ARQUIVOS DE ASMA, ALERGIA E IMUNOLOGIA

Desensitization immunotherapy for *Malassezia spp.*: experimental case report

Imunoterapia de dessensibilização para Malassezia *spp.: relato de caso experimental*

Raphael Coelho Figueiredo[1], Caroline Braga Barroso[2], Livio Melo Barbosa[2], Márcia Gabrielly Teles de-Macedo[2], Peniel Leite Rocha[2], Andréa Maria de Araújo Mendes[2], Lara Milena Santos Silva[3], Elaine de Lima de-Almeida[1]

ABSTRACT

Pityriasis versicolor is a infection caused by *Malassezia* yeast species, which, despite simple management, involves a high risk of recurrence and chronicity, and there are few effective therapies for resistant strains. Desensitization for *Malassezia spp.* has been reported in the literature, but for atopic dermatitis, rather than pityriasis versicolor, making this an innovative report. The case presented herein is of a 28-year-old man who had typical manifestations of pityriasis versicolor in the face, cervical, dorsal, and axillary region for 4 years that were resistant to topical and systemic therapies. Once the ineffectiveness of traditional therapies had been determined, weekly *Malassezia* desensitization sessions were begun, progressively increasing first in dosage and then in frequency. After 11 months, the lesions had improved completely. In this case, immunotherapeutic techniques effectively treated pityriasis versicolor, although the evidence is as yet insufficient to support large-scale use.

Keywords: *Malassezia*, tinea versicolor, desensitization, immunological, case reports.

RESUMO

A pitiríase versicolor (PV) consiste em uma infecção fúngica ocasionada por leveduras de *Malassezia spp.*, que apesar de manejo simples, é uma doença com elevadas chances de recidiva e cronificação, além da pouca variedade de terapias efetivas para tratar cepas resistentes. Existem relatos na literatura sobre utilização de dessensibilização para *Malassezia spp.*, mas para o tratamento de dermatite atópica e não PV, conferindo caráter inovador ao relato em questão. O caso apresentado consiste em um paciente de 28 anos, do sexo masculino, com manifestações típicas de PV em região de face, cervical, dorsal e axilar, há 4 anos, com resistência aos esquemas terapêuticos tópicos e sistêmicos. Uma vez identificada a ineficácia das terapias tradicionais, foi iniciado o tratamento com dessensiblização para *Malassezia spp.*, em aplicações semanais, com aumento progressivo da dosagem e posterior aumento no intervalo das aplicações. Após onze meses de realização do novo tratamento, o paciente evoluiu com melhora completa das lesões. Conclui-se que a utilização de técnicas imunoterápicas para o tratamento de PV foi considerado eficaz no caso relatado, apesar de ainda não haver evidências que amparem sua utilização em maior escala.

Descritores: *Malassezia*, tinha versicolor, dessensibilização imunológica, relatos de casos.

1. Clinic of Allergy and Clinical Immunology (CAAIC) - Imperatriz, MA, Brazil.
2. Universidade Federal do Maranhão, School of Medicine – Imperatriz Science Center - Imperatriz, MA, Brazil.
3. Universidade Estadual da Região Tocantina do Maranhão – School of Medicine - UEMASUL - Imperatriz, MA, Brazil.

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Introduction

Pityriasis versicolor (PV), also known as tinea versicolor, is a common, superficial, and benign fungal infection caused by yeasts belonging to the genus *Malassezia* (formerly *Pityrosporum*). Fourteen species of *Malassezia* have been described so far; those mainly associated with to PV are *M. furfur*, *M. globosa*, and *M. sympodialis*.[1]

These pseudo-yeasts are dimorphic, saprophytic, lipid-dependent fungi found in the normal flora of the skin. However, due to the influence of endogenous and exogenous factors, such as hyperhidrosis, the use of topical oils, immunosuppression, endocrine disorder, malnutrition, genetic predisposition, etc., these fungi convert to a pathogenic mycelial form associated with the onset of typical clinical manifestations of PV.[2] The infection most commonly affects adolescents and young adults, since, due to increased androgenic stimuli in these stages of life, the sebaceous glands reach peak functioning, predisposing this population to fungal colonization.[3,4]

