IDENTIFICATION OF *MALASSEZIA* SPECIES AND ITS ASSOCIATION WITH CLINICAL MANIFESTATIONS OF ATOPIC DERMATITIS

Tran Cam Van*, Nguyen Van Hieu**

ABSTRACT

Introduction: Atopic dermatitis is a chronic, recurrent inflammatory skin disease that is characterized by an eczematous reaction. Few studies have investigated fungi in the pathogenesis of atopic dermatitis, however, there are different about distribution of *Malassezia* species. **Objectives:** To indentificate of *Malassezia* species and its asociation with clinical manifestations in Vietnamese atopic dermatitis patient. **Methods**: 178 patients who were diagnosed with atopic dermatitis and had a postitive direct examination of *Malassezia* at the National hospital of dermatology and venereology between July 2019 and June 2020. Specimens were taken with cellotape, then stained in 20% of potassium hydroxit combined with Parker™ blue black ink. All patient who had postive test were cultured on SDA and mDixon. For fungal samples, we selected pure colonies with morphological characteristics of yeast as follows about 1cm in diameter, round, cream or milky in color, smooth and glossy to detect the species. **Results**: From the samples of atopic dermatitis patients, we cultured and idenfified 41 cases. 5 species were found, in which *M. globosa* was the most common species, accounting for 39%, followed by *M. restricta* (19.5%), M. dermatis (17.1%), *M. furfur* (17.1%) and *M. sympodialis* (2.4%). **Conclusion**: On the skin lesions of Vietnamese patients with atopic dermatitis, *M. globosa* was the most common species with 39.0%.

* *National Hospital of Dermatology and Venereology*
** *Hanoi Medical University*
Responsible person: Tran Cam Van
Email: trancamvan.dl@gmail.com
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I. INTRODUCTION

Atopic dermatitis (AD) is a chronic, recurrent inflammatory skin disease that is characterized by an eczematous reaction. In recent decades, atopic dermatitis prevalence has been increasing worldwide, estimated at 15-20% of children and 1-3% of adults [1]. Many factors are involved in disease progression and severity, especially genetics, but the mechanism is still unclear. Dry skin and impaired skin barrier function play a major role responsing to triggers and external factors such as microorganisms. Using topical corticosteroids, immunomodulators, moisturizers and biological drugs can help treat disease, however, there are not effective. So, It is necessary to determine the role of some factors such as microorganisms, house dust or food.

Malassezia fungi is a yeast specialized to live on skin microflora. Few studies have investigated fungi in the pathogenesis of AD [1]. Nowicka et al (2019) detected 13 allergens from *M. furfur* and *M. sympodialis* [2]. Glatz et al research on 132 childrens and 67 adults, find a correlation between the fungi infection and the severity of disease [3]. However, there are different about distribution of Malassezis species. In Japan, Sugita et al (2003) showed that *M. globosa* and *M. restricta* are the common species with 93.8% and 87.5%, respectively [4]. In

Sweden, according to Falk et al (2005), the most species in lesion of AD patients was *M. sympodialis* with proportion with 46%, following *M. obtusa* (30%), *M. globosa* (28%) [5]. Thus, seven species of *Malassezia* were detected in skin lesions of patients with AD [2].

This study aims to contribute to role of fungi infection in the AD by indentification of *Malassezia* species in Vietnamese AD patient.

II. OBJECTIVES AND METHODS

The study was performed in 178 patients who were diagnosed with AD and had a postitive direct examination of *Malassezia* at the National hospital of dermatology and venereology between July 2019 and June 2020.

Hanifin and Rijka's standard was used to diagnosis AD. Then, direct examination from scales on skin lesion was carried out. Specimens were taken with cellotape, then stained in 20% of potassium hydroxit combined with Parker™ blue black ink. After 30 mins, we examined by microscopy (40X and 100X magnification), evaluated the number of yeasts per a microscopic field as follows negative if from 0 to 4 cells, postitve 1+ if from 5 to 14 cells, positive 2+ if from 15-29 cells, positive 3+ if from 30 to 39 cells, positive 4+ if above 40 cells. Direct examination microscopy results were considered positive when yeast cell and filament morphology was found or yeast cell morphology with a count of 3+ or 4+.

