ORIGINAL ARTICLE

Resistance of Oral *Candida albicans* Infection to Fluconazole and Nystatin among Healthy Persons after Treatment with Azithromycin and Hydroxychloroquine to Treat Suspected SARS-COV-2 Viral Infection

[1]Mahmoud R. M. El-Ansary*, [2]Amira R. El-Ansary

[1]Department of Immunology and Medical Microbiology, Faculty of Medicine, Misr University for Science and Technology, Giza, Egypt

[2]Department of internal medicine, Faculty of Medicine, Misr University for Science and Technology, Giza, Egypt

Key words:
COVID-19; Antimicrobial Resistance; Azithromycin; Hydroxychloroquine

****Corresponding Author:***
Mahmoud R. M. El-Ansary
Department of Immunology and Medical Microbiology, Faculty of Medicine, Misr University for Science and Technology, Giza, Egyp
Tel.: +201143666640
melansary2022@gmail.com
ORCID ID:
0000-0003-2458-1930

ABSTRACT

Background: *Oral candidiasis is considered the most common fungal infection in humans. Polyene or azole antifungal drugs can be used for Candida infection. In addition, Nystatin therapy is used in mild forms of oral candidiasis. However, the current literature reports conflicting results regarding azithromycin and hydroxychloroquine for COVID-19.* ***Objective****: We aim to evaluate the resistance of oral Candida albicans to fluconazole and nystatin in healthy individuals who have received therapy for suspected COVID-19 with azithromycin and hydroxychloroquine.* ***Methodology****: The current case-control study collected samples of oral candidiasis from two types of patients. The cases group (group 1) were patients with confirmed COVID-19; the control group (group 2) were healthy controls without previous COVID-19. The antifungal susceptibility was examined based on the principles of M44-A suggested by the Clinical and Laboratory Standards Institute (CLSI).* ***Results****: Thirty patients were included in the cases group. In terms of the antifungal susceptibility between the study groups, the study showed the details of fluconazole and nystatin resistance. Fluconazole resistance was detected significantly higher in 86.7% of the cases group compared with only 56.7% of the subjects in the control group (P=0.010). Similarly, nystatin resistance was significantly higher in 76.7% of the cases group, while the control group showed 23.3% drug resistance (P< 0.001).* ***Conclusion****: Treating COVID-19 patients with hydroxychloroquine and azithromycin negatively impacted the antimicrobial resistance (AMR) of oral candidiasis. Given the limited efficacy of both treatments, avoiding the broad-spectrum use of both agents in managing COVID-19 patients is advisable.*

INTRODUCTION

Candida species are widely distributed in nature and are normal microflora of the skin and mucous. They can cause opportunistic infection, especially among immunocompromised people. Oral candidiasis is considered to be the most prevalent fungus infection in humans. Over 80% of oral candidiasis infections are caused by *Candida krusei, Candida albicans, Candida glabrata, and Candida tropicalis* infection represents the most common species isolated from the oral mucosa, found in 30% to 50% of neonates, children, and adults.[1]

Cancer patients receiving chemotherapy and radiation therapy frequently develop oral candidiasis, representing an incidence of 90% to 95%.[2,3] Oropharyngeal candidiasis commonly affects people with acquired immunodeficiency syndrome (AIDS) as they are incompatible with highly active antiretroviral therapy.[4] When Candida penetrates the surface epithelium, it causes severe systemic manifestations associated with substantial morbidity and mortality rates 79%); hence, efficient diagnosis and management are required.[5,6]

Treating patients with oral *Candida albicans* infection often include polyene or azole antifungal drugs.[7] Azoles drugs, such as fluconazole, are associated with better solubility and less nephrotoxicity than polyenes; however, they are compromised because of azole resistance, especially with *Candida albicans*.[8] In addition, nystatin is used in mild forms of oral candidiasis and can be used in cases of azole resistance [9,10]. Several mechanisms of antifungal drug resistance have been studied, mostly in vitro.[11–13]

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), primarily reported in China in December 2019, is the cause of a worldwide pandemic, infecting more than 10 million persons, with a mortality rate of 1.4-6.9%.[14] Opposing results regarding the use of azithromycin and hydroxychloroquine have been

reported. A prospective study enrolling 11 patients found no significant clinical benefit for hydroxychloroquine and azithromycin in patients with SARS-CoV-2.[15] In contrast, another study found that hydroxychloroquine was associated with a significant viral load reduction on day 6 (70% [14/20]) compared to control (12.5% [2/16]).[16]

Therefore, the current study aims to assess the resistance of oral *Candida albicans* to fluconazole and nystatin among healthy persons after treatment with azithromycin and hydroxychloroquine for suspected COVID-19.

