NARRATIVE REVIEW

# Association of Candida Species with Novel SARS-CoV-2 and Biomarkers for Fungal Premalignant Oral Lesions

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## Abstract

Oral fungal infections can be caused by certain species of fungi among which candida albicans is the most implicated. Oral candidiasis is correlated with multiple conditions, such as coronavirus disease-2019, oral leukoplakia and oral erythroplakia. Tenascin is a glycoprotein and is present at the site of tissue injury and chronic inflammation, and tends to be over-expressed in cases of malignancy. Matrix metalloproteinase-9 belongs to a family of zinc-dependent endopeptidases and is involved in the degradation of extracellular matrix, leading to tissue invasion and metastasis. The current narrative review was planned to shed light on the fungal co-infections of coronavirus disease-2019 and molecular mechanisms of matrix metalloproteinase-9 and tenascin involved in the pathogenesis of fungus-associated oral leukoplakia and oral erythroplakia.

**Keywords:** Oral, fungal, COVID-19, Tenascin, MMP-9.

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## Introduction

Candida species belongs to the genus of yeasts and is one of the most common causes of fungal infections worldwide. Candida is detected on the skin and in oral cavity, throat, gastrointestinal tract (GIT), and vagina. In oral cavity it causes oral candidiasis. The most commonly isolated species that can cause oral candidiasis in humans is candida (C.) albicans. It is isolated in over 80% of fungal lesions. Oral candidiasis is defined as white patches on the buccal mucosa, tongue and the roof of the mouth. Symptoms include redness, loss of taste and pain while eating or swallowing. Predisposing factors for oral candidiasis include drug therapy consisting of prednisone and corticosteroids, blood dyscrasias, malignancy, malnutrition, endocrine disorders, immunological disorders and salivary changes, such as reduced salivary flow rate associated with salivary gland hypo-function and xerostomia.[1] The oral candidiasis has been classified as pseudomembranous candidiasis, erythematous candidiasis, hyperplastic candidiasis, denture-associated candidiasis and angular chelitis.[2] The lesions of oral candidiasis progress towards leukoplakia and erythroplakia if they are left untreated over a long period of time. The current narrative review was planned to explain the fungal co-infections associated with coronavirus disease-2019 (COVID-19), and signalling of matrix metalloproteinase-9 (MMP-9) and tenascin in the pathophysiology of fungus-associated oral leukoplakia and erythroplakia. Electronic literature databases, such as PubMed and Google Scholar, were searched using medical subject heading (MeSH) terms, such as COVID-19, matrix metalloproteinase-9 signalling, tenascin, oral leukoplakia, erythroplakia, oral potential malignant disorder, oral candidiasis and fungus-associated premalignant lesions in various combinations. All papers in English language having full texts available were included. The papers that were published in any language other than English and whose full texts were not available were excluded. The literature search was done from February to June 2021.

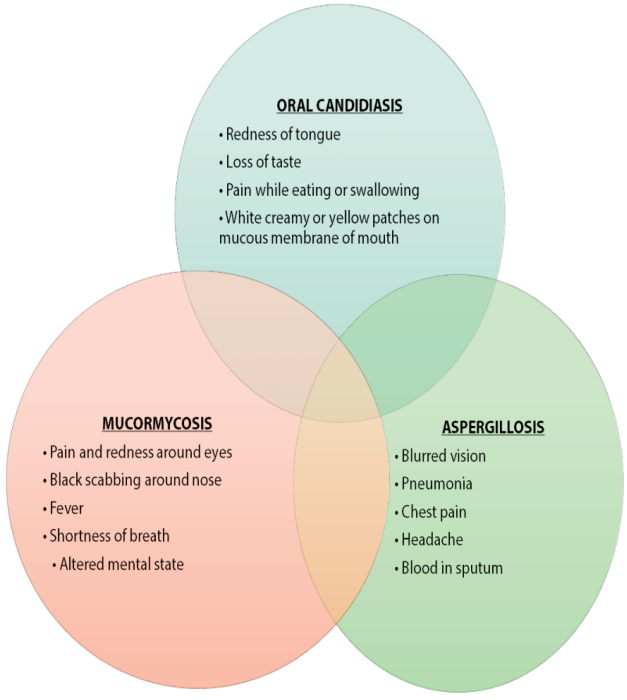


**Figure:** Signs and symptoms of three most commonly encountered fungal co-infections in coronavirus disease-2019 (COVID-19) patients.

