Oral Candidiasis Infections Associated with Covid- 19 Patients in West of Iraq

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Summary
Coronavirus is a devastating global public health disaster in the last years. It affected a large proportion of people around the world. Some of the co-infections associated with the global COVID-19 pandemic are the secondary mycotic infections that following COVID-19 disease. There are genetic and environmental factors that are involved in these infections. In this research, the main goal is to study the mycotic infection of 87 COVID-19 patients of those with comorbidities diseases such as DM, Hypertension, and other chronic disease. Also, to observe the relationship between the mycotic infections and the patients which treated with corticosteroid and broad-spectrum antibiotics. As well as identification of Candida by using an efficient and sensitive molecular method for the Internal transcribed spacer (ITS) gene associated with contagion. Fungal DNA was extracted from patients according to well-established procedures. Then, DNA was amplified by PCR techniques using specific primers for *ITS* exons. The results of this study indicate a significant association between species of fungi, (*P-value* = 0.000) and (*Chi-square* = 54.76). Also, this study shows a significant association between species of candida, (*P-value* = 0.000) and (*Chi-square* = 19.32). As well as the study mentions a significant association between species of fungi and recipient broad-spectrum antibiotics with an increased risk for COVID-19 contagion, (*P-value* = 0.044). In addition, our outcomes proved no significant association between fungi with smoking, DM, and hypertension, (*P-value* = 0.143), (*P-value* = 0.568), and (*P-value* = 0.070), respectively. Finally, no significant association appeared between fungi with an increased risk for COVID-19 contagion, (*P-value* = 0.170), (*Chi-square* = 0.053) about corticosteroid therapy. In conclusion the oral candidiasis was the most frequent infection among COVID-19 patients. However, our findings need more replication in the Arabic countries to confirm these outcomes.
Keywords: Covid- 19; Oral Candidiasis infections; Health

1. Introduction

The mycotic co-infections related to worldwide COVID-19 may be missed or misdiagnosed. Even though there are not many distributions, COVID-19 patients, particularly immunocompromised, have a higher likelihood of infection with invasive mycoses, Candida and Aspergillus infections in COVID-19 patients will require early identification by a proper workup (histopathology, culture, and PCR-based tests) to guarantee successful treatments. Candida albicans is the most common cause of oral candidiasis, which is a common opportunistic infection of the oral cavity. Candida infection is more common in certain age groups and has been linked to a wide range of risk factors for the development of the infection. These include acute candidiasis, chronic candidiasis, and angular cheilitis. Smoking, diabetes, Cushing's syndrome, cancer, and others immune deficiency status are all variables that might increase the risk of fungal infection. Select high-risk groups benefit from antifungal prophylaxis by reducing the prevalence and severity of infections. [4]

Mold infected in immunocompromised people, particularly those with diabetes mellitus, organ transplantation, and stem cell and hematological malignancies, can cause Candidiasis [6].

The secondary infections which called co-infections appear to be hospital acquired infections and they more commonly caused by bacteria and fungi, the higher rate of co-infections with bacteria and fungi in those patients with severe COVID-19 infections, who were also more likely to suffer complications and death. The finding of pathogens of secondary infection together with a low lymphocyte count, in addition to high levels of D-dimers, which shown to be associated with severity of COVID-19 infection. [35]

2. Methodology

Collection of Specimens

87 samples were collected from Heet Hospital. Oral samples were taken from Covid-19 patients. Covid-19 and oral Candidiasis occurs in people of all ages and both sexes between the ages of 10 and 83 years. Using sterilized cotton swaps samples were taken. The swapes was inoculation on SDA culture media for isolation of pathogenic Candida and subculture on CHROM Agar and microscopic examination with KOH 10% and lactophenol cotton blue.

Laboratory Diagnosis

Culture Incubation

All clinical samples of suspected lesions were infected with chloramphenicol on SDA Cycloheximide supplementation intermediate using a sterile inoculation ring and incubated at 25-30°C before being assessed. Negative cultures were after

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4 weeks, whereas positive cultures were inspected by macroscopic and microscopic identification.

Identification of Fungal Isolates

Macroscopic and microscopic analysis of culture isolates were used to discriminate between the fungi. The thorough examination is defined by the time of growth and is one of the types that isolates pigment producing cells in the absence of pigment dependent surface morphology in the medium. The growth of fungus using lactophenol cotton blue dye is examined microscopically. Nature assisted in the formation of the fungus and conidia (large and small conidia), Distinguish between these groups.