METHODOLOGY

Setting and patients:

The present study procedure was planned following the declaration of Helsinki's established principles and was approved by the Hospital's Ethical Committee at Misr University for Science and Technology (MUST). Participants were allocated from the University Hospital's Outpatient Dermatological Clinic. Samples from two different patient types with oral candidiasis were obtained for this observational investigation. Patients with confirmed COVID-19 who matched the following two major criteria comprised the first category (cases group): (1) Age above 12 years, (2) prior SARS-CoV-2 infection confirmed by PCR, (3) treatment with hydroxychloroquine and azithromycin were the first two criteria. The second kind, or control group, consisted of healthy patients who had never had COVID-19.

Sample collection:

We included participants who had not received antifungal therapy for the past three days to prevent any false negative results. Samples were collected using sterile swabs during the visit to the Outpatient Clinic. Samples were washed in distilled water and transported to the laboratory for further analysis. Samples were stored on Sabouraud Dextrose Agar culture media at 37°C. After 48-72 hours, the cultured plates were assessed. First, slides were developed from the structured colonies. *Candida albicans* organisms were identified by the standard methods if we found yeast cells.[17]

Antifungal susceptibility testing:

Antifungal susceptibility was evaluated based on the principles of M44-A proposed by the Clinical and Laboratory Standards Institute (CLSI). The standard strain of *Candida albicans* was ATCC 2091. The isolates were subcultured overnight at 37°C on Sabouraud Dextrose Agar (SDA). The organism was suspended in saline, and the turbidity was set at 0.5 McFarland standards. The plates were loaded with the media and incubated at 37°C with the discs on top. After 24 and 48 hours, the zone of inhibition was captured on camera. Fluconazole 10 mg/disk and 100 units/disk of nystatin were utilised (Sigma Aldrich, USA).[18]

***Statistical analysis*:**

Data were analysed using SPSS software, version 16. Chi-square was used to compare cases and controls regarding gender, Fluconazole resistance, and Nystatin resistance. In addition, T-test student was used for comparison between groups regarding age. A P-value of less than 0.05 was considered statistically significant.

RESULTS

During the study period, thirty patients were included in the case group. The patients were confirmed previous COVID-19 infection and history of hydroxychloroquine and azithromycin treatment during the management period of COVID-19 infection. The cases group showed an average age of 37± 9 years old. 36.7% of the included patients were males, and 63.3% were females. In the control group, the average age was 33 ± 8 years old. 46.7% of the included patients were males, and 53.3% were females. There was no significant difference between cases and control groups regarding age and gender distribution (P = 0.094 and P = 0.432, respectively). (Table. 1)

Table 1: Demographics Characteristics of the studied participants.

Parameters	Cases (n= 30)	Controls (n= 30)	P-value
Age, mean (± SD)	37 (9)	33 (8)	0.094
Gender			
Male	11 (36.7)	14 (46.7%)	0.432
Female	19 (63.3)	16 (53.3%)	

Regarding antifungal susceptibility between the study groups, Fluconazole resistance was detected significantly higher in 86.7% of the cases group compared with only 56.7% of the subjects in the control group (P = 0.010). Table. 2 shows the details of the fluconazole and nystatin resistance.

Table 2: Clinical data of the studied participants.

Parameters	Cases (n= 30)	Controls (n= 30)	P-value
Fluconazole resistance			
Sensitive	4 (13.3%)	13 (43.3%)	**0.010**
Resistant	26 (86.7%)	17 (56.7%)	
Nystatin resistance			**< 0.001**
Resistant	23 (76.7%)	7 (23.3%)	
Sensitive	7 (23.3%)	23 (76.7%)	

Similarly, nystatin resistance was significantly higher in 76.7% of the cases group, while the control group showed 23.3% of the drug resistance (P < 0.001) **Figure 1**.

