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Competition of SARS-COV-2 and *Candida albicans* on Angiotensin-Converting Enzyme 2 Receptor in COVID-19 Patients' Tongue

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ABSTRACT

Background and Aim: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) takes advantage of the mouth and nose mucosal cells' receptors to attach and make its way towards the lower respiratory tract. Researchers have not yet addressed the correlation of this virus with normal flora of oral cavity. *Candida albicans* (*C. albicans*) is one of the normal flora that occupies oral cavity and it is found to serve as a trap for some viruses. This study aimed to investigate the role of candida biofilm in the tongue of COVID-19 patients for protection against severity of the disease through the coverage of tongue ACE2 receptors.

Materials and Methods: A group of 56 patients called "Together to fight COVID-19" was formed through social media. The COVID-19 test results, hematological tests, and tongues photographs before and during the treatment were collected and evaluated. Data were analyzed by SPSS.

Results: Among the 56 patients who had tongue check-up for the presence of cheese-like coating of candida, 55 patients (83.3%) were positive even for the Robert Harrison's simple Candida home test. Among them, 90% were mild with COVID-19 infection, they recovered within 10 days. Other 10% were moderate to severe, recovered in 10-20 days. Tongue images observation identified red spots and enlarged papillae within the white layer.

Conclusion: Overall, a correlation was detected between the clinical phenotypes of SARS-COV-2 and the presence of *C. albicans* in the tongue of the patients. These data provide a link between SARS-COV-2 severity and *Candida albicans* biofilm.

Keywords: ACE2 Receptor, *Candida albicans*, COVID-19, SARS-COV-2, Tongue Microbiota

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1. Introduction

The presence of *Candida albicans* (*C. albicans*) as a normal flora in the buccal cavity and respiratory tract was detected not only in healthy people but also in increased levels in immune-compromised patients and especially during the viral infections **(1, 2)**. The biofilm of candida is a layer associated with candida community on the surface of cavities **(3)**.

The *C. albicans* is considered as normal flora in the skin, gastrointestinal tract, and female genital and oral cavity **(4)**. It is the causative agent for oral thrush, however, commonly presented with no symptoms. It forms pseudo-membrane in the tongue and nasopharyngeal cavity and in some cases it forms candida lesions and stays for long life with anti-fungal resistance mechanism **(5)**. It is implicated in mucosal surfaces, which cover mouth, pharyngeal cavities, gastrointestinal, genital, and urinary tracts. It causes systemic infection by invading different sites in the human body **(6)**.

Biofilm is produced by candida when yeast cells attach to a surface and undergo cell morphogenesis to start forming monolayer of the cells, which then develops buds and hyphae with polysaccharide as an extracellular matrix **(7)**. Unlike other types of candida, the growth of *C. albicans* biofilm is enhanced by the presence of saliva **(8)**. Biofilm matrix of *C. albicans* is composed of proteins, carbohydrates, lipids, noncoding DNA, and α-1,2 branched α-1,6-mannans binds to unbranched α-1,6-glucans, which together form Mannan-glucan complex **(9)**. The presence of *C. albicans* in the oral cavity may be shown by creamy lesions on tongue and buccal mucosa and may be extended to the nasopharyngeal cavity. These creamy layers can be easily scratched from tongue, and it is considered as a feature of the *C. albicans* presence in tongue even in the healthy people **(10)**. This layer is composed of mucous and mannans, which may cover all the surface of the tongue **(11)**.

Although candida can also infect healthy people and coexists in human body tracts, their presence in immune-compromised patients is increased. In fact, candida disease affects about half of the HIV-1 patients and about 90% of the patients with AIDS **(12, 13)**. Interestingly, the virus showed direct interaction with *C. albicans* and this link enhanced candida virulence and decreased viral activity **(14, 15)**.

