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Cytomorphometric Changes of Buccal Exfoliated Pap Stained Smears among COVID-19 Recovered Patients; Sudan 2022

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ABSTRACT

Introduction: COVID-19 is known to cause various changes in oral mucosa which can be detected by exfoliative cytology. The present study aimed to determine the cytomorphometric change of oral mucosa exfoliated Pap-stained smears for COVID-19 recovered patients at Khartoum state 2022.

Methods: This was a cross-sectional comparative, community-based study conducted at Khartoum State. Eighty (80) participants who recovered from COVID-19 enrolled in the study as cases and twenty (20) healthy participants as the control group.

Results: In the present study the ages of the participants ranged between 19-75 years. Mean was significantly different in cytoplasm diameter (CD), nucleus diameter (ND), and nuclear/cytoplasmic ratio (N/CR) between the patients who recovered from COVID-19 versus the healthy group (69.71µm versus 37.45µm, P. value= 0.001; 10.02µm versus 6.05µm, P. value= 001; and 28.9 µm versus 18.0µm, P. value= 0.016) respectively.

Conclusion: The marked changes in cytomorphometric parameters caused by infection with COVID-19 prove that infection with the virus causes changes in the buccal mucosa.

These findings will help in the screening and diagnosis of the disease.

KEY WARDS: Buccal, COVID-19, Cytomorphometry, Pap Smear, Recovered Patients, Sudan.

INTRODUCTION

Coronaviruses are a large family of viruses are known to cause respiratory infections such as Middle East Respiratory Syndrome (MERS), Severe Acute Respiratory Syndrome (SARS), and Severe Acute Respiratory Syndrome corona-virus2 (SARS-CoV-2) (1,2). World Health Organization (WHO) officially named (SARS-CoV-2) as coronavirus disease 2019 (COVID-19) (3). The disease spreads through close contact with symptomatic or asymptomatic infected people via droplets and aerosols generated by talking, breathing, sneezing, and coughing (4). The oral cavity plays a vital role in virus entry and transmission. Oral signs and symptoms include dry mouth, burning mouth, taste loss, Candida Albicans infection, dysgeusia and ulceration (5,6). The buccal mucosa is lined by non-keratinized stratified squamous epithelium and exhibits angiotensin converting enzyme2 (ACE2) and transmembrane serine protease2 (TMPRSS) expression (7,8). The virus consists of the envelope protein (E), spike protein (S), transmembrane protein (M), and nucleoprotein (N). The (E, M, and S) proteins have a role in virus entry, assembly, and pathogenesis. The(N) protein plays a major role in RNA replication, suppressing the host cell's innate immune response, and aiding in virions assembly (9,10). The principal mode entry

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of (SARS-CoV-2) is via binding of the viral spike glycoprotein (S) to ACE2 host cell receptor and activated by host cell-derived transmembrane serine protease2 (TMPRSS2) to gain entry for replication (11). The oral cavity is one of the main routes of virus entry, and the mucosa is the first site of infection and exposure to cellular changes (5). Several studies demonstrated the colonization of oral mucosa by (SARS-CoV-2) (7), and recently there are ongoing studies on oral mucosa and saliva as a rapid screening test for the disease (10). The study of cytomorphometric changes due to the virus will help in a better understanding of virus pathogenesis and diagnosis, however, no quantitative studies were conducted for exfoliated cells from the oral mucosa.
Therefore, the present study aimed to determine the cytomorphometric change of oral mucosa exfoliated Pap-stained smears for COVID-19 recovered patients at Khartoum state 2022.

MATERIAL AND METHOD

Study design/Setting:

This was a cross-sectional comparative, community-based study conducted at Khartoum State, Sudan from December 2021 - January 2022.

Participants:

Eighty (80) participants who recovered from COVID-19 enrolled in the study as cases and twenty (20) healthy participants who were never exposed to COVID-19 as the control group.

Inclusion Criteria:

Regardless of gender and age, all COVID-19-recovered patients were their infection confirmed by RT-PCR enrolled in the study.

Exclusion Criteria:

Diabetes Mellitus, women with menstruation, alcoholism, tobacco user, acute diseases such as acute respiratory infection, and the patient exposed to computed tomography were excluded from the study.

Sample collection:

Under sterile and antiseptic procedures each participant was requested to rinse his mouth with water and dried it with sterile gauze to remove excess saliva and debris. Using a moist wooden spatula with distilled water to obtain a smear from clinically normal appearing buccal mucosa for each group with gentle pressure for 2-3 minutes. Scrapping material was smeared on clean labelled slides. Slides were then fixed in 95% alcohol for 30 minutes. Air drying of the smears was avoided, and smears were stained using conventional pap stain. All quality control measures were adopted during the specimen's collection and processing. The smears were then stained with Papanicolaou stain as the following procedure. Demographical information was collected from the cases as well as the control group.