This dermatosis is marked by lesions that can manifest as spots or fine scaly plaques with variable changes in skin pigmentation, including hypopigmented, hyperpigmented and/or slightly erythematous regions. The neck, trunk, and proximal extremities are the most commonly affected sites, with the intertriginous areas and face being less common.[5] The change in skin color is the patient's main complaint, mainly because it involves social stigma.[6]

Topical antifungals are the first-line treatment for PV, with oral imidazoles or terbinafine used for more extensive manifestations.[7] However, despite being easy to treat in clinical practice, PV's recurrence rate is high – up to 80% within 2 years.[1,7] Thus, recurrence after treatment with adequate antifungal agents and "chronification" are major complaints.[8]

Therefore, new therapies should be considered, since few classes of antifungals are available and infections by resistant strains are increasing.[9] Alternative therapeutic protocols, such as subcutaneous immunotherapy for *Malassezia* desensitization or the administration of yeast adhesion factor inhibitors have been reported, although the literature on the subject is still limited.[10]

Desensitization immunotherapy was developed more than a century ago to stimulate the immune system of allergic patients, modulating their response to allergens and creating a kind of immunological tolerance.[11] To accomplish this, doses of the same allergen are introduced in gradually larger amounts, functioning as a specific therapeutic vaccine.[12]

To reduce the reactivity of allergen extracts, allergoids, molecules polymerized through chemical agents, such as glutaraldehyde or formaldehyde, or biological agents, such as transglutaminase, can be produced. Thus, desensitization immunotherapy is expected to interrupt and modify the natural course of the disease.[12]

With this in mind, the present study reports a case of recurrent PV treated with desensitization immunotherapy for *Malassezia* spp., a little-discussed antifungal therapy with innovative potential.

Case report

A male 28-year-old market analyst born in Imperatriz, Maranhão (northeastern Brazil) sought out our allergology service due to hypopigmented spots in the cervical region, the back, and the face, and hyperpigmented and erythematous spots in the axillary region for 4 years (Figure 1), including clinical improvement and subsequent recurrence after topical and oral antifungal therapy.

Lesion biopsy revealed mild acanthosis of the epidermis and focal vacuolar alteration of the basal layer, in addition to slight mononuclear infiltrate, confirming the diagnosis of superficial perivascular dermatitis. Traditional laboratory analysis, including direct mycological examination and culture with antifungigram, showed mycosis by *Malassezia* spp. resistant to several oral and topical antifungals, which indicated PV. The case was then forwarded to a professional dermatologist. During dermatological care, the patient still had erythematous papules in the cervical and both infra-axillary regions, with positive results for Zileri's sign, well-defined hyperpigmented macules in the groin, and onychomycosis in the nails. At that point, treatment consisted of oral terbinafine, topical isoconazole and fenticonazole, as well as a hydroalcoholic solution with 2.5% selenium sulfide. Although the lesions were monitored monthly, adjusting the medication as necessary, the patient still had active PV lesions in the cervical region and scaly lesions in both axillary regions.

The patient was followed up for 1 year by an allergist and 4 months by a dermatologist, which, in addition to the previous 4-year history of lesions, totaled 5 years and 4 months living with active PV,

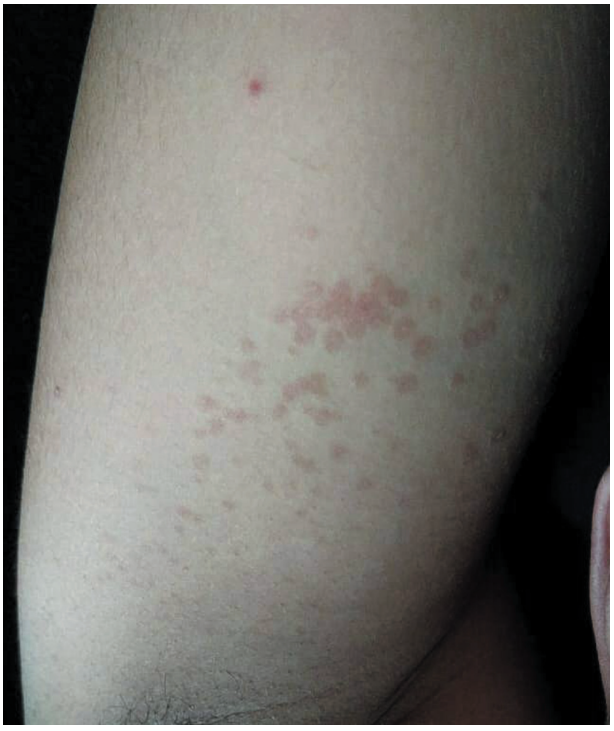

Figure 1
Stain-like lesions and erythematous papules in the right infra-axillary region

