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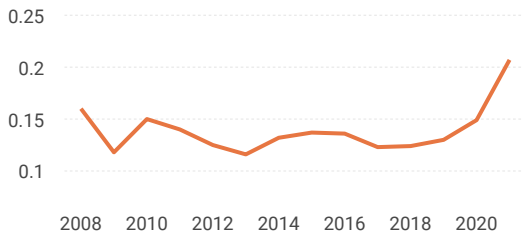
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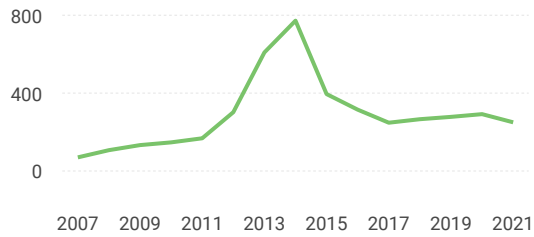
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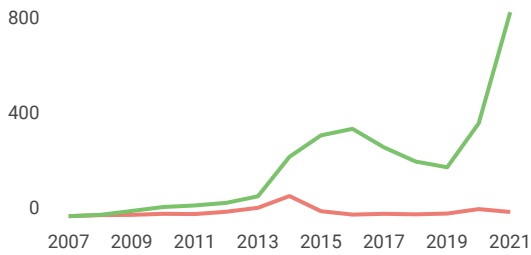
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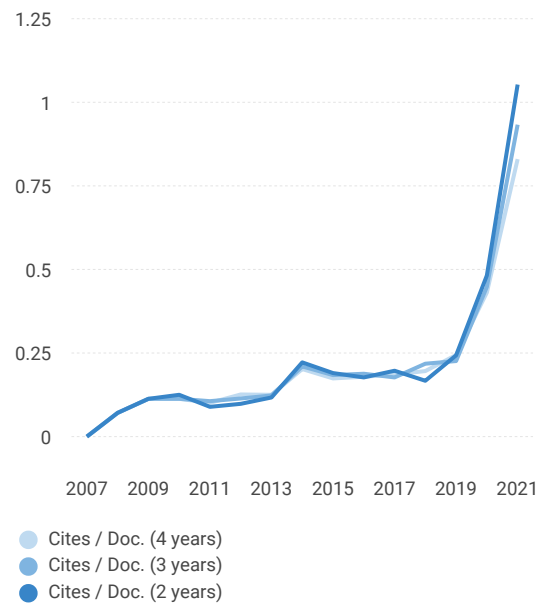
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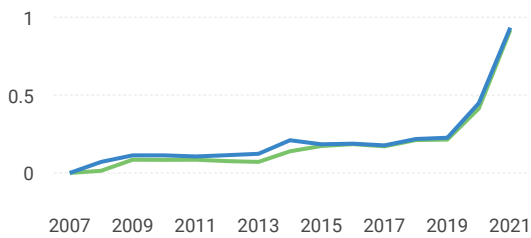
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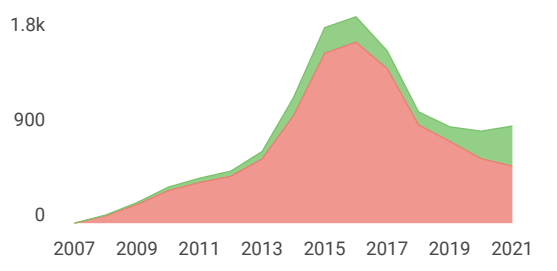
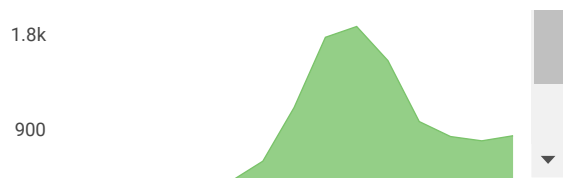
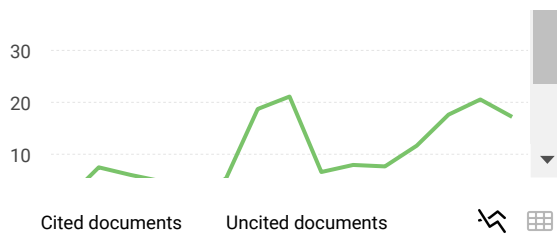
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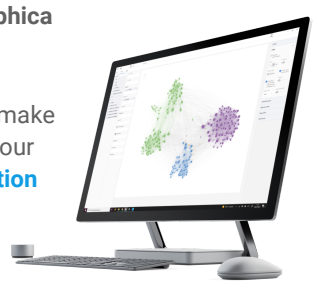
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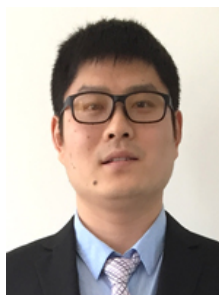
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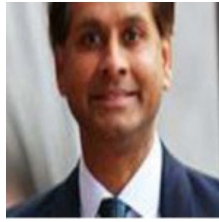
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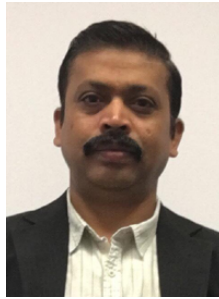


