Am. J. Trop. Med. Hyg., 107(1), 2022, pp. 110–112
doi:10.4269/ajtmh.22-0015

Role of Immunotherapy in *Pythium insidiosum* Keratitis

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Abstract. *Pythium* keratitis is a potentially devastating ocular condition. Incidence of *Pythium* keratitis has been reported in tropical and subtropical areas. In previous reports, there were no effective or standard treatments, and combinations of medication, immunotherapy, and surgery were proposed. *Pythium insidiosum* antigen immunotherapy (PIAI) showed an acceptable safety profile, but its efficacy is questionable in *Pythium* keratitis. This retrospective review included 10 eyes from 10 patients. All cases were confirmed diagnosis of *P. insidiosum* keratitis by culture and/or polymerase chain reaction. Three doses of PIAI were injected at 2-week intervals in all patients. The infiltration diameter ranged from 5.2 mm to total corneal involvement, and eight cases (80%) had hypopyon. Therapeutic penetrating keratoplasty (TPK) or scleral graft were undertaken in nine cases. Enucleation was done in one case on the first visit. A second TPK was undertaken in three cases, and two globes were saved. Two cases in the globe salvage group received voriconazole via eyedrops and intracameral injection. No case received either linezolid or azithromycin. Three of nine eye globes (33.33%) were saved. PIAI did not show efficacy in the treatment of *Pythium* keratitis. Radical surgery including resurgery in recurrence is an approved effective treatment. The recently reported medications may offer supportive management.

INTRODUCTION

Pythium insidiosum keratitis is a sight-threatening disease with high morbidity (globe loss). The efficacy of antimicrobial agents remains inconclusive. The most effective treatment is radical surgery including therapeutic keratoplasty, scleral graft, evisceration, and enucleation. The role of immunotherapy is still controversial.[1–3]

P. insidiosum antigen immunotherapy (PIAI) is prepared from antigens of *P. insidiosum.* The recommended schedule of immunotherapy was three injections of 100 to 200 μL (2.0 mg/mL) at 2-week intervals via the subcutaneous route.[4] The curative mechanisms are switching from Th2 to Th1 and the expression of mononuclear cells.[5]

PIAI has been reported to treat human pythiosis, such as arterial pythiosis. Arterial pythiosis has high mortality and limited effective therapeutic options. PIAI was successfully used to treat a young boy with arterial pythiosis. He had failed to respond to antifungal therapy but showed dramatic recovery after immunotherapy.[6] Wachiwanawin et al.[7] later used a new formulation of PIAI, either alone or combined with radical surgery, to cure four of eight (50%) Thai cases of terminal arterial pythiosis. The authors concluded that immunotherapy using PIA is a safe and effective method to treat pythiosis in humans.[7]

PIAI has been reported for *Pythium* keratitis in some studies. Results from previous studies for *P. insidiosum* keratitis are still inconclusive.[1,8]

The purpose of the present study was to evaluate the outcome of PIAI for *P. insidiosum* keratitis. The clinical courses of all 10 cases are described and discussed to clarify the role of immunotherapy combined with medications and surgeries.

MATERIALS AND METHODS

This is a descriptive retrospective study. Ten patients who had a culture- and/or polymerase chain reaction–confirmed diagnosis of *P. insidiosum* keratitis and were treated with PIAI at the KKU Eye Center, Srinagarind Hospital, Khon Kaen University, Thailand.

The study adhered to the tenets of the Declaration of Helsinki and was approved by the Khon Kaen University Ethics Committee for Human Research (HE561445).

All clinical records with a diagnosis to *P. insidiosum* keratitis who received a total of three doses of PIAI were reviewed.[4] Information regarding age, sex, medication, surgery, and immunotherapy were obtained. The main outcome measure was the global salvage rate (defined as the rate was that of the eyes that did not require either enucleation or evisceration) after immunization. All basic data including drugs of choice, surgical techniques and vaccination are summarized in Table1.

RESULTS

The mean (±SD) age of patients was 42.2 (±7.43) years (range: 30–51 years). All cases were confirmed of the diagnoses of *P. insidiosum* keratitis by cultures and/or polymerase chain reaction (PCR). Five cases (50%) were male and five were female. The mean (±SD) ulcer size was 7.54 (±1.89) millimeters. All ulcer locations were in the center and deep to the endothelium. There was a suspected limbal/scleral involvement in 1 case (No. 8). Onset of disease ranged from 7–18 days.