All patient who had postive test were cultured on SDA and mDixon, store in incubator at 32ºC and 40ºC, followed up within one week. For fungal samples, we selected pure colonies with morphological characteristics of yeast as follows about 1cm in diameter, round, cream or milky in color, smooth and glossy to detect the species.

III. RESULTS

From the samples of AD patients, we cultured and idenfified 41 cases, the following results:

Table 3. Culture results from scales of AD patient (n=41)

***Malassezia* species**	**n**	**%**
M. globosa	16	39.0
M. restricta	8	19.5
M. furfur	7	17.1
M. dermatis	7	17.1
M. sympodialis	1	2.4
***Malassezia* spp.**	2	4.9
Total	41	100

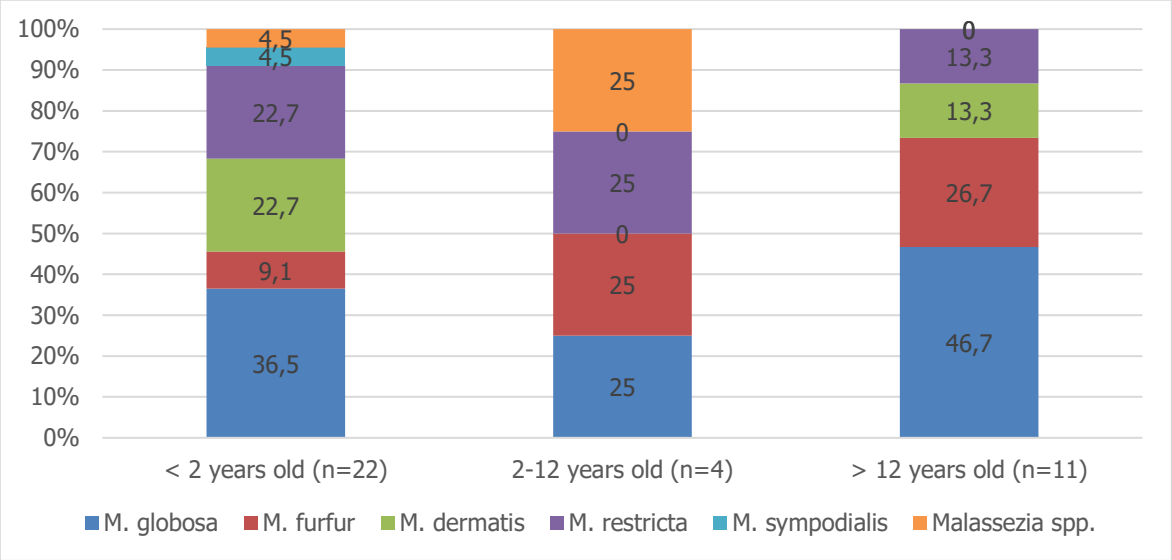

Figure 1. Distribution of Malassezia species by age group (n=41)

Table 4. Distribution of Malassezia species by sex (n=41)

***Malassezia* species**	**Male**		**Female**	
	n	**%**	**n**	**%**
M. globosa	12	41.4	4	33.3
M. restricta	5	17.2	3	25.0
M. furfur	4	13.8	3	25.0
M. dermatis	6	20.7	1	8.3
M. sympodialis	1	3.4	0	0
***Malassezia* spp.**	1	3.4	1	8.3
Total	29	100	12	100