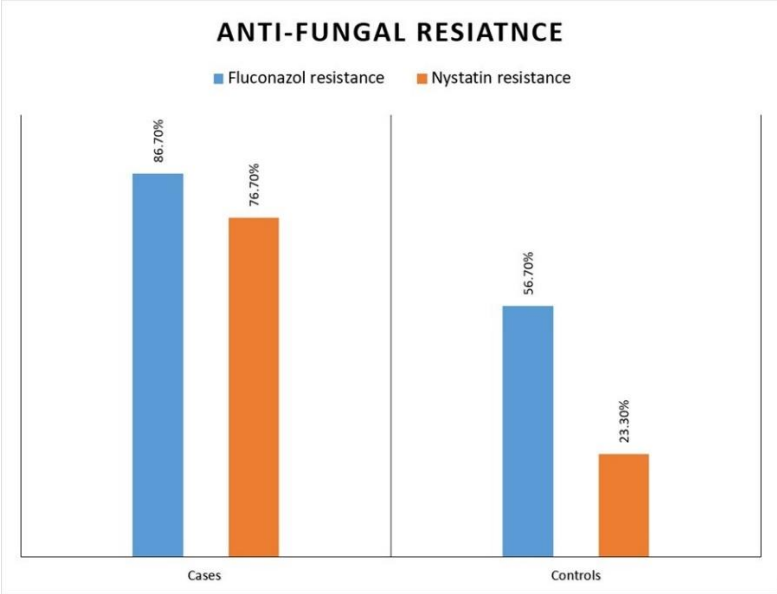

Fig. 1: Antifungal resistance in both cases and control groups

DISCUSSION

Antimicrobial resistance (AMR) is one of the long-term complications of the COVID-19 pandemic due to the suboptimal or inappropriate use of antimicrobials in the acute care setting.[19] Recent studies reveal that fungal and bacterial co-infection is minimal in COVID-19 patients; however, broad-spectrum antimicrobial prescribing rates and use are substantial.[20] These prescribed antimicrobial agents include antibiotics such as azithromycin, antimalarial agent as hydroxychloroquine, and antivirals like remdesivir, ritonavir, and lopinavir.[16,21] Many reports showed that these agents are used empirically without any confirmed diagnosis.[22–24] Azithromycin is also extensively used with hydroxychloroquine, according to the WHO, even though they are not currently approved outside of COVID-19 clinical studies.[25] The inappropriate use of these antimicrobial agents may lead to a serious condition, secondary bacterial infection, which was linked with a high mortality rate in patients with COVID-19.[26] Moreover, it was observed that patients with COVID-19 who were treated at crowded spaces like nursing homes and psychiatric hospitals were associated with highly resistant microbes, including vancomycin-resistant enterococcus, Methicillin-resistant Staphylococcus aureus, and carbapenemase-producing organisms, and fungi as Candida.[27]

Generally, the risk of invasive Candida infection is very high in patients with critical illness who received an extensive course of antibiotics. This risk is directly proportionate with the type, duration, and dose of antibiotic.[28] Jensen et al.[29] showed that patients who received high-dose antibiotics were associated with a higher rate of invasive oral Candida than those who received low-dose (HR= 1.9, 95% CI: 1.01 – 3.6). In addition, the risk was substantially higher in patients

who received antibiotics for three days than those who used the high-dose antibiotics for 1-2 days (HR= 3.8, 95% CI: 1.6 – 9.3). In COVID-19 patients, Wang et al.[30] showed that out of 69 patients with COVID-19, 29 were subjected to sputum culture, but only five patients were associated with co-infection; of them, two patients had *Candida albicans*. Invasive candidiasis was shown to be prevalent in 14.1% of COVID-19 patients with ARDS, according to research conducted in the United Kingdom.[31] In critically ill patients with COVID-19, three cases of candidemia were observed after receiving an IL-6 inhibitor, tocilizumab.[32] Moreover, several case reports have documented the Candida co-infection in patients with COVID-19.[33]

Our findings showed that fluconazole resistance was significantly higher in 86.7% of the cases group compared with only 56.7% of the subjects in the control group (p= 0.010). Similarly, nystatin resistance was significantly higher in 76.7% of the cases group, while the control group showed 23.3% drug resistance (P < 0.001). Shirvani and his colleagues [34] conducted another study to isolate Candida species from individuals with the COVID-19 disease and evaluate the susceptibility pattern of Candida species to routine antifungal drugs. The detected species showed significant resistance against Itraconazole and Fluconazole; however, the Amphotericin B and Voriconazole responded well. Their findings demonstrated that *Candida albicans* and *Candida parapsilosis* were the only detected species (96% vs 4%). In prior studies, multiple azole, multidrug, and fluconazole were found in 30%, 40%, and 100% of *Candida auris* isolates, respectively.[35,36] Oral candidiasis can travel from the oropharynx to the oesophagus or systemically through the bloodstream or upper gastrointestinal tract when therapy is not given or is ineffective, such as in fluconazole-resistant candida or immunocompromised people.[37] The resultant candidemia has been proven to cause considerable morbidity and death, with a 71–79% mortality rate.[38] An investigation in Spain found that COVID-19 individuals had candidemia, candiduria, and severe intraabdominal candidiasis.[39] As a result, it's critical to consider how these individuals will be treated in a hospital setting. The use of AuNPs, nanoparticles of gold, was proposed in cases of azole resistance in patients with invasive Candida.[40]

To the best of our knowledge, this study is the first study to assess the resistance of oral *Candida albicans* to fluconazole and nystatin among healthy persons after treatment with azithromycin and hydroxychloroquine for suspected COVID-19. However, we acknowledge that our study has some limitations, including the small sample size and the single-centre setting, which may hinder the generalizability of our findings. In addition, we studied only one species of *Candida albicans*; however, according to the literature, it is the most prevalent species in COVID-19 patients.