The most common topical medications used were natamycin 5%, amphotericin 0.15%, and ketoconazole 2%. Different topical medications depended on availability of each drug or the affordability for each patient. Linezolid and azithromycin, however, may not have been available to the patient at that time due to no publications to support this evidence. Two cases in the globe salvage group (this group included 2 patients who did not require either enucleation or evisceration) received voriconazole via eyedrops and intracameral injection. Eight of 10 cases (80%) received intracameral amphotericin B injection. Three cases (Nos. 2, 3, 7) received topical voriconazole. All medications were maintained after surgery, except in evisceration or enucleation cases.

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TABLE 1
Demographic data, treatments and outcome

No.	Age	Sex	Medications	Size (mm)	Hypopyon	Surgery (recipient size, day) and vaccine (day)	Final VA, last F/U (cause)
1	30	M	Top: Nat, Keto Ora: Itra	6.9	−	TPK (8.0 mm, D10), Vac (D13)	HM, 12 mo (graft failure)
2	50	M	Top: Nat, Keto, Vor Ora: Itra, Ter IC: Amp (D2)	5.2	+	SG (8.5 mm, D7), TPK (9.0 mm, D36), Vac (D37)	HM, 11 mo (graft failure)
3	51	F	Top: Nat, Keto, Vor Ora: Itra, Ter IC: Amp (D4, D13)	6.2	+	SG (8.5 mm, D4), TPK (9.0 mm, D21), Vac (D22)	HM, 8 mo (graft failure)
4	40	F	Top: Nat, Keto Ora: Itra IC: Amp (D1, D5)	7.1	+	SG (8.0 mm, D2), Vac (D5), Evis (D7)	−, 9 mo (refused second TPK)
5	33	F	Top: Amp, Keto Ora: Itra IC: Amp (D5, D11)	6.1	+	TPK (8.5 mm, D7), Vac (D8), Evis (D14)	−, 8 mo
6	33	F	Top: Nat, Keto Ora: Itra IC: Amp (D1, D25)	7.2	−	TPK (8.0 mm, D1), Vac (D15), TPK (8.5 mm, D25), Evis (D29) (complete Vac at D43)	−, 8 mo
7	48	F	Top: Nat, Keto, Vor Ora: Itra IC: Amp (D3, D13)	9	+	TPK (9.0 mm, D9), Vac (D17), Enu (D24) (* complete Vac at D45)	−, 11 mo (refused second TPK)
8	47	M	Top: Nat, Ampho Ora: Itra IC: Amp (D4)	Total cornea	+	Enu (D6), Vac (D11)	−, 6 mo
9	42	M	Top: Nat, Ampho Ora: Itra IC: Amp (D1, D3)	9.2	+	TPK (9.0 mm, D3), Vac (D6), Enu (D15)	−, 7 mo
10	48	M	Top: Nat, Ampho Ora: Itra	6.5	+	PK (8.0 mm, D6), Vac (D8), Enu (D11)	−, 9 mo

Amp = Amphotericin; Enu = enucleation; Evis = evisceration; F/U = follow-up; HM = hand motion; IC = intracameral; Itra = itraconazole (200) twice daily; Keto = ketoconazole 2%; mo = months; Nat = natamycin 5%; Ora = oral; SG = scleral graft; Ter = terbinafine; Top = topical; TPK = therapeutic penetrating keratoplasty; VA = visual acuity; Vac (Dx) = first vaccination on day × (start from initial visit) then 2 weeks apart for a total of three doses; Vor = voriconazole. D0 is admission date (the first day patient presented for treatment). In the final column, − indicates that the globe could not be saved due to evisceration or enucleation. Size is the largest diameter of corneal lesions.