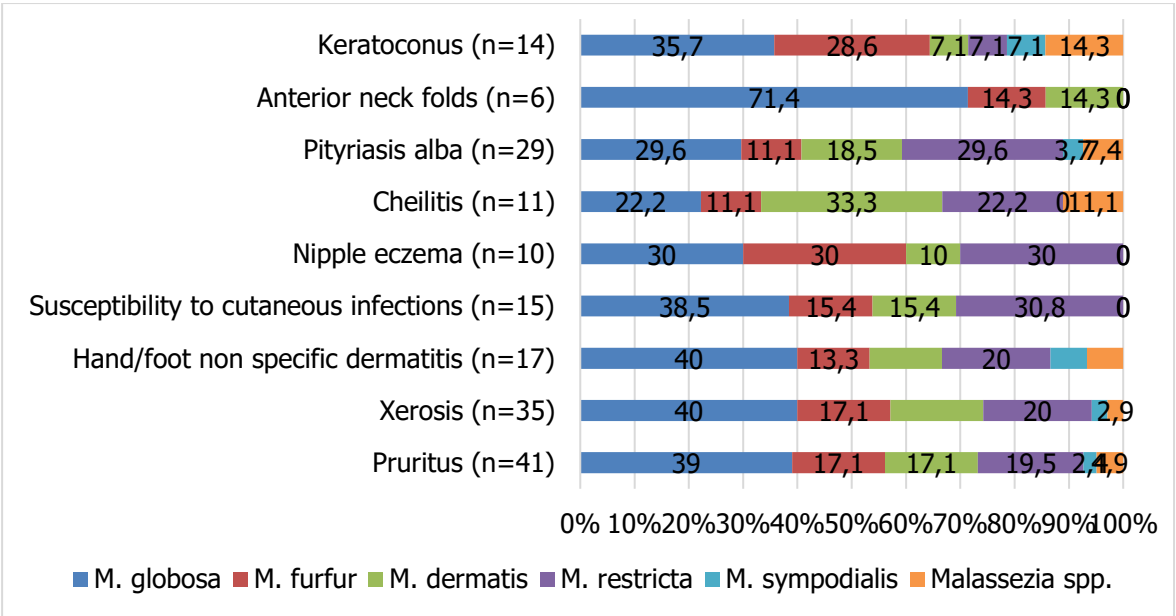

Figure 2*. Distribution of Malassezia species according to clinical features (n=41)*

Table 5. Distribution of Malassezia species according to disease progression and severity (n=41)

	M. globosa	***M. restricta***	***M. furfur***	***M. dermatis***	***M. sympodialis***	***Malassezia* spp.**	**Total**
Disease progression							
Acute	6 (60.0)	2 (20.0)	1 (10.0)	1 (10.0)	0	0	10 (100)
Subacute	7 (30.4)	4 (17.4)	4 (17.4)	6 (26.1)	1 (4.3)	1 (4.3)	23 (100)
Chronic	3 (37.5)	2 (25.0)	2 (25.0)	0	0	1 (12.5)	8 (100)
Disease severity							
Mild	2 (33.4)	2 (33.3)	2 (33.3)	0	0	0	6 (100)
Moderate	14 (40.0)	6 (17.1)	5 (14.3)	7 (20.0)	1 (2.9)	2 (5.7)	34 (100)

Table 6. Some factors related to species distribution of M. globosa (n=41)

Characteristic		***M. globosa***				**p**
		Positive		**Negative**		
		n	**%**	**n**	**%**	
Age group	< 2 years old	8	50.0	14	56.0	>0.05
	≥ 2 years old	8	50.0	11	34.0	
Gender	Male	12	75.0	17	68.0	>0.05
	Female	4	25.0	8	32.0	
Geography	City	4	25.0	8	32.0	>0.05
	Countryside	12	75.0	17	68.0	
Disease progession	Acute	6	37.5	4	16.0	>0.05
	Subacute - Chronic	10	62.5	21	84.0	
Disease serverity	Mild	2	12.5	4	16.0	>0.05
	Moderate	14	87.5	21	84.0	
Direct examination	yeast cell and filament	3	18.8	2	8.0	>0.05
	yeast cell	13	81.2	23	92.0	
Total		16	100	25	100	--

