Comparison of Minimal Inhibitory Concentration Level in Vitro of Itraconazole and Fluconazole against Malassezia furfur in Patients with Pityriasis Vesicolor in Makasar

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Abstract: Pityriasis Versicolor (PV), the second most common dermatomycosis in Indonesia, is a superficial fungal infection that is frequently reported in the tropics with warm temperatures and high humidity, as in Makassar. Recurrence and long course of disease in PV is most often caused by *M. furfur* species. Hence, oral antifungal is commonly used in extensive, recalcitrant and recurrent infections. The aim of this study is to assess the MIC of Itraconazole (ITC) and Fluconazole (FLC) against *M. furfur* of PV patients in Makassar. This is a multi-center cross sectional observational study with consecutive sampling of 21 isolates from PV patients in Makassar, with the identification of *M. furfur* from morphological (culture using modified Dixon agar) and biochemical criteria (catalase test and lipid assimilation test using Tween-20, 40, 60, 80, Cremophor El). Our study shows MIC for ITC and FLC against *M. furfur* ranged from <0.03-0.25 μg/mL and <0.03-2 μg/mL, respectively with MIC of ITC is lower than FLC. The regimen of ITC as systemic antifungal therapy for PV patients in Makassar, particularly caused by *M. furfur* might be a more effective option.

1 INTRODUCTION

Pityriasis Versicolor or tinea versicolor is a superficial fungal infection characterized by changes in skin pigment caused by colonization of lipophilic dimorphic fungi from the normal skin flora of the stratum corneum (Moniri et al, 2009). This disease is spread throughout the world but more often in the tropics with warm temperatures and high humidity, as in Makassar(Muhammad et al, 2009). In the tropics the prevalence is 30-40% and the frequency becomes higher in summer. Pityriasis Versicolor is the second most common dermatomycosis after dermatophytosis in Indonesia(Krisanty et al, 2009) *Malassezia* is a lipophilic dimorphic fungus belonging to normal flora and can be isolated from skin scrapings that originate from almost all areas of the body, especially in areas rich in sebaceous glands such as the chest, back and head area (Krisanty et al, 2009). Identifies *Malassezia* species in 98 PV patients based on morphological observation and biochemical evaluation in which *M. furfur* (42.9%) was the most prevalent species.

Pityriasis Versicolor does not affect health significantly but has psychological and social implications. The optimal treatment of PV should consider the effectiveness of the drug, safety, cost, and patient complaints. Topical antifungal is the first line of therapy, but in some patients complain of unsatisfactory response, short-term success, and regular application or longer treatment periods especially for large lesions, prompt the consideration of systemic antifungal (Silva et al, 1998). Antifungal has its breakthrough since the discovery of azole group, which has been shown to be safer than previous antifungal agents. The azole agents exert its antifungal property by inhibiting the cytochrome P450, *14-alpha-demethylase* enzyme. In addition topical therapy has a high recurrence rate up to 60% in the first year and 80% increase in the second year (Hu and Bigby, 2010). Oral antifungal is commonly used in extensive, recalcitrant and recurrent infections, as it can penetrate keratin, while *M. furfur* thrive at the base of the keratin layer (Pantazidou and Tebruegge, 2007). Several studies have suggested that ketoconazole (KTC), itraconazole (ITC), and fluconazole (FLC) have been shown to produce high clinical and mycological cure rates in patients with PV.

ITC is a triazole group antifungal which is a powerful keratophilic and lipophilic agent, having a

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similar mechanism by inhibiting *14-alpha-demethylase* resulting in disruption of sterol synthesis in the cell wall of the fungus. In vitro ITC is not only active against *Malassezia* species and *Candida* species but is also active against dermatophyte and nondermatophyte fungi. The dose of ITC used for PV is 200 mg/day for 7 days, with a minimum accumulative dose of 1000 mg for effective therapy. Four weeks after initial therapy, a cure rate of 80-90% has been reported (Faergemann et al, 2002). FLC is another antifungal class of azole with a high absorption rate, in which optimum concentrations can be found in the skin several hours after being consumed in small doses. Benefits of FLC includes rare side effects, mostly available, and preferred as it requires only two or three weekly doses, compared with a 7-day regimen for ITC (200 mg/day) (Silva et al, 1998). In vitro susceptibility tests of the *Malassezia* species to KTC, Voriconazole (VRC), ITC and FLC were performed by Miranda et al. which reported that the *Malassezia* species are highly susceptible to the four azole preparations, but the susceptibility to KTC and ITC appears higher (Miranda et al, 2007).

This study was conducted to assess the minimal inhibitory concentration (MIC) antifungal ITC and FLC against *M. furfur* as the causative agent of PV in Makassar in vitro. Previous in vitro research in Makassar has not been done even though ITC and FLC are one of the most effective modes of PV therapy for recurrent cases after treatment with topical antifungal, safer than other antifungal, and readily available.

