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Zygomycosis of the Oral Cavity in a Main Referral Centre in Malaysia: A Case Series (1967-2021)

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ABSTRACT

Zygomycosis is a rare opportunistic fungal infection caused by fungi in the phylum Mucoromycota, subphylum Mucoromycotina and order Mucorales. Immunocompromised patients may harbour the disease by inhalation of the spores from the environment into the paranasal sinuses. Rhino-orbital-cerebral is the most common form which extends into the oral cavity, especially to the palate. We present twelve cases of zygomycosis diagnosed in the Stomatology Unit, Institute for Medical Research (IMR), Malaysia. Most of the patients are immunocompromised and presented as the rhino-orbital-cerebral form. Routine haematoxylin and eosin (H&E) staining were used for all cases while selected cases required additional special staining. The characteristic histological features of broad, non-septate, branched, amphophilic fungal hyphae are seen. We aim to report this rare occurrence to contribute to the literature and raise awareness about this condition among clinicians. To date, this is the first compilation of oral zygomycosis cases known to be reported in Malaysia.

INTRODUCTION

Zygomycosis is an opportunistic, invasive fungal infection which is caused by fungi in the phylum Mucoromycota, subphylum Mucoromycotina and order Mucorales. The genera *Actinomucor, Apophysomyces, Cokeromyces, Cunninghamella, Lichtheimia, Mucor, Rhizomucor, Rhizopus, Saksenaea, Syncephalastrum,* and *Thamnostylum* are regarded as the causative agents in the majority of cases of zygomycosis in humans [1]. Specifically, the genus *Rhizopus* is the most commonly isolated organism in the rhino-orbito-cerebral form, *Mucor* in the cutaneous form and *Rhizomucor* in pulmonary infections [1].

Within the environment, organisms in the order Mucorales are usually found in soil, compost, animal faeces, decaying vegetables, agricultural debris, or other organic matter. The major route of human infection is via inhalation of the spores from the environment [2]. Besides that, percutaneous and gastrointestinal modes of transmission have also been recorded, for example, in traumatized patients and by contamination of tongue depressors used in a clinical setting, respectively [3].

Clinically, zygomycosis is known to present in four forms which are rhino-orbital-cerebral, pulmonary, cutaneous and disseminated form. The most common clinical manifestation reported is the rhino-orbito-cerebral form which happens by inhalation of the spores into the paranasal sinuses and manifests as early symptoms consistent with sinusitis [4]. It may progress rapidly into the surrounding tissues causing erythema and eventually, blackish discolouration due to blood vessel thrombosis and tissue necrosis. This is the result of direct penetration of the fungus through the tissue or blood vessel wall, which further causes haematogenous spread. Besides that, the infection can spread via perineural invasion, although not as common as the former [5,6]. The zygomycosis infection may extend superiorly into the ocular region and inferiorly into the oral region, thus causing damage to both areas. Ocular involvement

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is suggested by the presence of pain, vision blurriness or loss of vision while oral signs and symptoms include painful, black, necrotic ulcerations of the hard palate [3].

Many studies have been reported worldwide including from Asian countries (India, Pakistan, Uzbekistan, Qatar, Jordan, Japan and Korea). However, in the South-East Asia region, cases were reported from Malaysia, Thailand and the Philippines only [7]. Two separate zygomycosis cases were reported in Malaysia involving the nasofacial region and the tongue [8,9]. As zygomycosis is rare, it is not a highly reportable disease, especially in developed countries. Among developing countries, the detection rate may be low as the diagnostic facility may not be well-equipped for the identification of this microorganism. To date, this is the first compilation of zygomycosis cases known to be reported in Malaysia.

We aim to report this rare occurrence to contribute to the literature and raise awareness about this condition among health practitioners. They may play an important role in early detection which is crucial due to the high morbidity and mortality rate of zygomycotic infection [10].

We present twelve cases of zygomycosis reported in the Stomatology Unit of Institute for Medical Research (IMR), Malaysia from the year 1967 to 2021. The Stomatology Unit is the main referral centre for histopathological consultation of oral diseases in Malaysia. The epidemiology, clinical presentation, histopathological features, differential diagnoses and brief management of zygomycosis will be discussed. This case series has been registered under the National Medical Research Register (NMRR-21-571-59239). Since it is only a case series, ethical approval was not required (NIH Guidelines For Conducting Research In Ministry of Health Institutions & Facilities 3rd Edition 2021).