C. albicans forms jelly-like glycol-polymer biofilm called mucin. This product can regulate the virulence of microbes in oral, pharyngeal, and vaginal areas **(16)**. It was found to play role as a trap for several bacteria and viruses *in vitro*. Bacteria like *Streptococcus pneumoniae* **(17)** and *Helicobacter pylori* **(18)** disaggregated in the presence of candida mucin biofilm. Viruses like HIV-1 **(19)** and influenza virus **(20)** were confirmed to be infective by the presence of candida mucin. Herpes simplex virus 1 (HSV-1) and Coxsackie B virus were found to be embedded in *C. albicans* biofilm and protected against antivirals like acyclovir and disinfectants like hypochlorite, as well as treatments with UVA1 355 nm laser **(10, 21)**. In addition, *C. albicans* and HSV-1 were isolated together in mouth samples of neutropenic patients **(22, 23)**.

This study was designed to evaluate if there is a correlation between severity of COVID-19 infection and the presence of *C. albicans* biofilm in mouth.

2. Materials and Methods

Study design and sampling

Quasi-experiment research was done due to the restriction and inability to follow COVID-19 patients. Samples were collected randomly from 17 June 2020 until 14 September 2020. In this study 56 patients (21 female and 35 male) were observed through internet connection by social media like Telegram, Skype, and WhatsApp. A group of social communication called "Together to fight COVID-19" was formed that included COVID-19 patients, their relatives, as well as our research team in Al-Zahraa College of Medicine, University of Basrah.

The inclusion criteria included: conscious patients who were able to open mouth and protrude tongue to allow capturing image to the tongue. The patients or their family were advised to take a close-up photograph of the patients' tongue from a distance of 10-20 cm. Photos with low quality were rejected and a higher resolution photo were requested. The exclusion criteria included: unconscious patients or those who were in coma; patients who were unable to open mouth or at risk of jaw dislocation; patients who had an ulcer in tongue, or who had oral cancer. Research team sent written information to explain how to determine Robert Harrison's simple Candida home test and how to get better photos for their tongue. Direct examination of the patients was excluded due to health directorate restriction.

The patients were asked to send their laboratory test results including hematological tests, complete blood count (CBC), PCR-COVID-19 test results, and high-resolution images of their tongues before and during the treatment, and the image for the Robert Harrison's simple Candida home test. The study was designed to evaluate the association between the tongue photos and the severity of the cases. The topography of the tongue images was analyzed according to the previous researches **(24, 25)**. The severity of COVID-19 was speculated by calculating the ratio of neutrophils/lymphocytes. The cases were considered as severe, moderate, and low with ratios of up to 9.9, 4.79, and less, respectively **(26)**.

Tongue mages were analyzed by the features like; color of the tongue, density of the creamy-like layer, red dots on the surface of white layer of the tongue if presence, and inflammation of sweet buds in the apex of the tongue that showed more red color.

Robert Harrison's simple Candida home test, also called "spit test", is a very accurate examination like blood and stool tests of candida, according to Soumya Parmar et al **(27)**. The patients were asked to rinse the mouth with saliva and spit into clean and transparent glass filled with water. After 10 min, a picture of the callus was taken from the side along the surface of the water. If there was candida overgrowth, string legs hanging down from the saliva and cloudy specks were observed, which may be the result of candida overgrowth.

The ethical implications of the research emphasized the agreement with the patient that the research team members would not share any picture of the patients with parties other than the responsible researchers. The researchers also recommended that the patients take close-up pictures of the patient's tongue so that the patient's face would not be visible.

Statistical analysis

Statistical analysis was performed by SPSS version 26 (SPSS, Inc. Chicago, IL, USA). Data were classified according to gender and age (years) and depicted as count and percentage, respectively. Hematological tests were also analyzed by chi-square and Fisher's exact test was performed to calculate the significant level. The values less than 0.001 were considered as significant (P<0.0001).