2 METHODS

This study is a multi-center cross sectional observational study with consecutive sampling that was performed in microbiology laboratory of Hasanuddin University Faculty of Medicine in 2013. Specimen of 21 samples were collected by skin scraping from the back or shoulder, upper arm, chest, face and neck of PV patients from Dr. Wahidin Sudirohusodo Hospital and Hasanuddin University Dermatovenereology Department's networking hospital in Makassar, of whom the diagnosis of PV was confirmed by Wood' s lamp, direct microscopic KOH preparation, culture and signed the informed consent. Then the specimen was planted on the modified Dixon agar plate, incubated at 32-34°C and was regularly assessed to confirm the growth of yeast until the 3rd week. Furthermore, the yeast was identified by their morphology, catalase test and lipid assimilation test (growth of yeast in the presence of Tween-20, 40, 60, 80 and Cremophor El).

The in vitro susceptibility test by determining MIC value of both antifungals was conducted using broth microdilution that was performed in accordance with the NCCLS guidelines in document M27-A2. The inoculum suspension was prepared by the spectrophotometric method obtaining a final inoculum of (0.5-2.5)x10^3 cells/mL. The final concentrations of the antifungal agents (ITC and FLC) were 128µg/mL which then diluted half in series and was inoculated to suspension hence obtaining concentrations of 128 µg/mL, 64 µg/mL, 32 µg/mL, 16 µg/mL, 8 µg/mL, 4 µg/mL, 2 µg/mL, 1 µg/mL, 0.5 µg/mL, 0.25 µg/mL, 0.125 µg/mL, 0.06 µg/mL, and 0.03 µg/mL with false positive and false negative control prepared. Growth of each various concentrations of all two drugs was recorded every 24 h for 5 days of incubation at 32 ◦C. Cell growth was compared with growth in a drug-free control. The MIC was defined as the lowest concentration of agent that produced none or 90% growth in comparison with the control. Data analysis was performed using SPSS. The Fisher Exact test was used to analyze the mean and distribution frequency of each drug with P value <0.05 is considered significant.

3 RESULT

Based on the morphological and biochemical characteristics, the 21 isolates were identified as *M. furfur*. MIC showed apparent differences in antifungal susceptibility against *M. furfur*. For all 21 isolates, the MIC for ITC ranged from <0.03–0.25µg/mL and <0.03–2µg/mL for FLC. The MIC ranged, MIC90 values and MIC percentage for *M. furfur* are presented in Table 1 and Figure 1.

Table 1. MIC comparison of ITC and FLC against *M. furfur* isolates

Antifungals	**MIC cumulative percentage (%) of 21 isolates *M. furfur***														
	128	64	32	16	8	4	2	1	0.5	0.25	0.125	0.06	0.03	<0.03	MIC90
ITC	0	0	0	0	0	0	0	0	0	0	14.3	19	38.1	61.9	0.25
FLC	0	0	0	0	0	0	4.8	14.3	19	28.6	28.6	42.9	61.9	33.3	2

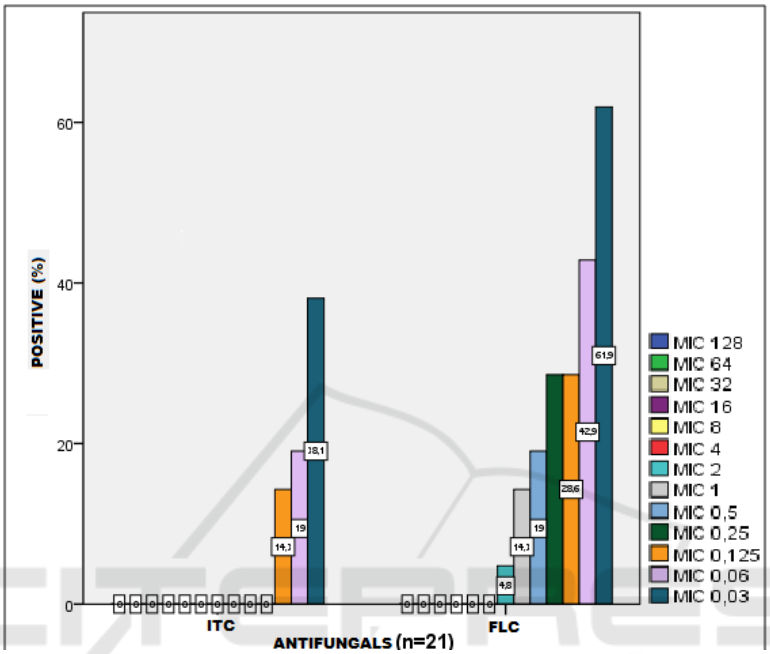

Figure 1. MIC comparison of ITC and FLC against *M. furfur* isolates.

4 CONCLUSION

In summary, our study shows MIC for ITC and FLC against *M. furfur* ranged from <0.03-0.25 µg/mL with MIC90 0.25 µg/mL and <0.03-2 µg/mL with MIC90 2 µg/mL, respectively. *M. furfur* isolates of our PV patients in Makassar are still sensitive to both antifungals, with MIC of ITC is lower than FLC. Thus, the regimen of ITC as systemic antifungal therapy for PV patients in Makassar, particularly caused by *M. furfur* might be a more effective option although further studies based on clinical trials are needed to confirm this.

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