Direct Microscope Examination

Microscopy is used to make the first diagnosis. The swap taken from growing culture and placed on a clean slide with 10% KOH added and a clean lid on top. Several times, the slide was passed through the flame of burner, then 5-30 minutes of incubation, and finally each slide was cleaned with light. Look for fungal hyphae,

Molecular Identification

DNA extraction method

Fungal DNA was extracted by using the Fungi/Yeast Genomic DNA Isolation Kit Provided by Norgen Biotek.

Methods of PCR for detection of specific genes

Primers Solutions

The primers were designed based on the National Center for Biotechnology Information NCBI and provided by the bioneer Company as a lyophilized product of various concentrations of picomol. Solution Final concentration of 10 pmol/µl was prepared separately by dissolving 10µl of stock solution for each primer and added to 90µl free nuclease distilled water un-ionic(ddH2O), mixed well and kept in (-20oC). They were mixed by vortex to homogenize before use. The sequences used in the study for (ITS gene) listed in Table (1).

Table (1): Sequence of PCR primer and molecular size of PCR products

Gene		Sequence of forward and reverse (Primer 3/-5/)	TM(C0)	Product (bp)	Reference
ITS	F	TCCGTAGGTGAACCTGCGG	52	550	[74]
	R	TCCTCCGCTTATTGATATGC			

Preparation PCR mixture

25µl of PCR reaction was composed of green master mix (Promega), primer solution, deionized water and template DNA with the following volume the PCR mixture used in the study.

Table:(2) PCR mixture used in the current study.

NO: Content of reaction mixture	Volume of reaction mixture for a single tube.	
1	Green master mix	12.5 µl
2	DNA template	3.5µl
3	Forwar primer (10 Picomol)	1 µl
4	Reverse primer (10 Picomol)	1µl
5	Nuclase free water	7 µl
	Total volume	25µl

SDA Culture Results

The whole 87 of the clinical samples, which was collected from the oral area of covid-19 patient, the grew fungi in the SDA culture, including 87 Candida spp According to the study, the highest relative percent occurrence was Candida.

Culture on CHROM Agar result

The positive culture on the SDA with candida was 87 sample, and when cultured on CHROOM AGAR to determine the species of candida, the result was 64 sample is Candida albicans, which had 73.56% of the total number, and 23 sample Candida tropicalis, which had 26.44% of the total number of candida, and our results go with the outcomes of the Iranian researcher) ,[9] Which found the highest percentage is the Candida albicans while the Candida tropicalis with low percentage, But he found more other species of Candida

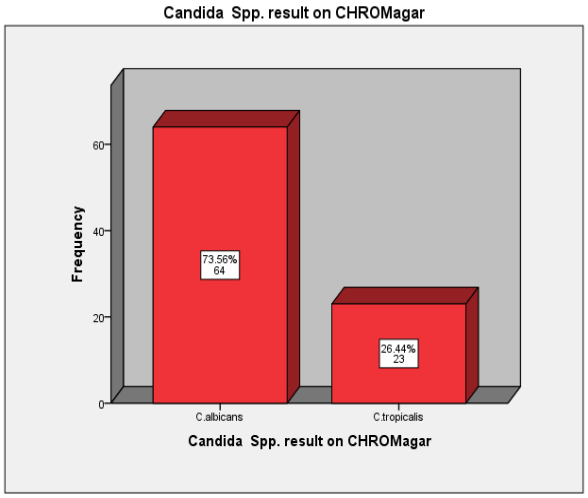


Figure 3 Frequency and percentage of candida spp. In covid-19 patients

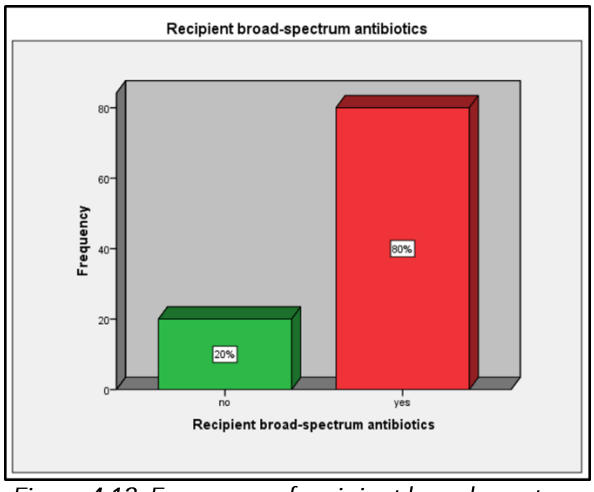


Figure 4.12. Frequency of recipient broad-spectrum antibiotics (candida app.,) in covid-19 patients.