CASE PRESENTATION

Twelve cases were retrieved from the computerized record in our unit from the year 1967 to 2021. These cases are summarized in Table 1.

Histopathology

Specimens from all cases were fixed in 10% formalin and processed in our laboratory for viewing under the light microscope. Routine haematoxylin and eosin (H&E) staining were carried out for all cases while selected cases required additional special staining (Periodic acid-Schiff (PAS) and/or Grocott methenamine silver (GMS)) to highlight the presence of the fungal cell wall. All sections showed similar microscopic features of broad, non-septate, right angle-branching, amphophilic, ribbon-like fungal hyphae admixed with necrotic debris, as shown in Figure 1. No other diagnostic methods were conducted for these samples in our laboratory.

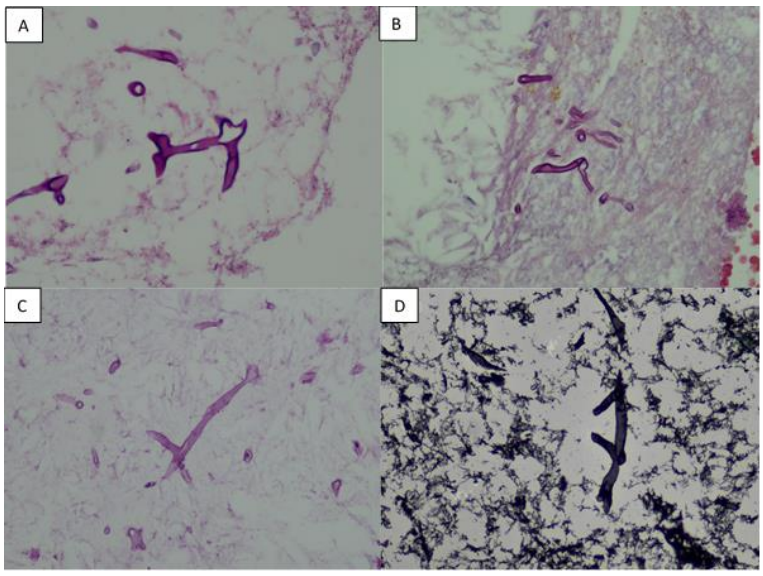

Figure 1. Broad, non-septate, right-angle branched, amphophilic, ribbon-like fungal hyphae. (A) H&E-stained section (60x magnification) taken from Case 5; (B) H&E-stained section (40x magnification) taken from Case 6; (C) PAS-stained section (40x magnification) taken from Case 6; (D) GMS-stained section (40x magnification) taken from Case 6.

Table 1. Clinical summary of the twelve cases reported.