including recurrences and unsatisfactory therapeutic results. The patient was thus sent back to the allergist to assess the benefits of desensitization.

The possibility of atopic dermatitis was ruled out due to the characteristics, location, and evolution of the lesions. Contact dermatitis was also excluded after a negative result in the patch test (the Brazilian standard battery; IPI ASAC Brasil®). A laboratory investigation was performed for immunodeficiencies, and the patient was negative for inborn errors of immunity.

Scrapings from the lesion were collected in the laboratory. To avoid other pathogens in the sample, the scraped surface was first disinfected with iodine solution (1% to 2% iodine tincture), which was removed with 70% alcohol and then left to dry prior to collection. After culture, 2 µg *Malassezia* spp. was isolated in 0.9% saline for a prick test to assess the patient's specific IgE response in a healthy control. The patient was positive for *Malassezia* spp. at 10 mm, with a positive control at 7 mm and negative control at 2 mm; the healthy control was negative for *Malassezia* spp.

In early April 2019, all pharmacological treatment was suspended and specific immunotherapy for *Malassezia* spp. began. The protocol differed in the number of applications, intervals, and dilution patterns from that used in allergen-specific immunotherapy for patients sensitized to house dust mites. The experimental treatment was approved by the Human Research Ethics Committee of the Federal University of Maranhão's University Hospital (opinion 5,375,840). The patient provided written informed consent for both the treatment and publication of the results. All data remained confidential and were anonymized under the responsibility of the researchers, in accordance with National Health Council Resolution 466/2012.

A slower (more gradual) desensitization protocol was planned, since this therapy was experimental and involved the risk of systemic reactions. The allergen was diluted in an aqueous solution of phenol + 0.9% physiological saline solution (IPI ASAC Brasil®). The weekly application began with 4 injections per visit, with progressively increasing doses: 0.1 mL, 0.2 mL, 0.4 mL, and 0.8 mL. Each week a different dilution was used, titrated in factors of 10 (ie, 1/10,000, 1/1000, 1/100, 1/10, 1/1), totaling 5 weeks. In the fifth week (1/1 dilution), an additional application was performed at a dose of 1.0 mL to prepare the patient for the maintenance phase (Table 1). During the maintenance phase, all doses were 1 mL with 1:1 titration. The initial maintenance schedule consisted of fortnightly or monthly injections, totaling 8 applications performed in this stage; this was followed by injections of 1 mL of the 1:1 concentration every 15, 21, or 30 days (Table 2).

Treatment began on April 2, 2019 and in a little over a month (May 9, 2019) the patient's lesions had improved by about 30%. After approximately 11 months (March 11, 2020) of desensitization, the recurrent PV was completely resolved in the cervical region, dorsum, groin, and both infra-axillary regions (Figure 2), with no adverse reactions. At the end of the protocol, given that the condition had resolved, a skin prick test was not performed to demonstrate immunological desensitization.

Discussion

Although PV generally does not involve risk of death or systemic impairment, it can cause substantial aesthetic and social discomfort for patients. Unfortunately, PV treatment failures are common due to fungal resistance, the long duration of treatment,

and the considerable side effects of antifungals.[13] Therefore, alternative and innovative antifungal strategies should be investigated as the key to future therapy, especially for cases of recurrent PV.[14]

Subcutaneous immunotherapy changes several types of antibodies specific to the injected antigen, causing serum antigen-specific IgG levels to increase. These remain increased during therapy and for several weeks or months after its end. The presence of *Malassezia* yeast cells in the skin stimulates higher production of interleukin-8 (IL-8) and interleukin-1.[15]