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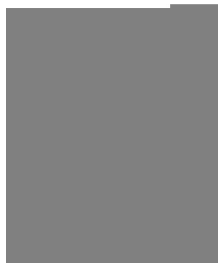
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Nystatin Profile on *Candida* Species in HIV/AIDS Patients with Oral Candidiasis: A Phenomenology Study

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Abstract

Oral candidiasis is the main symptom that often appears in patients with Human Immunodeficiency Virus (HIV) / Acquired Immune Syndrome (AIDS). Recent studies reported that some bacteria causing oral candidiasis are resistant to antifungal drugs. Describing nystatin profile against candida species in HIV / AIDS patients with oral candidiasis. Twenty-nine subjects were divided into 2 groups based on sex (23 male subjects and 6 female subjects). Subjects carried out tissue culture procedure and were tested for sensitivity to fluconazole and nystatin. The analysis was conducted by comparing sex and type of infecting bacteria. Statistical analysis used chi-square test, fisher, or ANOVA with 95% CI with $p < 0.05$. The average age of male and female subjects was 43.15 ± 3.67 years and 40.02 ± 10.23 years, respectively, with age range of 18-65 years. Recurrent oral candidiasis in male and female patients was 65.22% and 83.33%, respectively ($p = 0.079$). Subjects were resistant to fluconazole as much as 77.50% in men and 61.54% in women ($p = 0.823$). On the other hand, subjects sensitive to nystatin were 92.50% in men and 92.31% in women ($p = 0.167$). Fluconazole was resistant to *Candida albicans* (68.00%) and non-*Candida albicans* (78.57%) ($p = 0.048$), while nystatin was sensitive to *Candida albicans* (92.00%) and non-*Candida albicans* (92.86%) ($p = 0.791$). Most subjects were resistant to fluconazole, while the majority of subjects were sensitive to nystatin.

Keywords: Nystatin, fluconazole, oral candidiasis, *Candida albicans*, HIV/AIDS.

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Abbreviation: HIV: human immunodeficiency virus; CA=Candida albicans; NCA=Non-candida albicans; AIDS: acquired immunodeficiency syndrome; WHO: world health organization; ARV: antiretrovirals; and SDA: Sabouraud Dextrose Agar.

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INTRODUCTION

Oral candidiasis is one of the first clinical signs of AIDS found in 50% to 95% of HIV/AIDS patients. The condition is mostly caused by *Candida albicans*, which number is around 2-69.1% found in adult's oral cavity^{1,2}. *Candida albicans* is not the only species causing candidiasis, but also other species including *Candida glabrata*, *Candida krusei*, *Candida tropicalis*, *Candida parapsilosis* and *Candida dubliniensis*. *Candida* species is a commensal microorganism found in oral mucosa. However, this species becomes a predisposition factor causing oral candidiasis^{3,4}. Early treatment of oral candidiasis, according to WHO, includes administration of topical antifungal agents, such as nystatin, amphotericin B, miconazole, and clotrimazole. Those agents can be given in oral candidiasis case without complication^{2,5}.

