Research Article

Use of clotrimazole eye Suspension for fungal keratitis as monotherapy or sequentially with amphotercin-B

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Abstract

Aim: To determine the efficacy of 1% clotrimazole eye drops suspension for treating fungal keratitis as monotherapy or sequentially with topical amphotercin-B.

Method: This therapeutic trial was conducted in Ibn Al-Haetham Teaching Eye Hospital/Baghdad between February 2008 and July 2009. The patients with culture proven fungal keratitis were divided into two groups, first group (presented between February 2008 and May 2008) were treated initially with 1% clotrimazole eye suspension. Second group (presented between May 2008 and July 2009) were treated sequentially with 0.1% amphotercin-B eye drops (induction phase) for 2-3 weeks, then switched to 1% clotrimazole eye suspension (maintenance phase). The results in the sequential group were compared with a previous study of using 0.1% amphotercin-B eye drops as monotherapy for treating fungal keratitis.

Results: Thirty-two patients with culture proven fungal keratitis were enrolled in this study. Eight cases treated initially with clotrimazole suspension, only two of them had favorable response. While 24 patients treated sequentially and 20 of them had favorable response. Comparing the sequential therapy with a previous study used 0.1% amphotercin eye drops as monotherapy, showed that mean time for therapy was not significantly different in non-severe cases (4.1/4 weeks), while it was shorter in severe cases (9.5/12 weeks).

Conclusion: Use of clotrimazole eye drops as monotherapy is not effective in treating fungal keratitis. While Sequential therapy of using topical amphotercin-B during induction phase followed by 1% clotrimazole suspension as clearance, or maintenance phase is an effective and cheaper therapy for treating fungal keratitis.

Key words: *fungal keratitis, clotrimazole, amphotercin-B*

INTRODUCTION

Fungal keratitis is one of the major causes of ocular morbidity in the tropical regions.[1-3] Trauma with organic materials is the main predisposing factor for this devastating disease.[4-6] In Iraq, with semitropical climate and agricultural community, fungal keratitis is an important cause of suppurative keratitis and visual disability.[7]

Polyene and azole compounds present first line of antifungal agents in the treatment of mycotic keratitis. Natamycin 5% suspension is the only topical ophthalmic antifungal compound approved by the Food and Drug Administration of the United States, and considered the drug of choice for filamentous fungal keratitis.[8] Natamycin and other specific antifungal eye drops are expensive and scarce in the developing countries. Therefore treatment of fungal keratitis poses a therapeutic dilemma for the ophthalmologists in these countries including Iraq.

The objective of this study is to determine the efficacy of 1% clotrimazole eye suspension, in the treatment of fungal keratitis as monotherapy, or sequentially with topical amphotercin-B.

PATIENTS AND METHODS

This is a retrospective therapeutic trial study performed at Ibn Al-Haetham Tteaching Eye Hospital (IAHTEH) between February 2008 and July 2009. Patients were recruited from those suffering from suppurative keratitis who presented consecutively to the hospital during the 17 months period of the study. A questionnaire form was constructed for data collection, every patient was asked about demographic features, duration of symptoms, predisposing factors, therapy taken prior to presentation, associated ocular and/or systemic diseases. Visual acuity at presentation was documented, and ocular examination was performed with Slit Lamp recording size and depth of the ulcer, and stromal suppuration. Height of hypopyon, if present, was also recoded. Scraping of the edges of the ulcer was performed using a sterile bend tipped needle, excluding perforated ulcers and those of less than 2mm in diameter. The materials were inoculated into, blood, chocolate, brain- heart infusion agars, and Sabouraud's agar without cyclohexamide. All media were incubated at 370C, except Sabouraud's agar incubated at 300C. Direct smears were taken for Gram's staining and 10% KOH mounts. A diagnosis of fungal keratitis was considered if corneal scrapings revealed fungal elements in the smears, if fungus grew in more than one media or on several 'C' streaks in one solid medium. Identification of isolated fungi was done according to their macroscopic and microscopic morphology. Patients with lab proven fungal keratitis were enrolled as potential participants for this study.

