatives. Among the positive cases, 15% (3) had tested positive during the last previous test. No significant difference was found for general informations (p ≥ 0.05) (**Table I**).

Among the COVID 19 positive cases, 9 (45%) were symptomatic. Of these, 25% (5) had a sensation of loss of taste, and 5% (1) had a sensation of loss of smell (**Table II**).

Table I: Distribution of our population according to general informations

Variables	COVID-19 positive N=20 n (%)	COVID-19 negative N=20 n (%)	p
Age groups (years)			
[18-24[	7 (35)	9 (45)	0.520
[25-34[	8 (40)	7 (35)	0.744
[35-44[	4 (20)	4 (20)	0.311
[45-54[	1 (5)	0 (0)	
Gender			
Male	10 (50)	6 (30)	0.197
Female	10 (50)	14 (70)	
Marital status			
Single	18 (90)	14 (70)	0.114
Married	2 (10)	6 (30)	0.028
Ethnicity			
Beti	11(55)	12(60)	0.490
Bamileke	8 (40)	6(30)	0.510
Bassa	0 (0)	2(10)	
Fulani	1 (5)	0 (0)	
Religion			
Christian	19 (95)	20 (100)	0.311
Muslim	1 (5)	0 (0)	
Profession			
Teacher	1 (5)	2 (10)	0.548
Nursing staff	3 (15)	4 (20)	0.677
Tradesman	1 (5)	3 (15)	0.292
Student	12 (60)	10 (50)	0.525
Unemployed	4 (1)	1 (5)	0.119
Prior test			
Yes	19 (95)	15 (75)	0.077
No	1 (5)	5 (25)	
Result if previous test			
Positive	3 (15)	0 (0)	0.183
Negative		16 (85)	

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Table II: Distribution of our population according to oral symptoms

Variables	COVID-19 positive n (%)
General clinical form	
Symptomatic	9 (45)
Asymptomatic	11 (55)
Loss of taste	
Yes	5 (25)
No	15 (75)
Loss of sense of smell	
Yes	1 (5)
No	19 (95)

Salivary pH was lower in COVID-19 positive cases than in COVID-19 negative cases, i.e. 6.6 ± 0.5 vs. 7 ± 0.1 respectively (p=0.003). There were no significant differences between the total protein, sodium and potassium values of COVID-19 positive and negative cases (p ≥ 0.05) (**Table III**).

No statistical association was found between age, gender, hygiene habits, clinical presentation and salivary parameters (p ≥ 0.05) (**Table IV**).

Table III: Distribution of the population according to central dispersion measures of salivary parameters

Variables	COVID-19 positive			COVD 19 negative			p
	Mean ± SD	Min	Max	Mean ±S D	Min	Max	
pH	6.6 ± 0.5	6	7	7± 0.1	6.5	7	0.003
Total proteins (g/l)	9.8 ± 2.9	6.8	14.9	11±3.8	6.2	24	0,295
Sodium (mmol/l)	96.9	96.9	96.9	72.7±-	72.7	72.7	/
Potassium (mmol/l)	4.2 ± 2.5	0.19	8.7	3±1.7	0.7	6.3	0.086

Table IV: Factors associated with salivary biochemical parameters pH of COVID-19+ in our study

Variables	pH		Total proteins (g/L)		Sodium (mmol/L)		Potassium (mmol/L)	
	Mean±SD	p	Mean±SD	p	Mean±SD	p	Mean±SD	p
Age								
[18-24[	6.5±0.5	0.743	9.8±3.6	0.645	121.7±11.4	0.534	2.8±1.3	0.736
[25-34[	6.6±0.4		9.5±2.9		121.2±21.9		4.2±2.2	
[35-44[	6.8±0.5		11.0±2.8		131.5±16.9		6.9±3.5	
[45-54[	7		8.83		122.5		4.3	
Gender								
Male	6.8±0.4	0.753	10.3±2.9	0.453	124.7±12.3	0.243	4.6±3.3	0.946
Female	6.5±0.5		9.5± 3.1		122.5±20.3		4.0±1.8	
Brushing frequency								
1 time	6.3±0.4	0.563	9.1±2.93	0.453	118.9±13.9	0.213	3.4±1.4	0.384
2 times	6.7±0.4		10.2±3.03		125.5±17.9		4.6±2.9	
Clinical form								
Symptomatic	6.6±0.4	0.989	10.7±3.5	0.621	130.7±21.3	0.093	4.1±2.8	0.902
Asymptomatic	6.6±0.5		9.1±2.4		117.6±9.3		4.4±2.5	

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Discussion

The overall objective of this study was to evaluate some salivary biochemical parameters in a group of COVID-19 positive individuals compared to a group of healthy individuals in Yaoundé. The age group [18-24] was the most represented in our study.