In Indonesia, nystatin is an effective and affordable choice of antifungal for oral candidiasis³. The available doses of nystatin are 100.000 U/mL, and 400.000 – 600.000 U/mL for adults for 4 times a day for 7 – 14 days^{6,5}. In 2017, there were 261 out of 1020 patients with HIV/AIDS treated in Dr. Soetomo General Hospital, Surabaya, Indonesia, who experienced oral candidiasis. The number increased to 273 patients in 2018. From June – July 2018, there were 20 oral candidiasis patients treated with oral nystatin, but 30% of which returned to the hospital with the similar case.

Based on the above explanation, the authors conducted an in vitro test to measure nystatin resistance in oral candidiasis patients with HIV/AIDS.

METHODS

The subjects of this research were HIV/AIDS patients treated in Dr. Soetomo General Hospital Surabaya, Indonesia. The inclusion criteria were: HIV/AIDS patients diagnosed with rapid test/3 HIV testing methods^{7,8}, having diagnosed with oral candidiasis by clinical examination and 10-20% KOH test^{9,10}, and male or female patients aged >18 years. This study excluded subjects taking antifungal drugs in 2 weeks before test, and no colony growth found on culture examination with candida Sabouraud Dextrose Agar (SDA). The subjects must fulfill the informed consent.

This study employed an observational descriptive design carried out from August 2018 to

February 2019. The process of culture extraction was conducted in Dr. Soetomo General Hospital, Surabaya, Indonesia, and followed by culture examination that was carried out in Surabaya Health Laboratory, Surabaya, Indonesia. There were 29 patients who were consecutively sampled for the subjects in this research (Fig. 1). We also obtained 53 *Candida* isolates. The study protocol was in accordance with ethical procedure (0231/KEPK/IV/2018).

We first examined the subject's culture^{9,10} that was taken from oral tissue swab. The positive *Candida* was grown in SDA at 37°C for 48 hours¹¹. We used CHROMagar *Candida* (CHROMagar *Candida*, France) as the SDA medium. The growing *Candida* specimen were identified using cornmeal agar and tween 80 that were incubated at 42-45°C^{12,13}. We also conducted carbohydrate test to identify *Candida* species¹⁴. Furthermore, we conducted resistance test using disc diffusion method on Mueller Hinton agar with 2% glucose and methylen blue. The isolate of *Candida* species was implanted on the agar, then a paper disk containing nystatin or fluzonazole was placed on top of it. We made a 24-48-hour-observation to look for an inhibition zone around the paper disc. The diameter of inhibition zone was measured using caliper (Rosco Diagnostica, Taastrup, Denmark). We interpreted the inhibition zone diameter using CLSI¹⁵. This study used nystatin with a dose of 100,000 UI/ml (pharma chemistry Ltd, Bekasi, Indonesia) and fluconazole at a dose of 2 mg/ml (pharma chemistry Ltd, Bekasi, Indonesia)

We assessed demographic and clinical data of patients. The collected data were then processed using IBM SPSS Statistics software version 23.0 (IBM Corp., Armonk, NY, USA). The statistical analysis used chi-square, fisher, or ANOVA with 95% CI ($p < 0.05$).

RESULTS

Twenty-nine patients were divided into two groups based on their sex that consisted of 23 male subjects and 6 female subjects. The average age of male and female patients was 43.15 ± 3.67 years and 40.02 ± 10.23 , respectively. They were divided into several age groups, where most subjects were found in the age range of 36 – 45 years (8 subjects; 27.59%), and followed by age group of 56 – 65 years (7 subjects; 24.13%). Most

subjects had high school education background (48.27%), and unemployed (12 subjects; 41.37%) (Table 1). Most patients were Javanese (48.28%), and followed by Madurese (44.83%).