Case	Age	Gender	Race	Site	Clinical presentation	Medical history	Clinical differential diagnosis
1	59	M[a]	Malay	Left hard palate, upper left buccal sulcus	Total loss of vision of left eye, painful swelling, erythema with central black pigmented area, ulcer	Diabetes mellitus	Rhinocerebral zygomycosis
2	59	M	Chinese	Hard palate	Numbness, epistaxis, halitosis, ulcer	Diabates mellitus Corneal transplantation rejection (on oral prednisolone 20 mg qid)	Zygomycosis
3	69	F[b]	Chinese	Left buccal sulcus	Swelling	Not available	Not available
4	56	F	Malay	Tongue	Necrosis	Diabetes mellitus (Diabetic ketoacidosis secondary to acute infection)	Necrotic tongue secondary to chronic tongue biting
5	66	F	Malay	Maxilla	Swelling	Diabetes mellitus, hypertension, dyslipidemia	Necrotic bone
6	51	M	Malay	Left maxilla	Non-healing extraction socket	Diabetes mellitus, actinomycosis	Actinomycosis
7	74	M	Chinese	Right maxillary tuberosity	Swelling and necrotic bone	Diabetes mellitus, hypertension, dyslipidemia	Osteomyelitis, osteonecrosis, cemento-osseous lesion
8	55	M	Chinese	Maxilla	Large fistula over right infraorbital region, blackish discoloration, destruction of right maxillary sinus wall with OAC[c], destruction of floor of right orbit with minimal herniation of orbital content into maxillary sinus.	SCC[d] (underwent radiotherapy)	Osteoradionecrosis, recurrent SCC
9	38	F	Malay	Right maxillary sinus	Fever, right eye pain, epiphora, chemosis, loss of vision of right eye.	Diabetes mellitus	Not available
10	24	M	Indian	Bilateral orbital area	Fever, pus from right eye, frontal region, right ethmoidal and right infratemporal space, necrotic soft tissue and bone over frontal region and right orbital region, swelling of right eye, swelling and punctum over palate with pus discharge.	Post- alleged MVA[e] sustained right orbital blow out fracture with subluxation of right globe into sinus, bilateral lung contusion, left facial palsy, anaemia, *Pseudomonas aeruginosa* and *Candida* spp. infection	Necrotic bone
11	42	M	Bangladeshi	Left palate	Blackish necrotic tissue on left palate	COVID-19	Zygomycosis
12	61	M	Malay	Right maxilla	Non-healing extraction socket, exposed bone at right maxilla, blackish discolouration and sloughing	COVID-19, diabetes mellitus, hypertension, ischemic heart disease and single functioning kidney	Osteomyelitis, zygomycosis

[a]M: Male; [b]F: Female
[c]OAC: Oro-antral communication
[d]SCC: Squamous cell carcinoma
[e]MVA: Motor vehicle accident

DISCUSSION

The terms "zygomycosis" and "mucormycosis" have been used interchangeably across the decades. With the advent of molecular phylogenetic techniques, the terms "mucormycosis" and "entomophthoromycosis" have been proven to be more accurate in terms of specifying the true lineage of this fungal infection [11]. However, the term "zygomycosis" is still suggested to be used for centres where cultures are unavailable [11]. In view of the unavailability of the culture method, the name "zygomycosis" is consistently used throughout these 54 years in our laboratory.

A systematic review and meta-analysis of case reports concluded that the median age for those affected with zygomycosis was 51 years, with an age range from 39 to 61 years [4]. Our cases presented a slightly wider age range, from 24 to 74 years old, with a median of 57.5 years. Unlike an epidemiological study by Roden *et al.* which reported a mean age of 38.8 years, our study reported a mean age of 54.5 years [12]. This large gap might be because our cases consisted of mostly the elderly, compared to the wider age range in the previous study [12]. Most of our cases are males (8 out of 12 cases). This finding concurs with others [4, 12].

Malaysia, being a multiracial country, comprises three main ethnicities which are Malay, Chinese and Indian, in descending order of distribution [13]. In line with this, most of our patients were Malays (n=6, 50.0%), while Chinese came second (n=4, 33.3%), followed by Indians (n=1, 8.3%). To our knowledge, no studies highlighting the racial distribution of zygomycosis have been published in the literature.

This life-threatening infection usually happens in immunocompromised hosts including those with uncontrolled diabetes mellitus, steroid therapy, bone marrow or solid organ transplantation, haematological malignancy, iron overload and trauma [2]. A global epidemiological study showed that diabetes mellitus is the most common risk factor in the Asian population whereas haematological malignancy and transplant patients are highly recorded in Western countries [7]. One should also be aware that during this COVID-19 pandemic era, there has been an unusual rise in this opportunistic infection, possibly linked to the virus's ability to reduce insulin secretion, deteriorate insulin resistance and increase serum levels of free iron. Furthermore, it has been found that glucocorticoids, lopinavir-ritonavir and remdesivir used in the management of COVID-19 patients may worsen glucose control, thus rendering them at higher risk of developing zygomycosis [14]. Two of our cases (Case 11 and 12) were in line with this new finding regarding the relationship between this "black fungus" and COVID-19. Case 12 was further complicated by the patient having concurrent uncontrolled diabetes mellitus. Besides that, in the present case series, seven other patients had underlying diabetes mellitus, similar to other reported cases [15,16]. Case 2 in this report had a concurrent diagnosis of diabetes mellitus and underwent high-dose steroid therapy for corneal transplantation rejection. In case 8, the patient was a known case of squamous cell carcinoma (SCC) and had radiotherapy which suppressed his immune system. However, it does not mean that immunocompetent hosts are excluded from this disease as a case of a young immunocompetent host with zygomycosis was reported in India [16]. In fact, in case 10, even though the patient was initially immunocompetent, severe maxillofacial trauma might have caused him to be more susceptible towards developing zygomycosis.