Papanicolaou staining procedure (Pap stain):

The smears were hydrated through descending concentrations of ethanol, 90%, 70%, and distilled water for 3 minutes for each. Then the nucleus was stained with Harris's hematoxylin for 4 minutes, bluing for 4 minutes, counterstained with orange G6 for 4 minutes, differentiated with 95%alcohol, followed by cytoplasmic stain with EA50 (eosin azure 50) for 4 minutes, differentiated with 95%alcohol. Finally, the smears were dehydrated through ascending concentration ethanol for 3 minutes for each, cleared and mounted.

Cytomorphometric Analysis:

The smears were examined under x40, the cells were selected by moving the microscope stage in a "Z" shape to avoid recounting the same cell. The cells were identified based on morphology and staining characteristics. Nuclear, cytoplasmic diameter, and nuclear/cytoplasmic ratio were obtained by the Optika optical microscope camera using a digitizer cursor in both axes and about ten (10) well-defined, unfolded, good stained cells were counted. The means for the nuclear diameter, cytoplasmic diameter, and nuclear/cytoplasmic ratio were calculated for each sample and expressed in micrometres.

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Data Analysis:

The data were analyzed using Statistical Package for the Social Sciences 20 (SPSS Inc., Chicago).

The differences in cytomorphometric between the two groups were tested by independent sample T-test. A P. value of <0.05 was considered significant.

RESULTS

In the present study, the ages of the participants ranged between 19-75 years. There were 52 males and 36 females (Table 1). Mean was significantly different in cytoplasm diameter (CD), nucleus diameter (ND), and nuclear/cytoplasmic ratio (N/C R) between the patients who recovered from COVID-19 versus the healthy group (69.71 µm versus 37.45µm, P. value= 0.01; 10.02µm versus 6.05µm, P. value= 001; and 28.9 µm versus 18.0µm, P. value= 0.016) respectively (Table 2 and Figure 1). There were no significant differences in cytomorphometric analysis according to gender (Table 3). Although, no significant correlation between the duration of infection and cytomorphometric changes of COVID-19 recovered patients, all cytomorphometric parameters significantly direct correlated with female age (Table 4).

Table 1. Descriptive statistics for the study population

variables	Age			Gender		Total
	Minimum	Maximum	Mean	Male N (%)	Female N (%)	80
Case	19	75	33	48 (60%)	32 (40%)	
Control	19	67	28	16 (80%)	4 (20%)	20

Table 2. Mean (STD) of cytomorphometric analysis (µm) in the study population:

Variables	Population	Mean (STD)	Minimum	Maximum	95% Confidence Interval of the Difference		P. value
					Lower	Upper	
Cytoplasm diameter	Case	69.7 (54.3)	29.00	403.0	7.96737	56.50763	0.001
	Control	37.45 (7.6)	20.00	52.00			
Nucleus Diameter	Case	10.02 (4.1)	5.000	25.00	2.06607	5.88393	<0.001
	Control	6.05 (2.0)	4.000	14.00			
Nuclear Cytoplasmic/Ratio	Case	28.97 (19.5)	2.000	105.0	2.11379	19.83621	0.016
	Control	18.00 (7.4)	11.00	46.00			

Table 3. Mean (STD) of cytomorphometric analysis (µm) in patients recovered from COVID-19 according to gender

	Gender	Mean	Std. Deviation	P. Value	95% Confidence Interval of the Difference	
					Lower	Upper
	Male	78.1042	-3.35688	0.090	-3.3568	45.44022
Cytoplasm diameter	Female	57.0625	-.20948			
Nucleus Diameter	Male	10.6875	-2.731	0.081	-.20948	3.52198
	Female	9.0313	3.71122			
NC/R	Male	31.4167	22.17132	0.173	-2.731	14.939
	Female	25.3125	14.36042			

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Table 4. Correlation between duration and female age of recovered patients and cytomorphometric changes

Case		Cytoplasm diameter	Nucleus Diameter	NC/R
Duration	Pearson Correlation	.063	.010	-.020
	Sig. (2-tailed)	.576	.928	.858
	N	80	80	80
Female Age	Pearson Correlation	.560	.369	.415
	Sig. (2-tailed)	.001	.038	.018
	N	32	32	32
Male Age	Pearson Correlation	.277	.156	.281
	Sig. (2-tailed)	.058	.289	.054
	N	48	48	48