The subject's clinical condition was as follows: 27 subjects (93.10%) had lesions on the tongue, 1 subject (3.45%) in the mucous membrane, and 1 subject (3.45%) in the corner of the lips (Figure 2). Most subjects were recurrent

oral candidiasis patients (male = 65.22% and female = 83.33%) ($p = 0.079$). Some subjects had a history of systematic antifungal treatment (male = 17.39% and female = 16.67%) and topical antifungal (male = 34.78% male and female = 50.00%), with $p = 0.002$. Most subjects used antiretroviral (ARV) as much as 86.96% in men and 10.34% in women ($p = 0.518$; Table 1).

Table 1. Demographic and Clinic Characteristics of patient Gender

Variable	Sex		p
	Male (n=23)	Female (n=6)	
Age (mean ± SD)	43.15 ± 3.67	40.02 ± 10.23	-
Education (%)			
Not attending school	2 (8.70)	2 (33.33)	-
Junior high school	7 (30.44)	1 (16.67)	
Senior high school	11 (47.83)	3 (50.00)	
Undergraduate/Diploma	3 (13.04)	0 (0.00)	
Ethnic (%)			
Java	10 (43.48)	4 (66.67)	-
Madura	11 (47.83)	2 (33.33)	
Other	2 (8.70)	0 (0.00)	
Oral candidiasis (%)			
Recurrent	15 (65.22)	5 (83.33)	0.079
First infection	8 (34.78)	1 (16.67)	
Treatment history (%)			
Systemic antifungal	4 (17.39)	1 (16.67)	0.002*
Topical antifungal	8 (34.78)	3 (50.00)	
ARV treatment (%)	20 (86.96)	3 (50.00)	0.518

SD=standard deviation; ARV=antiretroviral; *significant 0.05

Table 2. Comparison of culture results on male and female subjects

Variable	Sex		p
	Male (n=40)	Female (n=13)	
Bacterium (%)			
Candida albicans	18 (45.00)	7 (53.85)	0.035*
Non-Candida albicans	22 (55.00)	6 (46.15)	
Fluconazole (%)			
Sensitive	9 (22.50)	5 (38.46)	0.823
Resistance	31 (77.50)	8 (61.54)	
Nystatin (%)			
Sensitive	37 (92.50)	12 (92.31)	0.167
Resistance	3 (7.50)	1 (7.69)	

*significant 0.05

Table 3. Comparison of fluconazole and nystatin sensitivity tests in the *Candida albicans* and non-candida albicans groups

Variable	Bacterium		p
	CA (n=25)	NCA (n=28)	
Fluconazole (%)			
Sensitive	8 (32.00)	6 (21.43)	0.048*
Resistance	17 (68.00)	22 (78.57)	
Nystatin (%)			
Sensitive	23 (92.00)	26 (92.86)	0.791
Resistance	2 (8.00)	2 (7.14)	