The palate is the most common oral site involved in the rhino-cerebral-orbital form. Besides necrosis, other oral findings include swelling, non-healing extraction socket, oro-antral communication and halitosis [3]. In our study, most of the cases (n=11, 91.7%) presented as the rhino-orbital-cerebral form involving the palate except in case 4 where the patient developed necrotic tongue secondary to traumatic biting and diabetic ketoacidosis. This concurs with the finding from an epidemiological study in which rhino-cerebral-orbital form is found to happen more commonly in diabetic patients [12]. In the literature, most oral zygomycosis cases also affect the maxillary bone [17,18]. Zygomycosis should be included in the differential diagnoses in patients with suspiciously delayed healing of extraction sockets as reported in cases 6, 12 and in other reported cases [19,20].

Roden et al. (2005) reported in a review that the majority of zygomycosis patients with malignancy will have their pulmonary region affected. However, in case 8 where our patient has underlying malignancy, the patient developed the rhino-cerebral-orbital form instead, which is deemed to be the second most common manifestation in cancer patients [12].

Even though zygomycosis is rare on the tongue, there is an interesting case reported in an infant

with Down Syndrome diagnosed with zygomycosis as a consequence of wooden tongue depressor usage [21]. One of our patients (case 4) developed tongue zygomycosis due to chronic biting which is comparable to the cutaneous form. Among the numerous possible causes of traumatic injuries to the skin, penetrating trauma is recorded as the most common cause for the cutaneous form of zygomycosis, similar to the finding in case 4 [12].

Radiographic imaging such as computed tomography (CT) scan is advised to reveal the extent of tissue involvement. Extension into the maxillary sinus could be observed as periosteal thickenings or effacement of the sinus walls [6]. If there is a suspicion towards the destruction of the eyes or brain, magnetic resonance imaging (MRI) is strongly recommended because of better sensitivity than a CT scan [22]. Data regarding the usage of imaging in our cases were unavailable since we accept the specimens to focus on the histopathological interpretation.

The destructive clinical presentation on the palate may alert the clinician towards other aggressive differential diagnoses such as oral SCC, salivary gland tumour, necrotizing sialometaplasia, osteonecrosis and other invasive fungal infections. The most common malignancy of the oral cavity, which is SCC, usually affects the tongue, floor of the mouth and gingiva, different from oral zygomycosis which is mostly present on the palate [23]. It is however important to not exclude SCC in the differential diagnosis of a non-healing palatal lesion as it is possible to arise there [24]. Besides the different site predilection between oral SCC and zygomycosis, SCC is usually preceded by a painless ulcer and white or red patch, unlike zygomycosis which is presented as extensive necrosis [25]. This difference in clinical presentation also applies to salivary gland tumours which mostly present initially as a painless mass, even though it happens more commonly at the palate, similar to zygomycosis [26]. Another close mimic is necrotizing sialometaplasia. Both conditions may appear as destructive palatal lesions, but necrotizing sialometaplasia is a self-limiting condition with a rapid onset and remains more localized [27]. In the presence of exposed bone, the differential diagnosis includes osteonecrosis. However, it may be helpful to note that osteonecrosis is more common in the mandible because of the relatively lower blood supply compared to the maxilla [28]. The distinction between zygomycosis and other invasive fungal infections may be achieved by histopathological examination or culture studies. In case 8, besides being diagnosed with zygomycosis, the patient also has osteoradionecrosis of the mandible. This shows the importance of multiple biopsies taken at different sites, especially when the clinical picture differs.

Widespread involvement of the oral cavity should alert the clinician of a possible concurrent diagnosis which may change the treatment plan. In case 6, the patient was suspiciously resistant to treatment for actinomycosis. Zygomycosis infection was only detected after a second biopsy was taken. Without the second biopsy, it would be impossible to justify starting the patient with high-dose antifungal therapy. An interesting case of overlapping zygomycosis and myiasis was even reported in the literature [29].