IV. DISCUSSION

Our results show *M. globosa* was the most common species at 39.0%, followed by *M. restricta* (19.5%. In Japan, Sugita et al revealed both *M. globosa* and *M. restricta* were common species in AD with proportion with 93.8% and 87.5% [4]. In Sweden, according to Falk et al, the most species was *M. sympodialis* (46%), following *M. obtusa* (30%), *M. globosa* (28%) [5]. Our results were similar to Sugita et al but different from Falk et al. We supposed that the distribution of species depends on climate as *M. globosa* ussually found in tropical climates. This is also consistent with the study of Nowicka et al [2]. The rate of fungal detection by culture with following species *M. globosa* (16 to 28% in Sweden), *M. sympodialis* (32 to 51% in Canada), *M. restricta* (3 to 22% in Korea), *M. furfur* (4 to 21% in Korea), *M. slooffiae* (3

to 7% in Sweden), *M. obtusa* (10 to 30% in Canada and Sweden), *M. dermatis* (6,5% in Korea). Two species only detect by PCR techniques are *M. japonica* (58%) and *M. yamatoensis* (14%). We detected 5 of these 7 species, except *M. obtusa* and *M. slooffiae*. Although there is a difference in prevalence among these study, *M. globosa* is a common species on the skin of AD patients in tropical countries, including Vietnam.

❖Age group

In a study on healthy subject by Gupta et al, there was a diference in the distribution of *Malassezia* species between the age groups, especially, *M. globosa* was found in all age group, *M. restricta* was only found in group of 15 to 40 years old and *M. sympodialis* was not found in children [6]. Falk et al only detect three species included *M. obtusa*, *M. globosa* and *M. slooffiae* in adults [5]. Prohic demonstrate that *M. globosa* isolated in group of 36 to 50 years old, also *M. restricta* in group of 21 to 35 years old [7]. According to Nakashima et al recognized *M. globosa* and *M. restricta* were two common species assosiated with disease serverity [8]. Although, Glatz et al confirmed there was a correlation between *Malassezia* species and disease derverity in adults, also not children because lipids which need to growth of *Malassezia* in children is lower than adults [1].

❖Disease progression

The detection rate of *M. globosa* in acute group was 60.0% higher than in chronic group and subacute group. Han et al study on 16 patient with chronic AD and determine four species included *M. globosa*, *M. restricta*, *M. sympodialis* and *M. furfur* [9]. In this study, *M. globosa* was the most frequently isolated species, but, *M. furfur* which was found allergens was the low rate. Hans supposed *M. globosa* and *M. restricta* are asociated with the onset or exacerbation of AD because of their high frequency on the skin lesions. According to Johansson et al [10], acute skin lesions of AD is associated with Th2/Th0 cytokines, whereas, chronic skin lesions with Th1 cytokines. The author has demonstrated that *M. furfur* is involved in positive reactions, which often appear in acute skin lesions. In our study, there was one of seven patient with acute AD found *M. furfur*. Beside, each species of fungus has a suitable growth pH such as *M. furfur* at pH=8.0, also *M. globosa* at pH=6.0.

❖Disease serverity

There was no difference in the distribution of *Malassezia* strains in the mild disease group. In the group of moderate disease, *M. globosa* was the highest proportion with 40%. Cho et al found out that genotype $(GT)_{10}$ of M. globsa was related with the severity of AD [11]. Although the colonization of *Malassezia* depends on lipid composition, water content and skin surface pH, *M. globosa* has a genotype that is closely related to the disease serverity. This is different from Rup et al [12]. The positive culture rate in group of SCORAD score under 40 points was higher than group of SCORAD score above 40 points. So, the author suggested this is related to the inflammatory process and chemical mediators with antifungal activity. We supposed that *M. globosa* may be associated with severe AD or exacerbation of AD.

V. CONCLUSION

On the skin lesions of Vietnamese patients with AD, *M. globosa* is the most common species with 39.0%, followed by *M. restricta* (19.5%).

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