CA=*Candida albicans*; NCA=Non-candida albicans; *significant 0.05

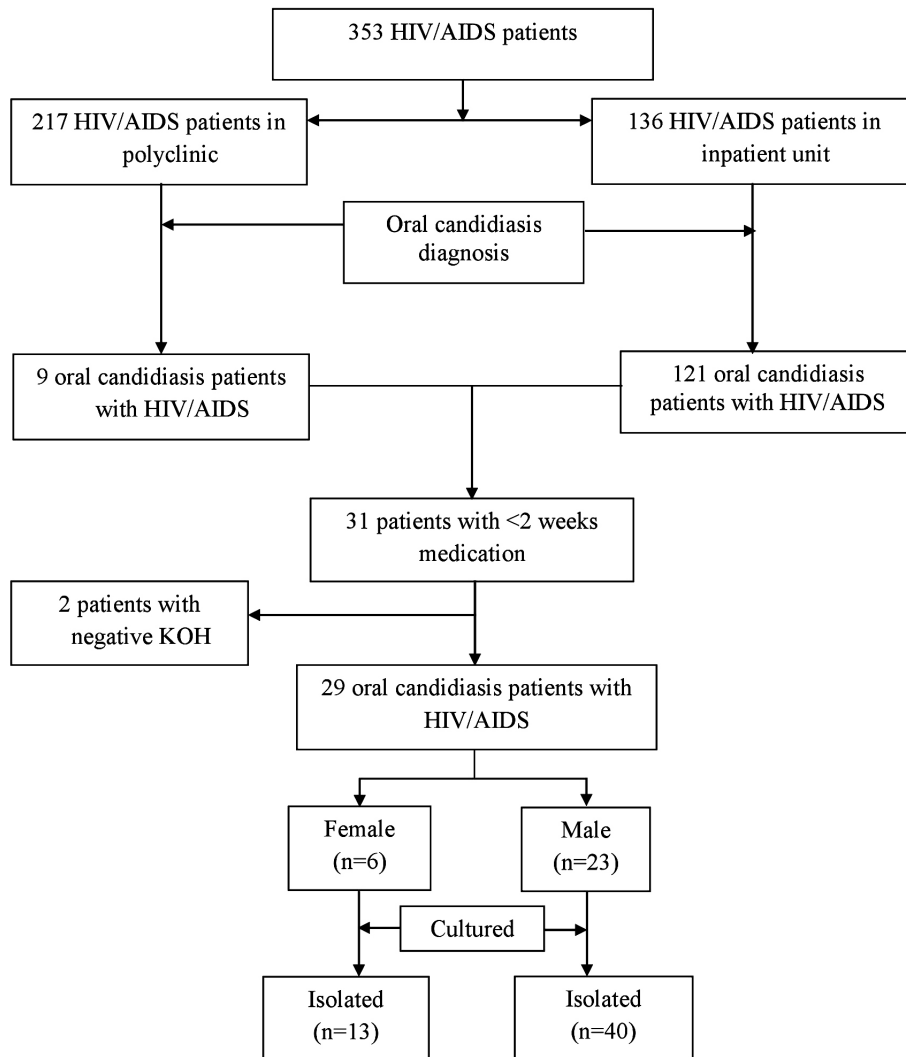


Fig. 1. Flowchart Diagram of Subject Sampling



Fig. 2. Clinical picture of the subject on the first day of hospital admission

Culture comparison based on the sex found that most subjects were infected with non-*Candida albicans* bacteria as much as 55.00% in male subjects, while most female subjects were infected with *Candida albicans* bacteria as much as 53.85% ($p = 0.035$). Most subjects were resistant to fluconazole as much as 77.50% in men and 61.54% in women ($p = 0.823$). The majority of subjects were sensitive to nystatin as much as 92.50% in men and 92.31% in women ($p = 0.167$; Table 2).

Most *Candida albicans* bacteria were resistant to fluconazole (68.00%), and most non-*Candida albicans* bacteria were also resistant to

fluconazole (78.57%) ($p = 0.048$). Most *Candida albicans* bacteria were sensitive to nystatin drugs (92.00%), while non-candida albicans bacteria were mostly sensitive to nystatin drugs as much as 92.86% ($p = 0.791$; Table 3).

DISCUSSION

The demographic data of this research included age, educational level and occupation. The highest age range was found in the adult group (63%). According to the data of Directorate General PPM & PL of the Ministry of Health in 2016, there were more than 50% HIV/AIDS patients were young adults and productive age groups with age group of 25-49 years old¹⁶. The finding of this study supported that adults included in productive and sexually active groups were more likely to engage in unprotected sexual behavior that was prone to HIV transmission¹⁷.

HIV/AIDS infection is a disease that has a huge social impact. Ninety percent of HIV/AIDS patients are likely to have oral cavity diseases that will have impact on the life quality, including occupational sector. This study found that 45% of the subjects were unemployed, and 9 subjects (45%) were high school graduates. HIV/AIDS patients with a low educational and socio-economic background have a bad oral health that makes them prone to oral cavity diseases¹⁸.

This study found that most patients were infected HIV due to heterosexual behavior (65%). This finding reflected the general condition of HIV/AIDS in East Java, in which the virus is mostly transmitted through heterosexual behavior (69.6%), followed by narcotics (21.9%)¹⁹. All patients in this study had white patches in their oral cavity, and being diagnosed with pseudomembranous oral candidiasis. This finding was consistent with a study conducted in India in 2013, in which pseudomembranous candidiasis was found in 72% of the subjects²⁰. The ARV administration to HIV/AIDS patients could significantly reduce oral candidiasis. Some patients in this study previously had ARV therapy, while the new HIV/AIDS patients had not received the therapy yet^{21,22}. Fungal infection was still found in most patients with antifungal therapy history, both systemic and topical²³.