A case was graded as severe if the suppuration or the ulcer had greatest diameter of >=6mm (or surface area of >=25 mm2), if the ulcer involved the deep one half of the cornea, or if there was a posterior corneal abscess. All other cases were graded as non-severe.

Treatment: Patients enrolled in this study were divided, into two groups. Patients in the first group (presented between February 2008 and May 2008) were treated with 1% clotrimazole suspension, and if there was a favorable response, same treatment continued until complete healing. While if treatment failure was recorded, topical 1% clotrimazole suspension was discontinued and topical 0.1% amphotercin-B was administered.

Patients in the second group (presented between May 2008 and July 2009) were treated initially with topical 0.1% amphotercin-B (induction phase). After 2-3 weeks, amphotercin-B was discontinued and 1% clotrimazole eye suspension was initiated (maintenance or clearance phase) till end of the therapy. The induction phase (topical 0.1% amphotercin-B) was given for two weeks in non-severe cases and for three weeks in severe cases. Eye drops were given initially every hour during waking time for first few days with gradual decrease in the frequency of instillation.

The outcome was monitored as follows:

- Signs of favorable response were decreasing in the ulcer's size, blunting of its margins, disappearance of satellite lesions, rounding out of the feathery margins, decreasing in the suppuration, and decreasing in the anterior chamber reaction.

-Signs of failure of treatment were increasing in the ulcer's size or suppuration with increasing in the anterior chamber reaction after one week from initiating the treatment.

-Healing of keratitis means no fluorescein staining, replacements of stromal suppuration by corneal scar, no anterior chamber reaction, no perforation, no adherent leukoma, and no anterior staphyloma. Healing stage may be reached either with good visual outcome (localized scar with improvement of vision from the baseline level), or with poor visual outcome (large vascularized dense scar without improvement of vision from the baseline level).

Total time of therapy; which is the time taken for signs of inflammation to subside and discontinue antifungal therapy, was recorded in every case.

The results of the second group (sequential therapy) were compared with a previous study (historical controls) of using 0.1% amphotercin-B eye drops as a monotherapy for treating fungal keratitis13. Both studies were conducted at the same hospital, took the same time (14 months), patients were examined and followed by the same ophthalmologist, and followed the same examination protocol, including the classification criteria. Clotrimazole eye suspension drops had been prepared at Pharmacy College/ University of Baghdad.

The study followed the principles of the Declaration of Helsinki. Institutional review board and ethics committee approval was granted by the Scientific Committee of IAHTEH.

RESULT

During the 17 months of this study, 32 patients (32 eyes) with lab proven fungal keratitis were registered. At presentation, 20 cases (62.5%) were severe, while 12 cases (37.5%) were non-severe. Mean age of the patients was 39.4 years ranged between 16-65 years, with no significant sex prevalence (male/female ratio: 1.1/1). Most patients were from rural areas (81%) with agricultural work (69%) (table 1).

Table 1. Demographic characteristics, of patients presented with severe and non-severe fungal keratitis.

	Non-severe	Severe	Total
Number	12	20	32
Mean age	38.1	40.2	39.4
Male/Female	6/6	11/9	17/15
Residence Ruler/Urban	9/3	16/4	25/7

Trauma with organic material was the leading predisposing factor. Patients presented with severe keratitis had longer duration of complaints and most of them had used several medications prior to presentation (table 2).

Aspergillus species and Fusarium species were the main isolated fungi (table 3).

Table 2. Predisposing factors, drugs used prior to presentation, duration of symptoms

	Non-severe	Severe	Total
Predisposing factors: Trauma by organic material Trauma induced by traditional healers* Previous cataract surgery with use of topical steroids	 8 0 0	 13 5 3	 21 5 3
Medications received by patients before presentation Antibiotics eye drops Sub-conjunctival injections of antibiotics Steroids eye drops Antiviral ointment	 12 0 0 0	 20 17 6 4	 32 17 6 4
Mean duration of symptoms before presentation(days)	12.5	28.3	22.4

***Some villagers with conjunctivitis go to these pretended traditional healers for remedy of foreign body sensation in their eyes. They remove the presumed foreign body by a dirty piece of cloth, or sometimes by their tongues**.