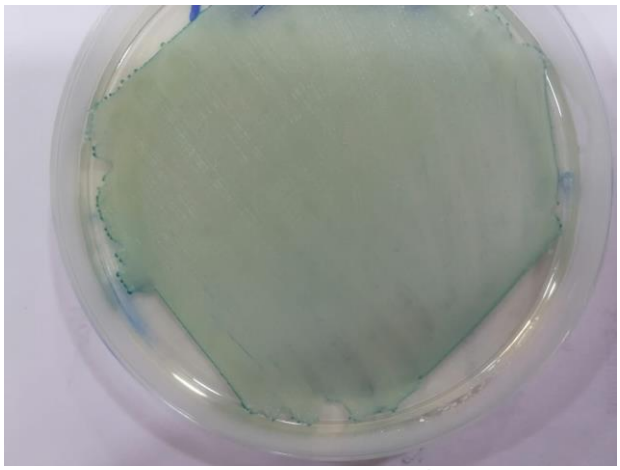
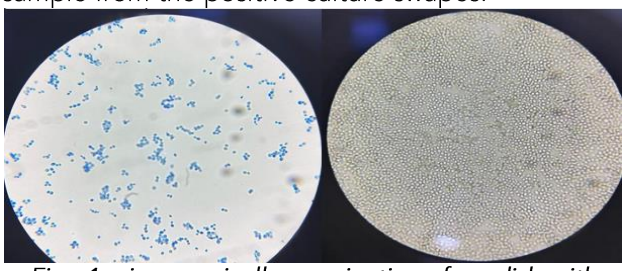


Fig. candida Spp. based on the specific color colonies.

Microscopic Examination of Specimens

Culture's positive examples There were 87 positive cases also with 87 positive cases using a direct KOH 10% and also with Lactophenol Cotton Blue, which this sample was taken directly from the positive culture fungi, why? Because they do not examine directly from the mouth, they examine after taken the sample from the positive culture swapes.



Figar1.microscopically examination of candida with Lactophenol Cotton Blue and KOH10%

Germ tube test for diagnosis for candida

The 87 sample of candida was tested with gem tube test to confirm the diagnosis of the fungi, The results were positive for all samples as confirmation of the culture results and the results of the microscopic examination.

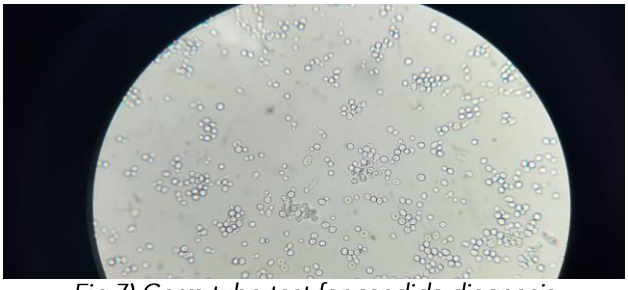


Fig.7) Germ tube test for candida diagnosis

Molecular Study

DNA extracted from 30 samples was analyzed by gel electrophoresis (Figure 4.20) and DNA fragment coding for the fungal ITS gene was amplified from the genomic DNA of candida. Gel electrophoresis analysis showed the bands of the amplified gene with the predicted size of 550 bp. We did a PCR test for these random samples as confirmation of the results of previous tests. The results of the PCR were (26) of the samples positive and (4) samples were negative as shown in (Table 3.35) and (Figure 4.22). The (3) negative results which present in non-target band size were anther fungi (not candida). And the last negative result which was without DNA band probably related to the failure of the probe to hybridize to the amplicon, or due to an error in the work steps or as a result of sample contamination. Our outcomes it goes with the outcomes of research White PL et al. (2004) [156].