Well-stained haematoxylin and eosin (H&E) sections may reveal the characteristic broad, mostly non-septated hyphae with right-angle branching, surrounded by a narrow zone of neutrophils. Besides that, angioinvasion, perineural invasion, suppurative necrosis, purulent and granulomatous inflammatory reaction; and partial engulfment by multinucleated giant cells may also be present [3,6,10]. These features, if seen in the sections examined, may prompt the pathologist to pursue other investigations such as Ziehl-Neelsen special staining to rule out acid-fast bacilli in the presence of granulomatous inflammation. H&E stain alone is usually sufficient to identify the fungal elements. In selected zygomycosis cases, additional staining with PAS and/or GMS might also be needed to assist in the identification of the fungal cell wall, especially when it is sparse or obscured by blood vessel thrombosis, extensive necrosis, haemorrhage and inflammation [3]. However, the organisms might stain faintly positive or appear negative with PAS and GMS due to fragmentation and necrosis of the fungal hyphae [10].

Confusion might arise when attempting to differentiate between zygomycosis and aspergillosis histopathologically as both may present as branching hyphae with septations, although zygomycotic organisms are rarely septated. The hyphae in zygomycosis appear broader and branch at a right angle compared to *Aspergillus spp.* which are narrower and characteristically dichotomous in branching at an acute angle. Both organisms can be detected by GMS special staining but zygomycotic hyphae usually appear paler than the fungal hyphae in aspergillosis [10]. It is important to distinguish between these two entities since failure to do so will adversely affect the patient's management. In

general, aspergillosis patients benefit more from voriconazole while zygomycosis patients respond better to a high dose of amphotericin B [30].

A disadvantage of histopathological examination alone is the inability to identify the zygomycete organism up to the genus and species level as it appears morphologically similar. For academic and research purposes, specific identification may be achieved by culture studies while immunohistochemistry (IHC) is not widely used. Many of the antibodies that are used to detect the antigen nowadays are unable to specify the specific organism present in the tissue due to the cross-reaction with multiple fungi. Other alternative tests such as immunofluorescence, *in situ* hybridization (ISH), PCR-based methods and laser microdissection has been introduced but not widely employed yet [10]. All cases presented here were diagnosed through histological investigation alone. Culture studies were not performed since we specialize in histopathological examination of slides only.

A global guideline for the management of zygomycosis by the European Confederation of Medical Mycology in cooperation with the Mycoses Study Group Education and Research Consortium has recommended prompt treatment with surgical debridement and immediate initiation of systemic antifungal monotherapy such as amphotericin B, isavuconazole or posaconazole for weeks to months [22]. Evidence supports the continuation of treatment until correction of the immunosuppression and proof of complete response based on imaging are achieved. This, however, is based on the practitioner's best judgement and may vary between patients [29,31]. In general, the outcome relies on the patients underlying condition, affected site and antifungal treatment. A review article reported a 3% survival rate for untreated cases, 61% for patients treated with amphotericin B only, 57% for cases treated with surgery only and 70% for cases treated with a combination of antifungal medication and surgery. [12]. Even though the zygomycosis infection is treatable in some patients, there is a possibility to develop further complications such as cavernous sinus thrombosis, disseminated infection and osteomyelitis. The patients might also be left with devastating functional and aesthetical deformities. Due to the extensive oral destruction, oral zygomycosis patients might need maxillary obturators as part of the reconstruction and rehabilitation process [29,31]. For our cases, we do not have data available on the treatment which was carried out since we are on the receiving end, only dealing with histopathological examination of the cases.

CONCLUSION

Zygomycosis may present clinically as very alarming to the patient and healthcare practitioners. Hence, it should be considered in the differential diagnoses of suspiciously destructive oral lesions, particularly on the palate. The importance of thorough history taking, patient examination, investigation and a complete biopsy request form, along with an effective multidisciplinary approach could not be emphasized enough. The later the treatment is started, the worse the prognosis will be for the patient.

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DECLARATION OF INTEREST

Authors declare no conflict of interest.

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