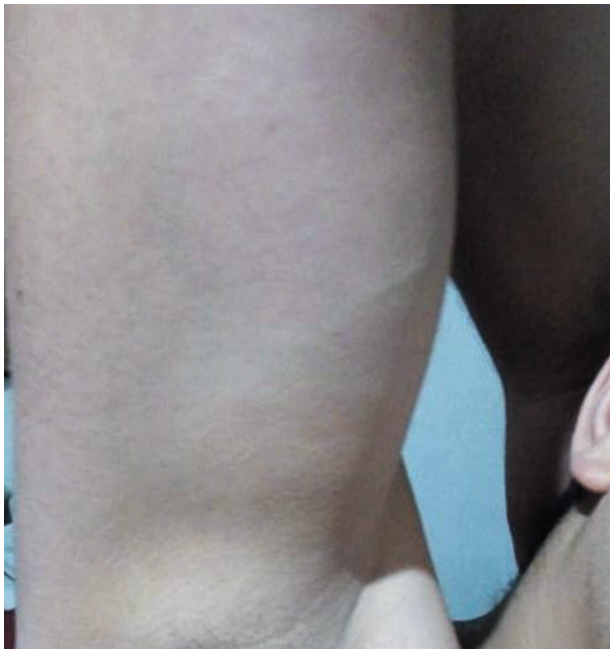

Figure 2
Right infra-axillary region after treatment, with no apparent lesions

In the present case, the patient did not respond well to topical agents, which are the treatment recommended in the literature. Similar cases were observed in a study of the main topical and systemic antifungal treatment regimens for recurrent PV; these treatments were not completely successful since the lesions remained.[9,14]

Although an elevated inflammatory state is not characteristic of PV, there is evidence of interaction between the species and the innate and specific immune response. Thus, since the antifungal immune response is physiologically marked by activation of the IL-23/IL-17 axis, in addition to controlling fungal growth, it may also be involved in certain immune-mediated pathological manifestations.[15]

This is relevant for the clinical picture described in this case report, since its recurrent nature after conventional treatment calls for new therapeutic approaches. The ideal approach for this case would be a less toxic therapy involving a more targeted antimicrobial spectrum. Several experimental treatments for fungal diseases have been described in the literature, such as monoclonal antibodies, immunotherapy with cytokines,

Table 1
Weekly treatment schedule

Week	Dilution	Dose			
1	1/10.000	0.1 mL	0.2 mL	0.4 mL	0.8 mL
2	1/1.000	0.1 mL	0.2 mL	0.4 mL	0.8 mL
3	1/100	0.1 mL	0.2 mL	0.4 mL	0.8 mL
4	1/10	0.1 mL	0.2 mL	0.4 mL	0.8 mL
5	1/1	0.1 mL	0.2 mL	0.4 mL	0.8 mL

Table 2
Fortnightly schedule

Fortnight	1	2	3	4	5	6	7	8
Dilution	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
Dose	1.0 mL	1.0 mL	1.0 mL	1.0 mL	1.0 mL	1.0 mL	1.0 mL	1.0 mL

vaccines, and antimicrobial peptides, which are new biopharmaceuticals capable of preventing or treating fungal infections. Antifungal peptides stand out in this list due to their specificity, selectivity, and tolerance.

The best option in the present case was desensitization for *Malassezia* spp. However, we could find few reports or descriptions of desensitization for *Malassezia*-type fungi, and they are generally for atopic dermatitis[10] rather than PV. Thus, the present case has experimental value and promising results.

However, despite its effectiveness, this desensitization protocol is challenging due to its complexity, requiring laboratories trained in fungal isolation and immunologists trained in dilutions and desensitization. Hence, access to this type of therapy remains limited.

Another limitation is that the treatment's degree of protection cannot be measured, which is necessary to predict relapses and the desensitization time necessary for lasting remission. However, the patient has been under clinical observation since the end of desensitization in March 2020 and, at the time of publication, has suffered no recurrence.

We conclude that desensitization to *Malassezia* spp. effectively treated PV in the present case. However, this method is still limited and is not feasible for large-scale use. More extensive studies are needed to confirm its effectiveness for recurrent PV and rule out side effects.

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Corresponding author:
Raphael Coelho Figueiredo
E-mail: formimp@hotmail.com