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Oral histoplasmosis in Malaysia: A retrospective analysis of cases reported in Stomatology Unit, Institute for Medical Research during 1995-2016

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Abstract. Histoplasmosis is a systemic fungal infection caused by inhalation of *Histoplasma capsulatum*, which is mainly found in bird and bat droppings. Oral manifestation of histoplasmosis may be the only initial manifestation of the disease or associated with chronic disseminated histoplasmosis. The first review of oral histoplasmosis among Malaysian population from 1967 to 1994 (27 years) revealed the occurrence of 37 cases, reported by Ng and Siar in 1996. This current study is the updated overview of oral histoplasmosis cases in Malaysia. The objective of the study was to review and describe clinical and demographic profile of oral histoplasmosis in Malaysia and to correlate histopathological features of oral histoplasmosis with patient's immunity status. We reviewed oral histoplasmosis cases diagnosed in Stomatology Unit, Institute for Medical Research (IMR), Kuala Lumpur from 1995 until 2016. The data was retrieved from the Oral Pathology Information system (OPIS) Stomatology Unit, IMR, which is the largest oral pathology database in Malaysia. Information regarding patients' sociodemographic data, medical illness, clinical presentation, histopathological features, and referring healthcare institutions was extracted from the clinical information which accompanied the biopsy request form. A total of 39 cases of oral histoplasmosis were identified from 1995-2016. Majority of them were male (89.7%). The age ranges from 29 to 85 years with mean age of 57.8 years. Almost half of them were Malays (51.3%), followed by Chinese (33.3%), Indians (7.7%), and other races (7.7%). The most common sites of oral histoplasmosis were tongue, gingiva, palate, and alveolar ridge. The main clinical presentation was ulcer (61.5%) whereas 38.5% presented clinically as swelling. 17.9% of patients were seropositive for human immunodeficiency virus (HIV), 12.8% had tuberculosis, 10.3% had diabetes mellitus, and 2.6% with hepatitis C. The incidence of oral histoplasmosis should raise suspicion of hidden immunodepression and may be the first manifestation of acquired immunodeficiency syndrome (AIDS). Early recognition and diagnosis is crucial to reduce risk of morbidity and mortality.

INTRODUCTION

Histoplasmosis is a systemic fungal infection caused by *Histoplasma capsulatum*, a thermally dimorphic fungus that exists in yeast form in host tissue at 35-37°C and develops into hyphae in soil environment at temperature below than 35°C (Goodwin *et al.*, 1980; Kauffman *et al.*, 2011). *Histoplasma capsulatum* can be divided into three varieties: *H. capsulatum* var. *capsulatum*, a human pathogen found in America; *H. capsulatum* var. *duboisii*, a human pathogen found in Africa; and *H. capsulatum* var. *farciminosum*, a pathogen of horses and mules found in Northern Africa and Middle East (Brandt *et al.*, 2011). This mycotic infection is endemic along the Mississippi and Ohio River valleys in the United States and in certain areas of South-East Asia, including Indonesia, Malaysia, Thailand, and Vietnam (Wang *et al.*, 1996; Kauffman *et al.*, 2011).

This disease is typically acquired through inhalation of airborne microconidia, often after disturbance of environmental sites or

soil containing organism (Kauffman *et al.*, 2011). Soil heavily contaminated with bird or bat droppings is a great reservoir for the histoplasma organism (Panackal *et al.*, 2002). Activities that have been identified as sources of histoplasma infection include construction, renovation, demolition, farming, exposure to chicken coops, and exploration of caves (Cottle *et al.*, 2013). The contributing factors like moist climate and areas of bird and bat droppings provide a suitable environment and habitat for the fungal organism (Liam *et al.*, 1990). Patients with history of travel and residing in endemic areas, hobby of raising birds, or exposed to contaminated soil containing bird or bat excreta were likely to be infected by *H. capsulatum*.

Histoplasmosis has been recorded among immunocompromised patients, such as those with Acquired Immune Deficiency Syndrome (AIDS). Pan *et al.* (2003) in their study show that among the Asian population China alone has 75% of the cases reported to occur along the Yangtze River. The most common underlying cause for histoplasmosis was HIV infection. The diagnosis of histoplasmosis in immunocompromised patients is a diagnostic challenge as weakened host immune system may predispose to various opportunistic infections. Chu *et al.* (2006) reported that it is the most common cause for hospitalization and death among endemic mycoses in the United States of America (USA).