The first group included 8 patients (table 4), was initially treated with 1% clotrimazole suspension. Two patients presented with non-severe keratitis had a favorable response, and the same treatment continued until complete healing was gained (after 4 weeks), with small corneal scars and improvement of vision from the base line. The causative fungi were Fusarium species. The other six cases (five with severe keratitis and one with non-severe keratitis) failed to respond to 1% clotrimazole. The causative fungi were Aspergillus species (4 cases), Scopulariopsis specie (1 case) and Fusarium specie (1 case). Clotrimazole suspension was discontinued and topical 0.1% amphotercin-B was initiated, but all these cases responded poorly to amphotercin-B and progressed to perforation with anterior staphyloma (four cases), or endophthalmitis that required evisceration (two cases).

Table 3

Isolated fungi from patients presented with severe and non-severe keratitis.

Fungi isolated	*Non-severe*	*Severe*	*Total*
Aspergillus sp.	7	10	17
Fusarium sp.	4	7	11
Pencillium sp.	0	1	1
Curvularia sp.	1	0	1
Alternaria sp.	0	1	1
Scopulariopsis sp.	0	1	1

In the second group (table 5), 24 patients were treated initially with amphotercin–B (induction phase), there was favorable response in 19 patients (9 with non-severe keratitis, 10 with severe keratitis). After two weeks in non-severe and three weeks in severe cases, treatment was changed to the maintenance therapy of 1% clotrimazole suspension. Induction phase (amphotercin–B) was stopped and maintenance phase (1% clotrimazole suspension) started while the keratitis was still active (positive staining, positive stromal suppuration, and active anterior chamber reaction). Favorable response continued in all patients on the maintenance therapy of 1% clotrimazole suspension, and the keratitis healed after 4-10 weeks from initiating the treatment with improvement of vision in 11 cases, while in 8 cases there was no improvement of vision from the base line level. The causative fungi were Aspergillus species (10 cases), Fusarium species (4 cases), Curvularia species, Alternaria species (two cases for each), and Pencillium species (one case). Five cases presented with severe keratitis, Fusarium species (4 cases), and Aspergillus species (one case), had failure of treatment on the initial therapy (amphotercin–B 0.1%), which was discontinued and 1% clotrimazole eye suspension was initiated. Four cases showed no favorable response and the keratitis progressed to perforation followed by anterior staphyloma (4 cases), and endophthalmitis that required evisceration (one case). One severe case of Fusarium species that showed no favorable response on amphotercin–B 0.1%, responded well on 1% clotrimazole suspension and healed with opaque vascularized cornea with no improvement of vision from the base line level. The results were compared with a previous study[9] (historical control) included 22 patients with fungal keratitis managed with 0.1% amphotercin eye drops as monotherapy (table 6). Both studies carried at the same

institution and took the same period. Numbers of patients increased in the current study, due to increased in the referral cases with suspected fungal keratitis to IAHTEH, and most of these referral cases were severe at presentation. Baseline characteristics of the patients, and type of fungi isolated were not significantly different between the two groups. Mean time of therapy for non-severe cases was not significantly different in both groups (4/4.1 weeks). While in severe case, mean time of therapy was shorter in the sequential therapy group (9.5 weeks), compared with the monotherapy group (12 weeks). Use of 1% clotrimazole suspension was not irritant and free from local side effects in all patients who used it.