3. Results

The severity of the COVID-19 disease was assessed based on the patients' CBC (Table 1) as mild/moderate or severe according to the previous research **(28)**. The results showed that 39 (69.6%) patients were mild/moderate with a median age of 56 years, and 17 (30.4%) were severe with a median age of 61 years old. The age of the patients ranged from 36 to 61 years old. The hematological parameters showed significant differences between mild/moderate and severe groups (Table 1). In COVID-19 patients, a set of hematological parameters like HGB, RBC, HCT, Lym, Mono, Eos and PLT decreased, whereas WBC, Neu, MCV, NLR, PLR, MCV and MCH increased. The differences among the groups remained significant over time (P<0.001). A 17 (30.35%) of the samples were considered as severs cases according to the Nneutrophils/lymphocytes ratio, which showed >5, while mild and moderate cases were showed <5 according to Toori et al **(29)**.

Table 1. Hematological parameters of the mild/moderate and severe COVID-19 patients

Parameters	Total (N=56)	Mild/Moderate (N=39)	Severe (N= 17)	P-value >0.001
Gender(F/M)	21/35	12/29	9/6	
Age (Years)	48.9±12	56±12.6	61±13.5	>0.001
WBC 10^9/L	9.12±4.41	8.59±4.01	11.46±5.32	>0.006
RBC 10^{12}/L	4.28±0.58	4.36±0.46	3.89±0.88	>0.001
HGB g/L	132.22±16.64	134.5±12.1	122.3±27.3	>0.004
Neut 10^9/L	7.41±4.34	6.69±3.83	10.51±5.10	>0.013
Lymph 10^9/L	1.18±0.79	1.34±0.78	0.50±0.39	>0.001
Mono 10^9/L	0.49±0.25	0.50±0.25	0.43±0.27	>0.001
Eosin10^9/L	0.04±0.06	0.04±0.06	0.00±0.01	>0.017
Baso10^9/L	0.02±0.01	0.02±0.01	0.02±0.02	>0.077
ImGr%	1.42±1.85	1.45±1.97	1.30±1.23	>0.005
HCT%	0.49±0.49	0.52±0.53	0.36±0.27	>0.004
MCV	92.11±3.33	91.88±3.43	93.11±2.64	>0.009
MCHC g/L	335.99±6.61	335.8±6.88	337.0±5.25	>0.083
NL	12.02±13.94	7.93±8.36	29.9±18.7	>0.001
PLT	316.46±309.28	238.8±196.0	655.6±457.4	>0.002
MCH, pg	30.95±1.28	30.85±1.35	31.37±0.82	>0.16
PLT 10^9/L	220.42±70.82	222.7±73.01	210.6±60.43	>0.001

F/M=Female/Male; WBC=While Blood Cell Count; RBC=Red Blood Cells Count; HGB=Hemoglobin level; Neut=Neutrophils; Lym=Lymphocytes; Mon=Monocytes; Eos=Eosinophiles; Bas=Basophils; ImGr=Immature granulocyte; HCT= Red Blood Cells specific volume; NL=Neutrophil lymphocyte; PLT=Platelets; MCV=Mean Cell volume; MCH, pg/cell= Mean Cell Hemoglobin Concentration (picogram/cell); MCHC g/L=Mean Cell Hemoglobin Concentration (gram/Liter).