Clinically, histoplasmosis may present in various forms, particularly acute and chronic pulmonary infections, disseminated histoplasmosis, and mediastinal fibrosis. The amount of fungus inhaled and host immunity status are two crucial factors in determining the clinical presentation and extent of the disease (Goodwin *et al.*, 1980; Chinn *et al.*, 1995; Kauffman *et al.*, 2011). The inhalation of spores by immunocompetent hosts usually results in asymptomatic or self-limited pulmonary infection (Goodwin *et al.*, 1980; Kauffman *et al.*, 2011). Immunocompromised individuals may have a developing generalized infection that involves multiple organs. Although the lungs may be the only site of involvement, the risk of extrapulmonary sites is unavoidable when the fungi travel through the reticulo-endothelial system. Oral manifestation of histoplasmosis is generally rare. The oral involvement may present as initial manifestation of the disease or as an associated chronic disseminated infection (Goodwin *et al.*, 1980).

MATERIALS & METHODS

A descriptive study was conducted to identify clinical and demographic data of oral histoplasmosis among the Malaysian population. The data was retrieved from the Oral Pathology Information system (OPIS) Stomatology Unit, Institute for Medical Research (IMR), which is the largest database for oral pathology cases in Malaysia.

All cases of oral histoplasmosis diagnosed in the Stomatology Unit, Institute for Medical Research (IMR), Kuala Lumpur from 1995 until 2016 (21 years duration) were selected. Inclusion criteria for selected oral histoplasmosis includes histologically confirmed histoplasma microorganisms whereby the yeast cell wall positively stained for Periodic acid Schiff (PAS) and Grocott's Methenamine Silver (GMS) stain.

Information regarding patients' sociodemographic data (age, gender, race), medical illness/comorbidities, clinical presentation, histopathological features, and referring healthcare institutions was extracted from the clinical information which came with the biopsy request form. The compiled data was grouped accordingly and analysed with descriptional statistics. This study will give an overview of sociodemographic data, geographical distribution, clinical presentations and histological features of oral histoplasmosis in Malaysia.

RESULTS

Thirty-nine oral histoplasmosis cases were identified and subsequent data pertaining to sociodemographic data, clinical

presentation, site of distribution, existing co-morbidities, risky habits, and histopathological features were analysed [Table 1]. Out of 39 patients, 35 of them were males (89.2%) and the remaining 4 patients were females. The findings reveal wide age spectrum that ranges from 29 to 85 years old, with mean age of 54.8 years. Interestingly, there were ten patients aged 40 years and below with 6 males (17.1%) and 4 females (100%). Three of these patients had existing tuberculosis and HIV infection, one patient had end stage renal disease with history of kidney transplant, one patient had Systemic Lupus Erythematosus (SLE) and another one was an intravenous drug user. Mean age for females was much younger which is 32.8 years compared to male patients of 57.5 years. About 61.5% of the lesions involved only single site of distribution with others affecting multiple sites. Site of distributions show predilections towards tongue, followed by gingiva, alveolar ridge, and palate. State of Selangor was reported of having the highest number of oral histoplasmosis cases (23.1%) and followed by Kuala Lumpur and Negeri Sembilan, each contributing 20.5% (Figure 1). The least number of oral histoplasmosis cases were recorded in the states of Perlis, Kedah, and Sabah. In terms of risky habits, two of the patients were intravenous drug user, one patient had multiple sexual partners, 9 patients had smoking habits, and 2 patients were alcohol drinkers. About 36% of patients had underlying immunosuppressive diseases for example AIDS, diabetes mellitus, history of kidney transplant, SLE, and adrenal tumour. Meanwhile, histopathological review of the cases revealed 64% (25) cases had histiocytic inflammatory responses (Figure 1: A and B) and 36%(14) had granulomatous inflammation (Figure 1: C and D).

Table 1. Sociodemographic data, clinical presentation, sites of distribution, immune status, and risky habits of patients infected with histoplasmosis

No	Characteristics	Subtypes	Gender		Total
			Male	Female	
1	Age range (years)		29–85	29–38	29-85
2	Mean age (years)		61.3	32.8	57.8
3	Race	Malay	18	2	20 (51.3%)
		Chinese	12	1	13 (33.3%)
		Indian	3		3 (7.7%)
		Others	2	1	3 (7.7%)
4	Clinical presentation	Ulcer	22	2	24 (61.5%)
		Swelling	13	2	15 (38.5%)
5	Sites of distribution	Focal	23	1	24 (61.5%)
		Multiple	12	3	15 (38.5%)
		tongue	13		13
		palate	8	1	9
		gingiva	7	2	9
		alveolar ridge	8	1	9
		buccal mucosa	7		7
		lip	2	2	4
		buccal sulcus	3	1	4
		fauces	1		1
		floor of mouth	1		1
6	Immunosuppression	Yes	12	2	14 (36%)
		No	23	2	25 (64%)
7	Risky habits	Intravenous drug user	2		2 (5%)
		Multiple sexual partners	1		1 (3%)
		Alcohol	2		2 (5%)
		Smoking	9		9 (23%)