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**Coronavirus disease and fungal infection:** COVID-19 is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and caused one of the major pandemics known in human history. Immunosuppression has been reported in COVID-19 patients which leads to a decreased count of cluster of differentiation- (CD4) +T cells and (CD8) +T cells. This in turn makes the COVID-19 patient susceptible to acquiring a fungal co-infection (Figure).[3] A news article published in local newspaper reported cases of opportunistic fungal infections in association with COVID-19 patients in Karachi, Pakistan. Most of these cases were related to mucormycosis, known as black fungus, and COVID-19-associated pulmonary aspergillosis (CAPA). In addition, as a post-COVID-19 complication, candidiasis was also seen in patients who had recovered from COVID-19.[4]

**Candidiasis in COVID-19:** Critically ill patients of COVID-19 treated with broad-spectrum antibiotics, parenteral nutrition or having prolonged neutropenia are at a high risk of acquiring candidiasis. A recent study reported that fungal culture was performed on the swabs taken from 99 COVID-19 patients. Of them, 5 cases were of fungal infections, including 1 case each of aspergillus (A.) flavus and C. glabratea, and 3 cases of C. albicans. Diagnostic techniques for candidiasis include a blood culture or culture of sample collected from the lesion in the oral cavity under sterile conditions, and a non-culture approach which includes mannan and anti-mannan immunoglobulin G (IgG) tests, C. albicans germ tube antibody (CAGTA) and polymerase chain reaction (PCR)-based assays. Treatment regime for patients infected with candidiasis includes echinocandin (caspofungin and micafungin), azoles (fluconazole and itraconazole) and amphotericin B.[5]

**Mucormycosis in COVID-19:** Mucormycosis was seen in COVID-19 patients who had a history of trauma, diabetes mellitus (DM) and prolonged neutropenia. Symptoms included pain and redness around the eyes, black scabbing around the nose, fever, shortness of breath (SOB) and altered mental state. Diagnosis of mucormycosis is based on the results of direct microscopy or plus fluorescent brighteners from sputum specimens. Skin lesions of non-septate mucorales hyphae with a variable width of 6-16µm are also a diagnostic criterion for mucormycosis. Amphotericin B lipid complex, liposomal amphotericin B and posaconazole oral suspension are given as first-line antifungal drug therapy.[5]

**Aspergillosis in COVID-19:** Aspergillosis infection, when accompanied with chronic obstructive pulmonary disease (COPD), caused serious life-threatening condition in COVID-19 patients. Symptoms of aspergillosis are blurred vision, chest pain, headache, pneumonia, and blood in the sputum. Diagnosis of aspergillosis is done with microscopic or histological intervention. Microscopic examination is done with the optical brightener methods, using calcofluor or blankophor fluorescent dyes. For the assessment of histological parameters, culture is done on fungus-specific media at 37$^{O}$C for 2-5 days. For treatment and prophylaxis of aspergillosis, triazoles (itraconazole, voriconazole, posaconazole and esaconazole) are recommended.[5]

**Table:** Fungal infections and their association with oral lesions.

| Fungal Infections | Premalignant lesions with Fungal Co-Infections | | |
|---|---|---|---|
| | Leukoplakia | Erythroplakia | COVID-19 |
| Candidiasis | ✓ | ✓ | ✓ |
| Mucormycosis | - | - | ✓ |
| Aspergillosis | - | - | ✓ |

COVID-19: Coronavirus disease-2019.

**Candida albicans infection in premalignant lesions:** C. albicans infection has been associated with the development of oral premalignant lesions as it was found to be an underlying cause of oral leukoplakia and due to its correlation with oral epithelial dysplasia (Table). Candida-infected oral leukoplakia has a higher rate of malignant transformation than non-infected leukoplakia.[6,7] Apart from leukoplakia, C. albicans has also been demonstrated in cases of erythroleukoplakia. Candida infection appears as secondary or superinfection in case of erythroleukoplakia, and studies suggested that the red component of erythroplakia diminished after treatment with antifungal drugs.[8,9]

**Inflammatory markers in premalignant lesions:**
Cytokines are a large group of proteins that are known to be involved in autocrine, paracrine and endocrine signalling as immunomodulating agents. Interleukin-1 (IL-1), IL-6, IL-8, cyclooxygenase-2 (Cox-2) and tumour necrosis factor-alpha (TNF-α) belong to the category of cytokines which contribute through their leukocyte chemotaxis activity to immunoregulatory processes. The function of IL-1beta, IL-6, IL-8 and TNF-α is to promote inflammation whereas IL-10 is anti-inflammatory in nature. By means of an arachidonic acid pathway, the two known isoforms of cyclooxygenase (Cox), Cox-1 and Cox-2, induce pain and inflammation.[10] Inflammatory markers, MMP-9 and IL-17, are specifically proposed as prognostic biomarkers in the development of oral squamous cell carcinoma (OSCC). It had been observed in a clinical study that supernatants and blood serums of patients with leukoplakia, erythroplakia and oral submucous fibrosis had increased levels of IL-17 and MMP-9, and exhibited a potential towards malignancy. Hence, the presence of IL-17 and MMP-9 is related with poor clinical outcomes, including decreased survival.[11] The expression of transforming growth factor-alpha (TGF-α) and epidermal growth factor receptor (EGFR) was up-

regulated in oral leukoplakia and oral submucous fibrosis. Therefore, EGFR and TGF-α are considered early biomarkers of malignancy in oral leukoplakia and epithelial dysplasia. Research has shown that in multiple cases of head and neck carcinomas, the levels of EGFR is increased, showing its potential role in a cascade of malignancy as it effects cell cycle progression, apoptosis, angiogenesis and metastasis.[12]