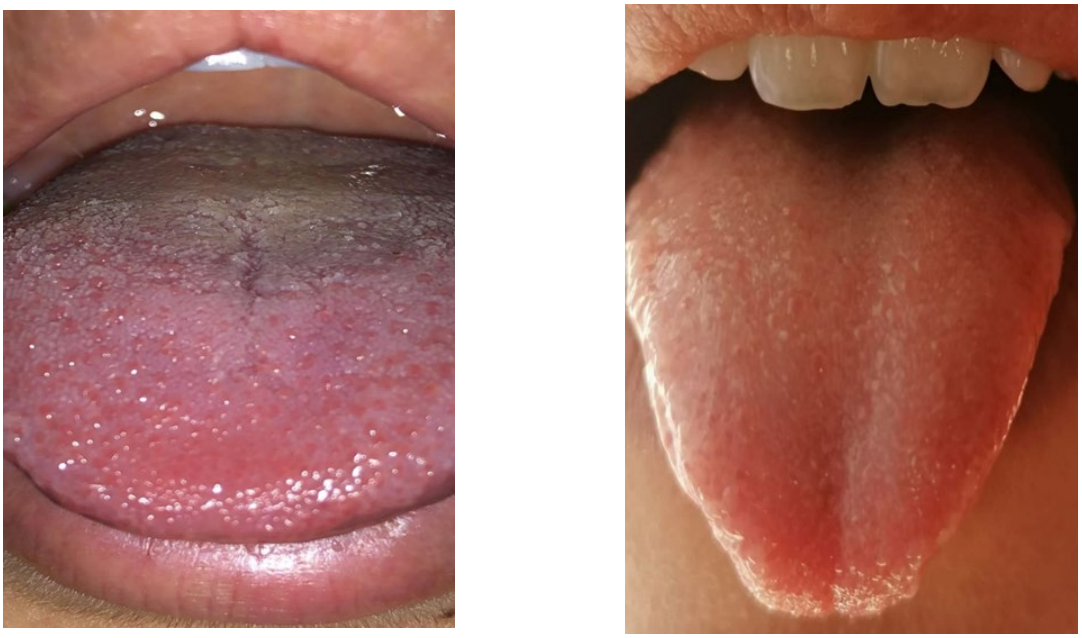
The tongue images were taken early morning before eating or drinking. The images showed different levels of white creamy layer in most of the patients with mild/moderate infection and the patients confirmed

the presence of this layer even long before the COVID-19 infection (Figure 1), which was in contrast to those who had severe COVID-19 infection (Figure 2). The tongues' images of those with severe infection appeared clear with no creamy-coated layers or little and some changes in their sweet buds in the apex of the tongue (Figure 3).

In addition, the images of the water glass for the Robert Harrison's simple Candida home test showed heavy mucoid legs from the surface of the water downward in cases with mild/moderate infection, while the severe cases showed less or no strings (Figure 4), which referred to the presence of candida biofilm in the mild/moderate cases according to the Robert Harrison's test.

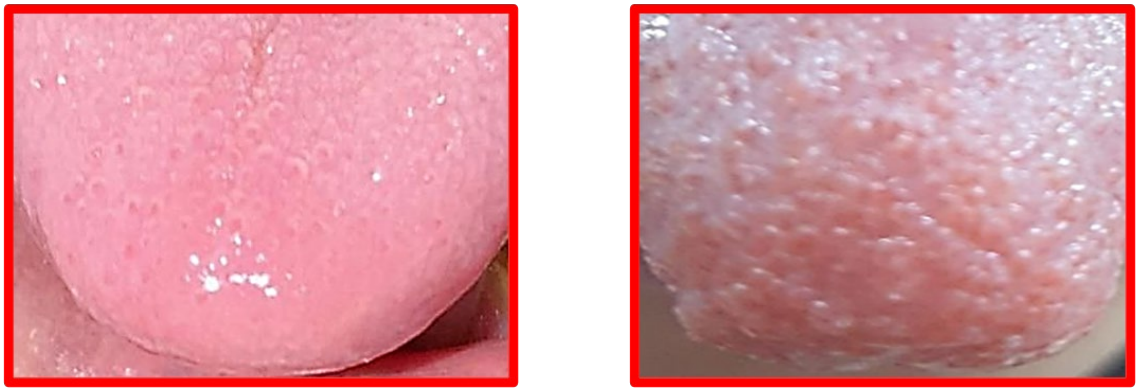
A B

Figure 1. White or creamy layer on the tongues of the patients. A) tongue of a 52-year-old patient and B) tongue of a 56-year-old patient both with moderate COVID-19 infections. Images were taken by the patients' mobile phones.