The corneal infiltration sizes at initial visit ranged from 5.2 mm to total corneal infiltration, and most cases (eight of 10, 80%) had hypopyon. Nine patients required TPK and/or scleral graft (enucleation was done at first visit in 1 patient due to total corneal infiltration). Trephination size ranged from 8.0 to to 9.0 mm (9.0 mm is the largest available trephination size in this hospital). The eye globes were saved in only 3 cases from 9 cases who received TPK and/or scleral graft (3/9, 33.33%) and enucleation was done as a primary surgery in one case. Saved eye globes were defined by no corneal or scleral infiltration and no inflammation. Even though a patient was scheduled for evisceration or enucleation during vaccination, all cases were continued with a full 3 doses at 2-week intervals. TPK or scleral grafts were undertaken in 9 cases. Enucleation was done in 1 case at first visit due to total corneal infiltration and the patient's needs. PIAI was applied after enucleation in this case because of suspected scleral involvement. Re-surgeries with TPK were done in 3 cases and 2 globes were saved. The indications for re-surgery were new infiltrations and increased hypopyon despite maximum topical medications and intracameral injections. There was no patient who was lost to follow-up.

DISCUSSION

The global salvage rate in previous reports varied from 42.31% to 89.13%.[1,2,9,10] In the present study, the global salvage rate was only 33.33% (three of nine cases). There are some possible explanations for lower rate of global salvage. Firstly, there was poor accessibility of some new medications that might have had effectiveness against *Pythium* keratitis; for example, voriconazole, linezolid and azithromycin.[11–15] Secondly, two patients could not wait for the second TPK due to corneal donor deficiency or financial difficulties. Second TPK was required in cases of recurrence, and it was reported to be from 12.50% to 53.30% in previous studies.[1,2,4,9,10] In this present study, two of three globes were saved after the second TPK, so re-surgery may increase the chance of saving the globe. Thirdly, the size of the trephine was limited, but this limitation cannot be explained in some cases with relatively large cuts compared with infiltration size (cases 5 and 10). Moreover, sample size of the present study is less than previous studies, so this rate may change with a larger number.

Immunotherapy for *Pythium* keratitis showed an acceptable safety profile, but the efficacy is questionable in both systemic and corneal pythiosis.[1,16] Even though adjunct vaccination has been reported to successfully treat arterial pythiosis, the sample sizes were relatively small, and it is not widely used.[5,7] In a previous study of *Pythium* keratitis, PIAI was reported to be used in at least one dose in 17 patients, and the globes were saved in 41.2% (seven of 17).[1] In the present study, only 33.33% (three of nine) globes were saved with the combination of all medications, surgery, and immunotherapy. These results suggest that the vaccine did not improve the global salvation rate. The first explanation may be from the avascular area of the cornea. Mononuclear cells and Th1 are the main mechanisms from PIAI. The corneal area is an avascular area, so it is more difficult for cells, including cytokines, to access the infection sites compared

with systemic organs. Second, most cases (80%, eight of 10) had hypopyon, so intracameral seeding was suspected, and this may indicate disease severity.[5,6,17] Finally, the time for reactivation of PIAI may be too long because the progression of *Pythium* keratitis is relatively rapid.

It has been reported that successful immunotherapy in systemic animal pythiosis depends on the chronicity of the lesions before therapy. All horses with lesions $<$ 15 days old were cured by an early PIAI, whereas those with chronic pythiosis ($>$ 2 months duration) eventually died.[4,7] In humans, one report indicated that the global salvage group (16 of 30, 53.33%) comprised significantly younger patients, and the first ocular surgeries were performed significantly sooner than in the nonglobal salvage group (14 of 30, 46.67%).[18] The globe removal cases, however, has an average chronicity less than the globe salvage cases in the present study. Therefore, chronicity may not be the only factor that demonstrates prognosis of globe salvage in human *Pythium* keratitis.

In summary, it was not able to show that immunotherapy was an effective treatment for *Pythium* keratitis in the present study. A larger sample size with a randomized control trial is needed to confirm this. Radical surgery including resurgery on recurrence is currently the only approved effective treatment.[1–5] Newly reported medications (ex. azithromyicin or linezolid) may provide supportive management.[1] New immunotherapies, however, are evolving in animal studies, and these may offer new hope for the treatment of *Pythium* keratitis.

Received January 7, 2022. Accepted for publication March 2, 2022.

Published online June 6, 2022.

Acknowledgments: We thank the patients for their participation, the nursing and medical staff for their assistance, the Department of Ophthalmology and the faculty for financial support, and Professor James A. Will for editing the manuscript via the Publication Clinic KKU.

Financial support: This study was funded by a grant from Research Affairs, Faculty of Medicine, Srinagarind Hospital, Khon Kaen University.

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