**Matrix metalloproteinases in premalignant lesions:**
Matrix metalloproteinases are considered a large family of zinc-dependent endopeptidases that are largely responsible for the degradation of all extracellular matrix (ECM) proteins and components of basement membrane in both physiological conditions (embryonic development, reproduction, angiogenesis, bone development, cell migration and wound healing) and pathological conditions (arthritis and metastasis).[13] MMP-9, known as gelatinase B, is the largest member of the gene family, and well-established evidence is available that proves the overexpression of MMP-9 in oral leukoplakia and erythroplakia.[14] Oral premalignant lesions, such as leukoplakia and erythroplakia, show progression towards oral cancer by degradation of collagen IV, elastin and fibronectin through the action of MMP-9.[15] MMP-9 has been the centre of attention as a potential biomarker of leukoplakia and erythroplakia, and has been quantitatively measured in saliva and serum of oral potential malignant disorder (OPMD). A study showed that MMP-9 directly targeted the meshwork of type IV collagen along with collagen type V, VII and X, fibronectin and elastin. This breakdown of meshwork of collagen facilitates the invasion and metastasis of tumour.[16]

**Activation of matrix metalloproteinases in premalignant lesions:**
Integrins along with cell adhesion receptors regulate the activation of MMPs, leading to their migration and invasion. Normal gingival epithelium and oral tissues with chronic inflammation generally do not express integrin-αvβ6 but its expression is seen in the malignant transformation of oral leukoplakia. The role of integrin-αvβ6 is to promote the migration of malignant keratinocytes, and it also up-regulates the expression of MMP-9.[17]

**Tenascin:** ECM plays an important role in cell adhesion, migration, proliferation, differentiation and gene expression.[18] The structure of ECM is protected by proteases. A study showed that degradation of ECM was observed in progression of premalignant lesions into oral squamous cell carcinoma (OSCC).[19] ECM is composed of four classes of components; collagen, glycoproteins, proteoglycans and elastin. Collagen is the main component of ECM and in pathological conditions, such as leukoplakia and OSCC, its activity becomes abnormal. Among the glycoproteins, fibronectin, tenascin and undulin are the basic constituents.[20] Tenascins are large glycoproteins that are present in embryonic and adult ECM. Out of the four family members, the two isoforms of tenascin, tenacsin-C and tenascin-W, are observed to be overexpressed in preneoplastic conditions.[21] Tenascin-C is a large ECM glycoprotein that has a very tightly controlled pattern of expression. In most healthy adult tissues, little or no tenascin is expressed. The role of tenascin is seen at the site of tissue injury and chronic inflammation. However, persistent expression of tenascin-C is associated with non-healing premalignant lesions and fibrotic diseases.[22]

**Tenascin in premalignant lesions:** A risk of malignancy is always associated with oral leukoplakia and according to statistics, 11% cancers develop from such premalignant lesions. According to a study, in cases of moderate dysplasia a minimal expression of tenascin was seen. However, when the degree of dysplasia ranged from mild to severe, a greater intensity and area of expression of tenascin has been observed. A homogenous pattern of tenascin expression appeared early in dysplastic changes in epithelium. In subjects with mild to moderate degree of dysplasia, the expression pattern of tenascin was seen to be reticular whereas in case of severe dysplasia it was observed to be fibrillar. Hence, it was concluded that this pattern of expression indicates changing degrees of dysplasia in oral epithelium.[23,24] A study showed that tenascin was usually present in the stromal connective tissue immediately adjacent to the oral basement membrane and its expression was enhanced in leukoplakia, erythroplakia, oral submucous fibrosis and OSCC. The increase in reactivity in leukoplakia is associated with the increase in degree of hyperplasia, hyperkeratosis and dysplasia of the lesion. Tenascin was seen to be consistently present around the tumour cells in well-differentiated tumours. However, the expression was low in poorly differentiated tumours. This suggested a different level of stromal reaction owing to the state of differentiation of tumour cells.[25]

## Conclusion

Fungal infections are caused by several fungi species. Among all the species, C. albicans is most commonly seen involved in oral lesions. Oral candidiasis, if not treated at the proper time, can lead to the development of oral premalignant lesions, such as leukoplakia and erythroplakia. Oral candidiasis has also been reported as a fungal co-infection in COVID-19. MMP-9 and tenascin play a vital role in the progression of fungal premalignant oral lesions by disruption of basement membrane.

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