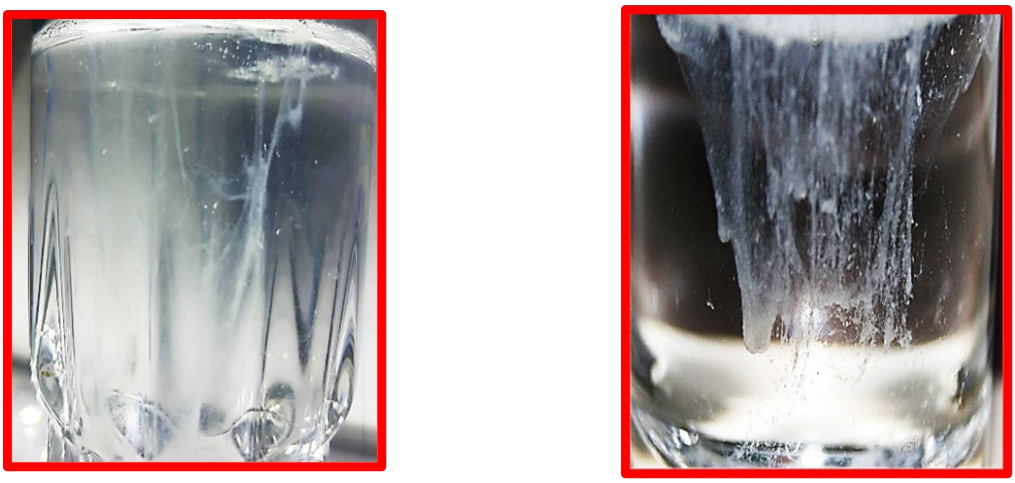
A B

Figure 2. Clear tongues of the patients, **A)** tongue of a 57-year-old patient and B) tongue of a 49-year-old patient both with severe COVID-19 infections. Images were taken by the patients' mobile phones.



A B

Figure 3. Sweet buds in apex of the tongues of the patients with severe COVID-19. A) Sweat buds of a 55-year-old women And B) Sweat buds of a 48-year-old women both with severe infections. Images were taken by patients' mobile phones.



A B

Figure 4. Robert Harrison's simple Candida home test images. **A)** Very little string legs from a 60-year-old patient with severe infection. B) Heavy mucoid legs downward from the surface of the water from a 55-year-old patient with mild/moderate infection.

4. Discussion

The study focused on the presence of white patches in the tongues of the patients with COVID-19 infection. Because of the restrictions applied by the governmental quarantine getting samples from the patients was impossible. Therefore, the study used the social media to contact patients and guided them to send images. Totally, 56 patients cooperated to send the images of their tongues, blood test results, and the Robert Harrison's simple Candida home test results.

The severity of COVID-19 is reflected by rising in values of neutrophils/lymphocytes ratio (NLR). Asymptomatic patients have NLR of 1.92, suggesting minimal immune response activation. In patients with mild disease, the NLR increases slightly to 2.08, indicating a modest elevation in inflammatory activity. However, a more substantial rise is observed in moderate cases, where the NLR reaches 4.79, signifying a higher degree of systemic inflammation. In severe COVID-19 cases, the NLR sharply rises to 9.9, reflecting significant immune dysregulation and a heightened inflammatory response. This progressive increase in NLR across disease stages suggests that NLR could serve as a useful biomarker for assessing the severity of COVID-19 and potentially predicting adverse outcomes **(29)**.

The results found that the patients how already had white creamy layer on their tongue, represented a feature of *C. albicans* infection and get mild to moderate severity of COVID-19 infection according to their CBC results. They confirmed the presence of these creamy white layers in their tongues long before COVID-19 infection. This may give a hint that *C. albicans* is present as a normal flora in the buccal cavity and respiratory tract of the healthy as well as immune-compromised persons and even during viral infection **(1, 2)**. It may cover the ACE2 receptors in the epithelial of the tongue and respiratory tract that have

been found to play a critical role in SARS-Cov2 attachment and penetration **(30-32)**. Consequently, *C. albicans* coverage prevents the interaction of the virus with the cellular receptors.