DISCUSSION

Treatment of fungal keratitis poses a therapeutic dilemma for the ophthalmologists in Iraq, due to unavailability of specific readymade antifungal eye drops. Some antiseptic agents were postulated to be effective in keratomycosis,[10,11] but trial of using chlorhexidine gluconate for treating fungal keratitis in Iraq was not encouraging.[12] During the last few years, in IAHTEH, cases of culture proven fungal keratitis were treated with topical 0.1% amphotercin-B prepared from vials for intravenous injections (Fungizone 50mg vials).[9] Fresh preparations had to be reconstructed every few days, and this was very difficult for patients with distant residence taking in consideration that the treatment has to be continued for several weeks. Therefore, there is a real need for an effective and more practical therapy for fungal keratitis in Iraq.

Azole derivates eyedrops had been used for treating fungal keratitis.[13,14]Clotrimazole, a derivative of imidazole, is widely available, effective against dermatomycosis, and it was also used for ophthalmic mycosis since the 1970s, but the data were derived from case reports or uncontrolled studies.[15,16]

Although the number of cases treated initially with clotrimazole suspension in this study was small (8 cases), but the results were not encouraging, and this agrees with a previous study used dermatological preparations of clotrimazole for treating 12 cases of fungal keratitis.[15] Clotrimazole suspension is probably not an ideal choice as monotherapy for treating fungal keratitis.

The current study shows that treatment with topical amphotercin–B in cases that had not responded to clotrimazole eye suspensions, appeared to make the fungi more resistant to amphotercin-B. On the other hand, sequential treatment with amphotercin–B followed by clotrimazole eye suspension has a promising result. Amphotercin-B seems to potentiate the effect of subsequent use of clotrimazole.

Combinations of azoles and polyene are frequently used for treating keratomycosis, but the nature of their interaction and the advantages of these combinations are still unclear. Data on the efficacy of combination therapy are sparse and consist largely of results from studies *in vitro* and experimental animal models.[17]

Studies based on animals models and in vitro data determined that sequential exposure to an azole followed by a polyene could be detrimental. Preexposure to an azole compound causes replacement of membrane ergosterol with a methylated sterol derivative to which amphotercin-B binds less well.[18,19] Experiments in vitro reported that sequential therapy with azole may reduce subsequent activity of amphotercin-B.[20] When an azole was first applied to the culture, strong antagonism upon subsequent exposure to amphotercin-B was observed.[21] Experiments in animals models shows that sequential therapy with azole and amphotercin-B has resulted in the attenuation of amphotercin-B activity after azole preexposure.[22] On the other hand, experiments in vitro on Aspergillus species shows that pretreatment with amphotercin-B and then with azoles resulted in greater synergistic effects than those obtained when the drugs were given simultaneously.[23]

Polyene-azole combinations have been used in humans with systemic fungal infections. Sequential therapy with polyene followed by an azole (fluconazole or itraconazole) has been used for treating fungal meningitis and it appears that pretreatment with amphotercin B during induction phase might aid the positive impact of subsequent azole activity during the consolidation, clearance, or maintenance phase.[24,25] Azoles may be used for long-term consolidation or clearance therapy in patients who have received induction therapy with amphotercin-B for invasive Aspergillosis.[26]

Simultaneous use of anti-fungal azoles with polyenes is still a controversy subject. Previous studies reported that concurrent use of azoles with amphotercin-B was synergistic, indifferent, or antagonistic against pathogenic fungi in vitro.[23,27] Experiments in vitro with Candida spp. reported that some azoles have antagonist effects on amphotercin-B activity[20, 28,29,30] while other study observed augmented activity of amphotercin B and antifungal azoles.[31,32] Combination of Amphotercin-B, clotrimazole, nystatin, and natamycin may synergistically prevent infections in corneal storage medium.[33] Prajina et al concluded that concurrent use of 5% natamycin and 2% econazole did not appear to

offer additional benefits over monotherapy with 5% natamycin.[34] While Sharma concluded that empiric antifungal therapy is discouraged.[35]

To our knowledge, this is first study of using sequential therapy for human fungal keratitis. 1% Clotrimazole eye suspension can be used as a cheap, non toxic, and stable topical antifungal eye agent to complete eradication of the fungi from the infected cornea after primarily short course of topical amphotercin-B. Further trials are recommended for sequential therapy of 5% natamycin followed by azoles (e.g. 1% clotrimazole suspension, fluconazole, or itraconazole).