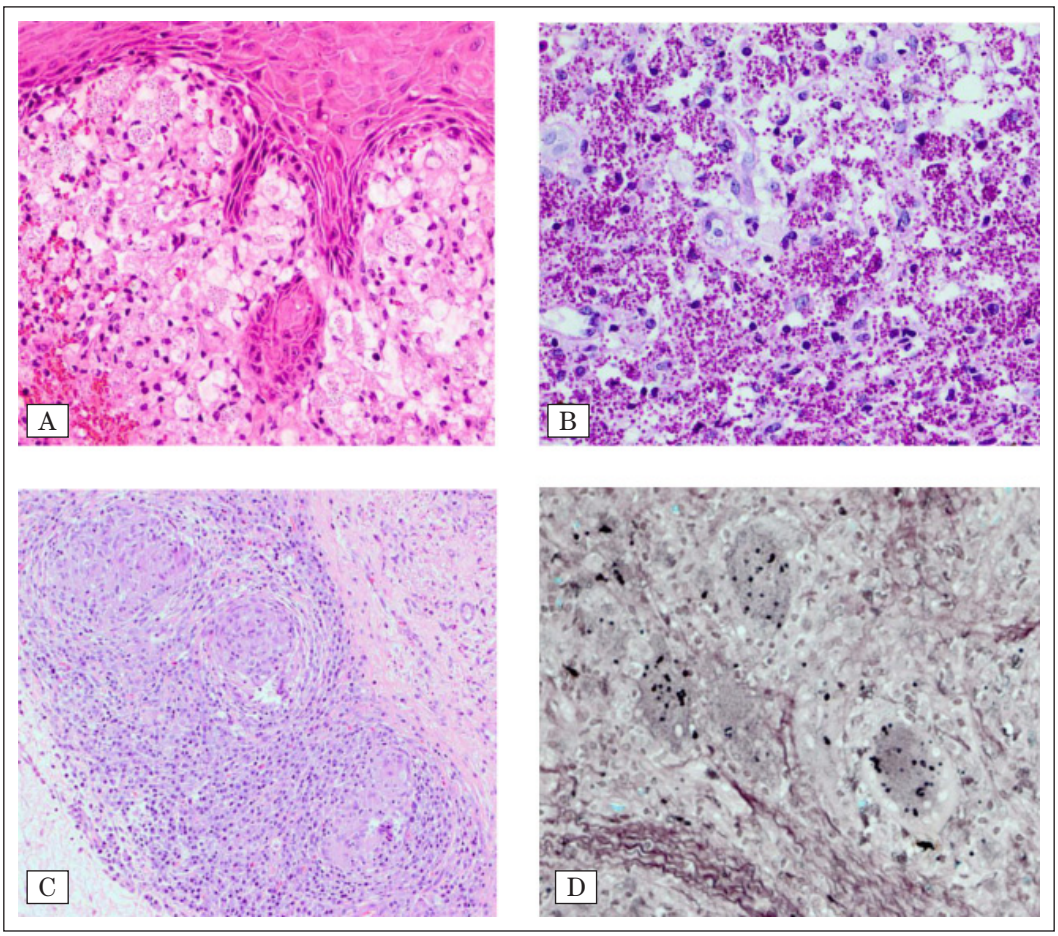

Figure 1. **A**. Photomicrograph shows beneath the surface epithelium, sheets of histiocytes containing clusters of small oval yeasts of *Histoplasma capsulatum* (Haematoxylin & Eosin stain 100x). **B**. Fungal organisms within histiocytes highlighted by Periodic Acid Schiff stain 400x. **C**. Multiple discrete nodules of non-caseating granuloma are evident in response to *Histoplasma* organism (Haematoxylin & Eosin stain 100x). **D**. Granuloma containing clusters of fungal organisms (Grocott's Methenamine Silver stain 400x).