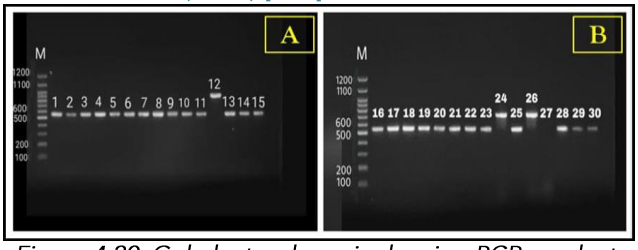


Figure 4.20. Gel electrophoresis showing PCR product with a band size of 550 bp. The products were run on 2% agarose. N: DNA ladder (1000 plus).

(Table 3.3) Shows the frequency of species of candida.				
	Observed N	Percent	Expected N	Residual
c.albicans	64	64.0	43.5	20.5
c.trobicalis	23	23.0	43.5	-20.5-
Total	87	87.0		

(Table 3.4) Species of candida and (P-value, Chi-square) test statistics.	
Species of Candida	
Chi-Square	19.322a
df	1
Asymp. Sig.	.000

(Table 3.14) Shows the comparison between fungal infection in both females and males.			
		Frequency	Percent
Gender	Female	36	43.6
	Male	51	55.4
	Total	87	99

3. Conclusions

According to the findings of the current study there are some of the following conclusions:

1- Oral candidiasis were the most common opportunistic among the cases.

2- The diagnosis of candida with PCR is more accurate than the other diagnostic methods.

3- Our results indicate that oral candidiasis under the effect of recipient broad-spectrum antibiotics is significantly associated with an increased risk for COVID-19 contagion. On the other hand, it is not significantly associated with the other factors.

4- As part of COVID-19, cases at high risk included those receiving broad-spectrum antibiotics, with acute respiratory distress syndrome, immunosuppressants, or corticosteroids.

5- Such a study has not been done in detail on our different Arab countries. And our findings need replication in Arabic countries confirm these outcomes on the increased impact of oral mycotic contagion within covid-19 patients.

5.2 Recommendations

1- Further studies with a large sample size are needed to study the correlations between oral mycotic infections among covid-19 patients.
2- This research should also be as an exam and expand the choice of various cure options and early detection of persons at high risk of relapse according to the patient's medical record and contagion history.
3- According to the results of our research, I recommend limiting the indiscriminate use of antibiotics, and also educating people not to take medicines without a doctor's prescription.
4- According to the results of our research, it is recommended not to take immunosuppressive drugs in cases of viral infections, except when necessary.
5- On the other hand, more examinations and other techniques are required to study and determine their influence on the clinical behavior of patients. Continues to perform the laboratory tests needed to confirm candidiasis and aspergillosis in our COVID-19 patients.
6- Finally, Dental specialists should know that unexplained oral candidiasis could propose T and B cell immunosuppression from viral disease.

References

Ali I, Alharbi OML. COVID-19: Disease, management, treatment, and social impact. Sci Total Environ. 2020 Aug 1; 728:138861. doi: 10.1016/j.scitotenv.2020.138861.

Song G, Liang G, Liu W. Fungal Co-infections Associated with Global COVID-19 Pandemic: A Clinical and Diagnostic Perspective from China. Mycopathologia. 2020 Aug;185(4):599-606. doi: 10.1007/s11046-020-00462-9.

Kubin CJ, McConville TH, Dietz D, Zucker J, May M, Nelson B, Istorico E, Bartram L, Small-Saunders J, Sobieszczyk ME, Gomez-Simmonds A, Uhlemann AC. Characterization of Bacterial and Fungal Infections in Hospitalized Patients with Coronavirus Disease 2019 and Factors Associated with Health Care-Associated Infections. Open Forum Infect Dis. 2021 May 5;8(6): ofab201. doi: 10.1093/ofid/ofab201.

R ajendra Santosh AB, Muddana K, Bakki SR. Fungal Infections of Oral Cavity: Diagnosis, Management, and Association with COVID-19. SN Compr Clin Med. 2021 Mar 27:1-12. doi: 10.1007/s42399-021-00873-9.

Nambiar M, Varma SR, Jaber M, Sreelatha SV, Thomas B, Nair AS. Mycotic infections - mucormycosis and oral candidiasis associated with Covid-19: a significant and challenging association. J Oral Microbiol. 2021 Aug 26;13(1):1967699. doi: 10.1080/20002297.2021.1967699.