This could be explained by the sampling environment being university. These results are similar to those of Voundi *and al* in 2020 in Cameroon, where the most represented age groups were [15-20[and [20-25[years] [10]. The average age of COVID-19 positive individuals was 27.65 ± 7.94 years with extremes of 18 and 48 years, and 28.45 ±6.5 years for COVID-19 negative individuals, with extremes of 20 and 39 years. This would be due to the majority presence of young adults in the student population, which is the most represented in our study: 12 (60%) of positive cases and 10 (50%) of negative cases. In our population, 95% (19) of the positive cases and 15 (75%) had already undergone a COVID-19 test prior to our study. This can be explained by the massive and free screening campaigns organized throughout the country by the Ministry of Public Health of Cameroon as a prevention measure for COVID-19. Thus the accessibility of the test was obvious. 11 (55%) of COVID-19 positive cases were asymptomatic: this result is close to that of a study conducted in Mali by Samaké *and al*, where the rate of asymptomatic cases was 50.4% [11]. Dysgeusia, a disorder in taste perception, whether or not associated with other symptoms, was experienced by 5 (25%) of positive cases. These results are similar to those of a study conducted by Alex Carignan *and al* in 2020 in Canada, in which the rate of dysgeusia in COVID-19 was 28% [12].

Concerning the biochemical parameters, the salivary pH was lower in the COVID-19 positive cases than in the negative ones, i.e. 6.6 ± 0.5 vs 7 ± 0.1 respectively (p=0.003). This could be due to the effect of non- or low-perceived hyposialia by cases of other non-assessed signs. In addition, a study by Jimenez *and al* in Brazil in 2020 suggested an association between COVID-19 and gastroesophageal reflux disease. The latter has the potential to cause a decrease in oral pH [13]. There were no significant differences between the total proteins, sodium and potassium values of COVID-19 positive and negative cases (p > 0.05). These results are in contrast to some data in the literature. Concerning electrolytes, Giuseppe et al. show in a study conducted in 2020 in the United States that Sodium and Potassium were significantly lower in COVID-19 positive cases than in negative cases [14]. This difference with our study would be due to the nature of the biological fluid which was serum in that study and salivary in ours. Also the nature of the reagents used would contribute. Neither age, were sex, hygiene habits nor clinical presentation associated with salivary pH, total protein, sodium and potassium in COVID-19 positive individuals (p > 0.05). For pH, this could be explained by the cross-sectional nature of the study, the youth of the study population. Gheorghes *and al* in 2021, on the other hand, demonstrated an association between hyponatremia and advanced age [15]. This difference would be due to the absence of elderly people in our study population. Dong Chen and al in 2020, during a study in China on potassium values in COVID-19 cases, observed an association with significant renal loss [16]. This difference would be due to the time of exposure, as this was a cohort study and ours a cross-sectional study.

Conclusion

COVID -19 positive individuals in spite of good oral hygiene habits present losses of taste and smell. COVID-19 positive individuals have an acidic salivary pH than healthy individuals. The evaluation of these parameters could contribute to the follow-up of oral pathologies in individuals with covid-19.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Authors' contributions

Voundi-Voundi E, Ngono and Pieme CA contributed to the design of the study; Voundi-Voundi E, Ngono MG and Nokam Abena ME organized the database; Nokam Abena ME Lowe JM and Medi-Sike C performed statistical analysis; Ngono MG performed the first draft of the article; Voundi-Voundi E, Ngogang MP and Pieme CA corrected sections of the manuscript. All authors contributed to the

revision of the manuscript, read and approved the submitted version.

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