DISCUSSIONS

The incidence of histoplasmosis with oral manifestations is an infrequent finding. Study by Goodwin *et al.*, 1980 revealed that oropharyngeal lesion is one of the common extrapulmonary sites in disseminated histoplasmosis. Oropharyngeal involvement occurs 66% in chronic disseminated histoplasmosis. On the other hand, the incidence of oral histoplasmosis in Malaysia is not clearly documented. We reported 39 cases of oral histoplasmosis from 1995-2016 (21 years' duration) while study by Ng and Siar in 1996 documented 37 oral histoplasmosis cases from 1967-1994 (27 years' duration). These findings show increasing trends of oral histoplasmosis in Malaysia with previously 1.4 cases per year to 1.9 cases per year. In our study, we found that oral lesions of histoplasmosis having predilection towards male with male to female ratio 9:1, a high incidence in late adulthood, and predominance towards single site involvement with tongue being the most affected site. These findings are comparable with study by Ng and Siar (1996) that reported predilection towards adult male with most common sites involving gingiva, tongue, and palate. Focal or multiple sites may be affected and

involvement of all oral sites has been reported (Chinn *et al.*, 1995). Ng and Siar (1996) also reported Malaysian Indian as the most frequent ethnic group having oral histoplasmosis. In contrast, our study reveals Malay as the most common affected ethnic group followed by Chinese, Indian, and others.

The clinical presentation may vary and is usually non-specific. In disseminated histoplasmosis, the common presentations are weight loss, high fever, respiratory complaints, hepatosplenomegaly, anaemia, and lymphadenopathy. Oral lesions may vary from ulcer, nodular to papillary lesions that often mimic squamous cell carcinoma (Chinn *et al.*, 1995). The compiled data demonstrates ulcer as the most common clinical presentation. This finding is in concordance with Ng and Siar (1996) study that showed 73% of oral histoplasmosis infections presents as an ulcer. Majority of patients complained of systemic symptoms like loss of weight, fever, sore throat, dysphagia, and pain on swallowing. With increasing incidence of AIDS over the years, opportunistic fungal infections such as candidosis, cryptococcosis, aspergillosis, blastomycosis, paracoccidioidomycosis, zygomycosis, and histoplasmosis have become more frequent. Histoplasmosis is recognized as one of the most frequently observed systemic mycosis that caused oral lesions in HIV-seropositive patients (Scully *et al.*, 1997).

In this study, out of 7 patients diagnosed with AIDS, 4 of them had existing tuberculosis, and 1 with hepatitis C. Kauffman (2007) has listed groups of patients at risk for disseminated histoplasmosis, to be immunosuppressed patients having defective cell-mediated immunity. This includes patients with AIDS, hematologic malignancies, solid organ transplant, hematopoietic stem cell transplant, those on immunosuppressive agents (corticosteroids, tumour necrosis factor antagonists), congenital T-cell deficiencies (Gamma interferon receptor deficiency, hyper-immunoglobulin M syndrome), and lastly in young patients (infants). Since 1985, disseminated histoplasmosis has been considered as one of the spectrums of infections that characterize AIDS in the population (Centers for Disease Control, 1985).

This study revealed that the most common differential diagnosis of persistent oral ulceration and swelling is oral squamous cell carcinoma or other types of malignancy. None of the clinicians raised the possibility of fungal infection such as histoplasmosis. The rare incidence of oral histoplasmosis may contribute to lack of awareness of the disease and may lead to misdiagnosis. Diagnosis of histoplasmosis is usually made by identifying the fungal organism by means of tissue biopsy and culture. The other techniques for identification of fungal organisms are in-situ hybridization, PCR based methods, and laser microdissection (Guarner and Brandt, 2011). The histologic diagnosis of histoplasmosis is achieved via the identification of the histoplasma organism usually as yeast forms within histiocytes or giant cell granulomas [Figure 1]. Under microscopic examination, *H. capsulatum* var. *capsulatum* is an oval yeast sized 2 to 4 µm with the basophilic yeast cytoplasm and surrounded by a clear zone of cell wall. The yeast cell wall is highlighted with GMS and PAS stains. This organism tends to be arranged in clusters because it is initially ingested by macrophages. On the other hand, African variant of *H. capsulatum* is larger is size, approximately 8 to 15 µm in diameter and may be pigmented (Brandt *et al.*, 2011).