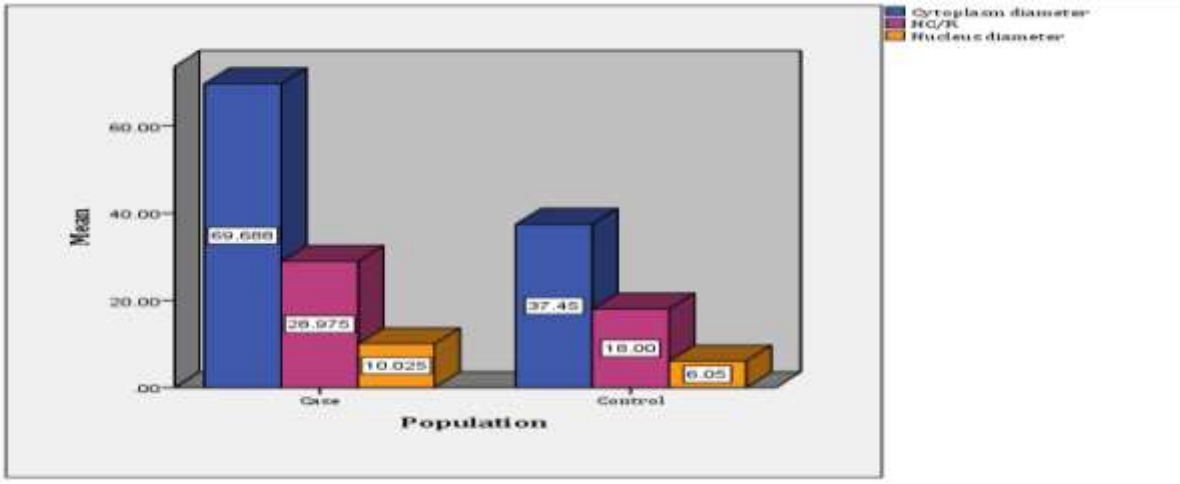

Figure 1. Means of cytomorphometric in the study population

Figure 2. cell measurement Cell Diameter, cytoplasm Diameter, and Nuclear/ Cytoplasmic Ratio:

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DISCUSSION

Perhaps this is the first study done in Sudan of this nature, however, several previous studies searched on cytomorphometric changes in the exfoliated cells from oral mucosa in patients with diabetes, tobacco users, smokers and alcoholics, and patients exposed to computed tomography and remarkable changes were founded (12–19). In the current study, Sudanese patients who have been exposed to the infection and recovered from the coronavirus were closely investigated concomitantly compared with a healthy population and their collected data was analyzed to reveal some of the mysteries of the disease in its behaviour. Coming to this end, in this study we found that significant changes were observed in the cell diameter, nuclear diameter and nuclear/cytoplasmic ratio in the different layers of the buccal mucosa. While most squamous cells of buccal mucosa have had low to moderate changes, serious and remarkable changes occurred to the outermost layer of superficial cells. This is becoming a certainty during the measurement of the cells' diameter because the overwhelming number of counted cells were detected to be superficial cells. It is worth mentioning that in the present study, the nuclear chromogenic pattern was found to be homogenous. This is bearing the normal characteristic of cell morphology obtained from buccal mucosa especially when these have been compared with our control study groups. When the literature has been scanned we could hardly come across morphometric and morphological changes studies that affected the squamous cells of the buccal mucosa among patients recovered from COVID-19 to compare with. But we did find published data by Zulkarnain and his coauthors in 2021 (20). They evaluated the cytological change of exfoliated epithelial cells in saliva from COVID-19 patients, and they found damage to the membrane and organelles of the superficial and intermediate cells. They correlate the severity of the cytopathic effect with disease duration. On the other hand, in the present study, the cytoplasmic diameter and N/C ratio in patients recovered from COVID-19 directly correlate to the age of females and this is probably due to hormones influence, and such finding reported by Sundharam, 2003 (21).

CONCLUSION

The marked changes in cytomorphometric parameters caused by infection with COVID-19 prove that infection with the virus causes changes in the buccal mucosa. These findings will help in the screening and diagnosis of the disease. The limitation of the present study is that we couldn't take a sample from patients with recent infections due to safety measures. Further study in this context is needed to understand the pathophysiology of the disease.

Ethical Statement:

Alfajr College Research Ethical Committee reviewed and approved the study. All participants were assigned the written informed consent form. All methods were carried out following relevant guidelines and regulations.

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Competing interests: None.

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