Bhatt K, Agolli A, Patel MH, Garimella R, Devi M, Garcia E, Amin H, Domingue C, Guerra Del Castillo R, Sanchez-Gonzalez M. High mortality co-infections of COVID-19 patients: mucormycosis and other fungal infections. Discoveries (Craiova). 2021 Mar 31;9(1): e126. doi: 10.15190/d.2021.5.

Akpan A, Morgan R. Oral candidiasis. Postgrad Med J. 2002 Aug;78(922):455-9. doi: 10.1136/pmj.78.922.455.

Roudbary M, Kumar S, Kumar A, Černáková L, Nikoomanesh F, Rodrigues CF. Overview on the Prevalence of Fungal Infections, Immune Response, and Microbiome Role in COVID-19 Patients. J Fungi (Basel). 2021 Sep 2;7(9):720. doi: 10.3390/jof7090720.

Hallur V, Prakash H, Sable M, Preetam C, Purushotham P, Senapati R, Shankarnarayan SA, Bag ND, Rudramurthy SM. Cunninghamella arunalokei a New Species of Cunninghamella from India Causing Disease in an Immunocompetent Individual. J Fungi (Basel). 2021 Aug 19;7(8):670. doi: 10.3390/jof7080670.

Tyrrell DA, Almeida JD, Cunningham CH, Dowdle WR, Hofstad MS, McIntosh K, Tajima M, Zakstelskaya LY, Easterday BC, Kapikian A, Bingham RW. Coronaviridae. Intervirology. 1975;5(1-2):76-82. doi: 10.1159/000149883.

J. S. M. Peiris, Richman, Douglas D, Whitley, Richard J, Hayden, Frederick J. Coronaviruses. Clinical Virology, wiley. 2016; pp. 1243–1265. doi: 10.1128/9781555819439.ch52.

Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL, Chen HD, Chen J, Luo Y, Guo H, Jiang RD, Liu MQ, Chen Y, Shen XR, Wang X, Zheng XS, Zhao K, Chen QJ, Deng F, Liu LL, Yan B, Zhan FX, Wang YY, Xiao GF, Shi ZL. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Mar;579(7798):270-273. doi: 10.1038/s41586-020-2012-7.

Li J, Li JJ, Xie X, Cai X, Huang J, Tian X, Zhu H. Game consumption and the 2019 novel coronavirus. Lancet Infect Dis. 2020 Mar;20(3):275-276. doi: 10.1016/S1473-3099(20)30063-3.

Siddell S, Wege H, ter Meulen V. The structure and replication of coronaviruses. Curr Top Microbiol Immunol. 1982; 99:131-63. doi: 10.1007/978-3-642-68528-6_4.

Gert-Jan Godeke, Cornelis A. M. de Haan, John W. A. Rossen, Harry Vennema and Peter J. M. Rottier. Assembly of Spikes into Coronavirus Particles Is Mediated by the Carboxy-Terminal Domain of the Spike Protein. J of Virol. 2000;74, (3):1566. DOI: 10.1128/JVI.74.3.1566-1571.2000.

Malik YA. Properties of Coronavirus and SARS-CoV-2. Malays J Pathol. 2020 Apr;42(1):3-11.

Kubo H, Yamada YK, Taguchi F. Localization of neutralizing epitopes and the receptor-binding site within the amino-terminal 330 amino acids of the murine coronavirus spike protein. J Virol. 1994 Sep;68(9):5403-10. doi: 10.1128/JVI.68.9.5403-5410.1994.

Belouzard S, Chu VC, Whittaker GR. Activation of the SARS coronavirus spike protein via sequential proteolytic cleavage at two distinct sites. Proc Natl Acad Sci U S A. 2009 Apr 7;106(14):5871-6. doi: 10.1073/pnas.0809524106. Epub 2009 Mar 24.

Sethna PB, Hofmann MA, Brian DA. Minus-strand copies of replicating coronavirus mRNAs contain antileaders. J Virol. 1991 Jan;65(1):320-5. doi: 10.1128/JVI.65.1.320-325.1991.

Sawicki SG, Sawicki DL. Coronavirus transcription: a perspective. Curr Top Microbiol Immunol. 2005; 287:31-55. doi: 10.1007/3-540-26765-4_2.