Goodwin *et al.* (1980) described three types of tissue reactions in histoplasmosis, which are diffuse histiocytosis, focal histiocytosis, and tuberculoid granulomas. Histiocytosis is defined as proliferation and recruitment of more macrophages, which in turn became histocytes in response to the presence of infective agent in the tissues. Diffuse histiocytosis is one of the characteristics of disseminated histoplasmosis and occur across the disease spectrum, ranging from mild to severe degree of parasitization. Focal histiocytosis usually occurs in moderate to severe degree of infection and often having area of central necrosis that are responsible for destructive lesions, clinically presented as oropharyngeal ulcers. When the number of microorganisms in tissue macrophages is

too small, this favours tuberculoid granulomatous reaction, indicating near normal tissue response and nearly normal immunocompetence. Thus, tuberculoid granuloma is usually not seen in disseminated histoplasmosis except in two conditions, which are in coexisting remnants of primary or acute reinfection histoplasmosis of which disseminated histoplasmosis is thought to have developed and in the mild form of disseminated histoplasmosis in which degree of mononuclear phagocyte parasitization is minimal.

Guarner and Brandt (2011) reported that various inflammatory responses towards histoplasma organism depend on host immune status. Histological features presenting as sheets of histiocytes containing yeast-like Histoplasma organism, may efface tissue architecture and produce necrotic areas indicating disseminated infection. However, in immunocompetent individual, non-caseating granulomatous inflammation with multinucleated giant cells are usually seen (Ge *et al.*, 2010; de Paulo *et al.*, 2013). In our study, from 25 patients with histiocytic inflammatory response, 9 (36%) of them had underlying medical conditions for example AIDS, history of kidney transplant, tuberculosis, ischaemic heart disease, Hansen's disease, diabetes mellitus, and SLE. For patients with histomorphology of granulomatous inflammation, 5 (35.7%) of them had hidden immunodepression such as AIDS, tuberculosis, diabetes mellitus, hepatitis C, and adrenal tumour. From our findings, not all of our patients with histiocytic inflammatory response have compromised immune status. In contrast, a few of our patients with granulomatous inflammation, which is closely associated with normal immunologic competence, had coexisting immunosuppressed conditions.

Two theories were proposed that might explain this variety of findings. Firstly, information regarding medical disease of patients was only obtained from the biopsy request form from the referring clinicians. No further follow up of patients' medical status was obtained thus the patients may also have underlying undiagnosed medical conditions or untold medical problems. Secondly, the current status of patients' disease activity and progress of treatment for instance, AIDS was not known. Horwath *et al.* (2015) stated that progressive disseminated histoplasmosis usually presents in those with low CD4 T-cell counts (<100 cells/mm^3) and typically occurs in patients not receiving highly active antiretroviral therapy (HAART). In addition, patients infected with tuberculosis and histoplasmosis at the same time usually presents histologically as granulomatous inflammation.

Histoplasmosis has restricted geographical distribution throughout the world. It is endemic in certain parts of America and is closely associated with soil richly contaminated with birds and bats droppings. In Malaysia, the endemic areas of oral histoplasmosis are not clearly delineated. In our study, most of the patient diagnosed with oral histoplasmosis was from the state of Selangor, but this could be due to the fact that our laboratory is the receiving centre of oral biopsy samples for the central zone of Peninsular Malaysia. The other theory includes the association with agricultural activities in these states and hobby or works related that exposed patient to soil contaminated with birds and bats excreta. Factors that cause high incidence of oral histoplasmosis in these states are not well understood.

The preferred treatment modalities are amphotericin B and itraconazole with fluconazole being the second line of antifungal drug. Amphotericin B is preferred for severe pulmonary or disseminated histoplasmosis whereas itraconazole is usually used for mild to moderate pulmonary or disseminated disease (Kauffman, 2007). Spontaneous remission of oral histoplasmosis has been reported. However, when not treated, this infection may result in death in AIDS patients (Adenis *et al.*, 2014).

CONCLUSIONS

Oral manifestation of histoplasmosis is rare in Malaysia but is showing an increasing trend for the past two decades. This disease

poses a diagnostic challenge and as such could lead to inappropriate management of the condition. In immunocompromised individuals, the diagnosis of oral ulcers is complex due to the possibility of various aetiologies. Health practitioners play a crucial role in diagnosis as they should recognize early clinical lesions of oral histoplasmosis so as to enable prompt initiation of therapy for prevention of further deterioration of the patient's health. Although majority of the patient in this study were immunocompetent, about one third of the patient had underlying immunodepression. This should raise suspicion of the possibility of hidden immunosuppression when oral lesions of histoplasmosis are seen.

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Conflict of interests

No conflict of interest is declared.

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