CONCLUSION

Treating COVID-19 patients with azithromycin and hydroxychloroquine negatively impacted the antimicrobial resistance of oral candidiasis. Given the limited efficacy of both treatments, it is advisable to avoid the broad-spectrum use of both agents in managing COVID-19 cases. Azithromycin and hydroxychloroquine had a subsequent negative impact on the antimicrobial resistance of oral candidiasis. The resistance to Fluconazole and Nystatin was detected significantly higher in cases groups compared with the control group. The study results recommend avoiding broad-spectrum use of Azithromycin and Hydroxychloroquine agents in the management of COVID-19 cases with Candida Albicans.

Recommendations

Further large-scale studies are recommended to investigate more species of candida and their response with broad-spectrum of antifungal agents.

Acknowledgment: We acknowledge all patients participated in this study.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

Conflict Of Interest: The author confirms no financial or personal relationship with a third party whose interests could be positively or negatively influenced by the article's content.

Funding Source: None (The author confirms no funding received to do this work)

REFERENCES

1. Akpan A, Morgan R. Oral candidiasis. Postgrad Med J 2002;78:455–459.
2. Maheronnaghsh M, Tolouei S, Dehghan P, et al. Identification of Candida species in patients with oral lesion undergoing chemotherapy along with minimum inhibitory concentration to fluconazole. Adv Biomed Res 2016;5.
3. Davies AN, Brailsford SR, Beighton D. Oral candidosis in patients with advanced cancer. Oral Oncol 2006;42:698–702.

4. Repentigny L de, Lewandowski D, Jolicoeur P. Immunopathogenesis of oropharyngeal candidiasis in human immunodeficiency virus infection. Clin Microbiol Rev 2004;17:729–759.
5. Meurman JH, Uittamo J. Oral micro-organisms in the etiology of cancer. Acta Odontol Scand 2008;66:321–326.
6. Pfaller MA, Diekema D. Epidemiology of invasive candidiasis: a persistent public health problem. Clin Microbiol Rev 2007;20:133–163.
7. Samaranayake LP, Keung Leung W, Jin L. Oral mucosal fungal infections. Periodontol 2000, 2009;49:39–59.
8. Cowen LE, Sanglard D, Howard SJ, et al. Mechanisms of antifungal drug resistance. Cold Spring Harb Perspect Med 2015;5:19–22.
9. Vazquez JA, Skiest DJ, Nieto L, et al. A multicenter randomized trial evaluating posaconazole versus fluconazole for the treatment of oropharyngeal candidiasis in subjects with HIV/AIDS. Clin Infect Dis 2006;42:1179–1186.
10. Badiei P, Aalborzi AA V, Davarpanah MA, et al. Distributions and antifungal susceptibility of Candida species from mucosal sites in HIV positive patients. Archives Of Iranian Medicine, 2010.
11. Akins RA. An update on antifungal targets and mechanisms of resistance in Candida albicans. Med Mycol Informa UK Ltd UK, 2005;43:285–318.
12. Kontoyiannis DP, Lewis RE. Antifungal drug resistance of pathogenic fungi. Lancet 2002;359:1135–1144.
13. Cannon RD, Lamping E, Holmes AR, et al. Efflux-mediated antifungal drug resistance. Clin Microbiol Rev, 2009;22:291–321.
14. Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72314 cases from the Chinese Center for Disease Control and Prevention. JAMA, 2020;323:1239–1242.
15. Molina JM, Delaugerre C, Goff J Le, et al. No evidence of rapid antiviral clearance or clinical benefit with the combination of hydroxychloroquine and azithromycin in patients with severe COVID-19 infection. Med Mal Infect, 2020;50:384.
16. Gautret P, Lagier J-C, Parola P, et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. Int J Antimicrob Agents, 2020;56:105949.
17. Sharon V, Fazel N. Oral candidiasis and angular cheilitis. Dermatol Ther 2010;23:230–242.
18. Li CH, Zhu ZH, Dai YH. Diaper dermatitis: a survey of risk factors for children aged 1-24 months in China. J Int Med Res 2012;40:1752–1760.
19. Rawson TM, Moore LSP, Castro-Sanchez E, et al. COVID-19 and the potential long-term impact on antimicrobial resistance. J Antimicrob Chemother, 2020;75:1681–1684.
20. Rawson TM, Moore LSP, Zhu N, et al. Bacterial and fungal coinfection in individuals with coronavirus: a rapid review to support COVID-19 antimicrobial prescribing. Clin Infect Dis 2020;71:2459–2468.
21. Cao B, Wang Y, Wen D, Liu W, Wang J, … Wei M. A trial of lopinavir–ritonavir in adults hospitalized with severe Covid-19. N Engl J Med 2020.
22. Huang C, Wang Y, Li X, Ren L, Zhao J, … Gu X. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395:497–506.
23. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet, 2020;395:1054–1062.
24. Abdelgawad HAH, Sayed A, Munir M, et al. Clinical Review of COVID-19; Pathogenesis, Diagnosis, and Management. Curr Pharm Des 2021;27:4232–4244.
25. Getahun H, Smith I, Trivedi K, Paulin S, Balkhy HH. Tackling antimicrobial resistance in the COVID-19 pandemic. Bull World Health Organ 2020;98:442.
26. Li J, Wang J, Yang Y, et al. Etiology and antimicrobial resistance of secondary bacterial infections in patients hospitalized with COVID-19 in Wuhan, China: a retrospective analysis. Antimicrob Resist Infect Control 2020;9:1–7.
27. Clancy CJ, Buehrle DJ, Nguyen MH. PRO: The COVID-19 pandemic will result in increased antimicrobial resistance rates. JAC-Antimicrobial Resist 2020; 49.
28. Hebert C, Villaran R, Tolentino J, et al. Prior antimicrobial exposure and the risk for bloodstream infection with fluconazole-non-susceptible Candida strains. Scand J Infect Dis 2010;42:506–509.
29. Jensen J-US, Hein L, Lundgren B, et al. Invasive Candida infections and the harm from antibacterial drugs in critically ill patients: data from a randomized, controlled trial to determine the role of ciprofloxacin, piperacillin-tazobactam, meropenem, and cefuroxime. Crit Care Med, 2015;43:594–602.
30. Wang Z, Yang B, Li Q, et al. Clinical features of 69 cases with coronavirus disease 2019 in Wuhan, China. Clin Infect Dis 2020;71:769–777.