A disc diffusion method was conducted to measure sensitivity of all *Candida* species to

nystatin. The results of sensitivity test were in the form of inhibition zone diameter. The criteria of susceptibility and resistance to antifungal agents were determined according to the interpretation of inhibition zone diameter for fungi by Rosco Diagnostica Company²⁴. This study found that neither *Candida albicans* nor *Candida non-albicans* species that resisted to nystatin. Nystatin currently becomes the primary therapy for oral candidiasis in patients with HIV/AIDS.

The resistance against nystatin was divided into two groups, namely intrinsic and extrinsic sensitivity. The extrinsic sensitivity shows a change in sensitivity pattern of *Candida* species, from sensitive to resistance against antifungal therapy. On the other hand, intrinsic sensitivity has occurred early on antifungal therapy. This study found some intrinsic sensitivities in a form of infection caused by *Candida krusei*, which occurred in 8% of subjects²⁵.

Some literatures stated that nystatin resistance is very minimum, but the therapy has side effects and toxicity. Nystatin generally works by distracting fungal cytoplasmic membrane and interacting with ergosterol. Ergosterol is important to maintain integrity and function of the enzymes of fungal membrane. Nystatin produces holes in cell membrane that becomes a way out for potassium ion and magnesium cellular components. This causes damages in proton gradient of cell membrane that leads to fungal cell death. Nystatin has a high binding capacity to ergosterol and low binding capacity to 3 hydroxy or oxysterol, such as fecosterol and episterol that becomes an important reason for nystatin resistance²⁶.

Although there is an increased in nystatin resistance, this remains a rare occurrence in fungal pathogenic isolates since nystatin could not be used for systemic fungal infection. Therefore, the indications are not as much as the azole group. The incident of nystatin-resistant strains may be largely not considered. Most fungal species are considered susceptible to nystatin. However, some of which intrinsically less susceptible to this antifungal agent, such as *Candida glabrata*, *Scedosporium prolificans* and *Aspergillus terreus*. Some species are also more susceptible to nystatin resistance, including *Candida lusitanae*, *Candida guilliermondii* and *Candida krusei*^{27,25}.

Factors such as recurrent oral candidiasis and history of antifungal usage are considered to cause differences of *Candida* species. Those factors are thought to be a predisposing factor that changes the type of *Candida* into *Candida* non-albicans. This may occur in patients with recurrent oral candidiasis as they are also exposed to antifungal medication thus supports the previous hypothesis. The characteristics of an antifungal drug are also factors that play a role in a difficult-to-treat infection. Fungistatic drugs will further encourage the formation of resistance compared to fungicidal drugs. Absorption, distribution and metabolism of a drug also contribute to the overall effectiveness of treatment based on the location of an infection. Antifungal drug dosages, including quantity, frequency, administration schedule and cumulative doses, can also play a role in treating a fungal infection. The administration of antifungal medication along with other prescription can also change the effectiveness of antifungal drugs. In addition, in the course of advanced HIV/AIDS, extensive fungal colonization was also found²⁷.

This study found a change in the spectrum causing oral candidiasis, since *Candida albicans* species were mostly found, while the number of *Candida* non-albicans was increasing.

CONCLUSION

In the invitro test, no *Candida* isolate was found to be resistant to nystatin. Therefore, oral nystatin is still effectively used as a standard therapy for HIV/AIDS patients with oral candidiasis.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

DM conceived the study. R contributed in study design. DM and YL collected data. CSM

participated in data analysis and interpretation. DM drafted the manuscript. R and YL revised the manuscript. All authors read and approved the manuscript for publication.

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DATA AVAILABILITY

The dataset used and/or analyzed during the current study are available from corresponding author on reasonable request.

ETHICS STATEMENT

The study protocol was in accordance with ethical procedure (O231/KEPK/IV/2018). All subject was received consent forms.

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