Candida biofilm has been shown to act as trap for the viruses like Herpes Simplex Virus 1 (HSV-1) and Coxsackievirus B5 (CVB5) in the cell culture **(13, 21)**. A study showed that *C. albicans* biofilm could protect HSV-1 from antiviral compounds like acyclovir or foscarnet or even protect the viruses from UV laser light 355 λ **(21)**. The adherence of *C. albicans* biofilm during HSV-1 and HSV-2 infections was also confirmed by Plotkin et al **(23)**. They noticed that the presence of bacteria like Staphylococcus had no effect in trapping viruses or biofilm formation, but it is related just with the presence of *C. albicans* **(23)**.

The evidence may give us an initial conformation of the role of *C. albicans* biofilm in covering the tongue and preventing the virus penetration to the tongue surface. In addition, manifestation of the tongue surface in COVID-19 severe cases attributed this effect to the attachment of the virus directly to the tongue surface and its penetration **(33-35)**. The presence of candida on tongue revealed infiltration of the tongue epithelial layer and keratinization **(30)**. This may cover or demolish the ACE2 receptors that are highly expressed in the cell surface of the epithelial layer of the tongue and mucosa of the oral cavity **(31)**.

Robert Harrison's simple Candida home test was performed to confirm the presence of mucoid layer in tongue, which may refer to the presence of candida biofilm, instead of culturing tongue smear samples because of quarantine and restrictions. The images from severe cases showed high mucoidal debris compared with others, who were mild/moderate patients. This may support the hypothesis that the presence of high level of *C. albicans* biofilm may prevent SARS-Cov2 from attachment to the receptors.

Tongue feature in patients with COVID-19 was shown to be related with severity of disease. Pang and colleague found that 75% of the patients with COVID-19 have greasy coting tongue, 53.3% of them showed mild and 87.5% showed sever disease **(36)**. Enlargement in tongue and red dots in tongue surface with yellow layer was identified by taking tongue images from 1487 patients ranging from 20 to 92 years, 61% of patients showed tongue enlarged and 97.9% showed red dots in the tongue surfaces, while white creamy layer was found in about 59.8% **(37)**.

This observation may explain why children are less infected with the COVID-19. In a study of 8866 cases in China, only 14 (0.15%) children younger than 10 years old got COVID-19 infection **(38)**. While another study referred that only 0.9% and 1.2% in the age groups of 10 and 19 years old of total 72314 patients were infected with COVID-19 **(39)**. This may be due to the intense presence of candida in children tongue compared to the adults. More than 80% of children carry candida in their tongue from an early age either due to their breast feeding or later due to their hygiene habit **(40)**.

Interaction of COVID-19 and *C. albicans* in mouth and saliva was confirmed before **(41)**. A reverse association was found between the presence of *C. albicans* and the presence of ACE2 receptors in COVID-19 patients, but they may overlook whether the presence of candida had occurred long before infection. Further research is needed to study the correlation between candida biofilm and severity of COVID-19 in a large size sample. In addition to morphological examinations, scraping specimens form tongue is necessaey to detect *Candida albicans* under immunoelectron microscopy to understand the way that the virus reacts in tongue surface like what was done by Tamiya et al **(42)**.

5. Conclusion

It was highlighted that tongues covered with white candida layers, hypothetically make it difficult for SARS-Cov-2 to attach to the epithelial layer of the tongue and start penetration, and if happened all these cases will be mild to moderate COVID-19.

6. Declarations

Acknowledgment

We thank all individuals who participated in the COVID-19 social group called "Together to fight COVID-19", which was managed by the Al-Zahraa College of Medicine, University of Basrah as coordinators.

Ethical Considerations

This research was approved by the Ethics Committee of the Al-Zahraa College of Medicine, University of Basrah under code number ID: Lab2.2023.E/T 35.

Authors' Contributions

Hussein K Abdul-Sada conceived and designed the experiments; Rasha N Jawad followed up on the patients' cases, asked them to conduct experiments on themselves, collected laboratory test results, and collected images of the tongue samples, Ihsan M Al-Badran analyzed laboratory data, and Rasha analyzed statistics and Hussein wrote the paper. All authors have read and approved the manuscript.

Conflict of Interests

Authors declare there is no conflict of interest at this work.

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