31. White PL, Dhillon R, Cordey A, et al. A national strategy to diagnose coronavirus disease 2019–associated invasive fungal disease in the intensive care unit. Clin Infect Dis 2021;73:e1634–e1644.
32. Antinori S, Bonazzetti C, Gubertini G, et al. Tocilizumab for cytokine storm syndrome in COVID-19 pneumonia: an increased risk for candidemia? Autoimmun Rev 2020;19:102564.
33. Ohashi N, Ideta Y, Takeda A, et al. Oral candidiasis caused by ciclesonide in a patient with COVID-19 pneumonia: A case report and literature review. SAGE Open Med Case Reports 2021;9.
34. Shirvani F, Fattahi A. Pulmonary Candidiasis Associated with COVID-19: Evaluation of Causative Agents and their Antifungal Susceptibility Patterns. Tanaffos 2021;20:29.
35. Al-Hatmi AMS, Mohsin J, Al-Huraizi A, et al. COVID-19 associated invasive candidiasis. J Infect Elsevier, 2021;82:e45–e46.
36. Chowdhary A, Tarai B, Singh A, et al. Multidrug-resistant Candida auris infections in critically Ill coronavirus disease patients, India, April–July 2020. Emerg Infect Dis 2020;26:2694.
37. Jeronimo LS, Lima RPE, Suzuki TYU, et al. Oral Candidiasis and COVID-19 in Users of Removable Dentures: Is Special Oral Care Needed? Gerontology 2022;68:80–85.
38. Antinori S, Milazzo L, Sollima S, et al. Candidemia and invasive candidiasis in adults: A narrative review. Eur J Intern Med 2016;34:21–28.
39. Garcia-Vidal C, Sanjuan G, Moreno-García E, et al. Incidence of co-infections and superinfections in hospitalized patients with COVID-19: a retrospective cohort study. Clin Microbiol Infect, 2021;27:83–88.
40. Rahimi H, Roudbarmohammadi S, Delavari H, et al. Antifungal effects of indolicidin-conjugated gold nanoparticles against fluconazole-resistant strains of Candida albicans isolated from patients with burn infection. Int J Nanomedicine 2019;14:5323.