This study highlights that treatment with topical amphotercin–B after using clotrimazole eye suspensions should be taken with cautious, and this is probably the same with the use of other polyenes and azoles. Further investigations required about this subject and for the simultaneous use of these antifungal agents.

We found newly infected eyes responded much better to the antifungal therapy (amphotercin–B or clotrimazole eye drops), and we agree with other studies,[36,37] in that patients with fungal keratitis who referred late were frequently with severe keratitis that was refractory to treatment.

Conclusions:

Use of clotrimazole eye drops as monotherapy is not effective in treating fungal keratitis. While Sequential therapy of using topical amphotercin-B during induction phase followed by 1% clotrimazole suspension as clearance, or maintenance phase is an effective and cheaper therapy for treating fungal keratitis.

Table 4

Group one: Cases of fungal keratitis treated initially with clotrimazole 1% eye drops.

Case No.	Fungi isolated	Severity at presentation	Initial Therapy	Response	Maintenance therapy	Response On Maintenance therapy	Total time of therapy (weeks)	Finial visual outcome
1	Fusarium sp.	Non-severe	Clotrimazole	favorable	Clotrimazole	favorable	4	Healing, improved V.A.
2	Aspergillus sp.	Severe	Clotrimazole	failure	Amphotercin-B	failure	12	Perforation, Anterior staphyloma.
3	Fusarium sp.	Non-severe	Clotrimazole	favorable	Clotrimazole	favorable	4	Healing, improved V.A.
4	Aspergillus sp.	Severe	Clotrimazole	failure	Amphotercin-B	failure	12	Perforation, Anterior staphyloma
5	Scopulariopsis sp.	Severe	Clotrimazole	failure	Amphotercin-B	failure	10	Perforation, Anterior staphyloma.
6	Aspergillus sp.	Non-severe	Clotrimazole	failure	Amphotercin-B	failure	10	Perforation, Anterior staphyloma
7	Aspergillus sp.	Severe	Clotrimazole	failure	Amphotercin-B	failure	12	evisceration
8	Fusarium sp.	Severe	Clotrimazole	failure	Amphotercin-B	failure	12	evisceration

Table 5: Group two: Cases of fungal keratitis treated sequentially with amphotercin-B and clotrimazole suspension

No.	Fungi isolated	Severity at at presentation	Initial therapy	Response on Initial therapy	Maintenance therapy	Response On Maintenance therapy	Total time of therapy (weeks)	Final visual outcome
1	Aspergillus	Severe	Amphotercin- B	favorable	Clotrimazole	favorable	8	Healing, improved V.A.
2	Fusarium	Severe	Amphotercin- B	favorable	Clotrimazole	favorable	10	Healing, unimproved V.A.
3	Aspergillus	Severe	Amphotercin- B	failure	Clotrimazole	failure	12	Perforation, Anterior staphyloma
4	Aspergillus	Non-Severe	Amphotercin- B	favorable	Clotrimazole	favorable	4	Healing, improved V.A
5	Fusarium	Non-severe	Amphotercin- B	favorable	Clotrimazole	favorable	5	Healing, improved V.A.
6	Aspergillus	Severe	Amphotercin- B	favorable	Clotrimazole	favorable	8	Healing, unimproved V.A.
7	Fusarium	Non-severe	Amphotercin- B	favorable	Clotrimazole	favorable	4	Healing, improved V.A.
8	Aspergillus	Non-Severe	Amphotercin- B	favorable	Clotrimazole	favorable	4	Healing, improved V.A.
9	Fusarium	Severe	Amphotercin- B	failure	Clotrimazole	failure	12	Perforation Anterior staphyloma
10	Aspergillus	Severe	Amphotercin- B	favorable	Clotrimazole	favorable	8	Healing, unimproved V.A.
11	Fusarium	Non-severe	Amphotercin- B	favorable	Clotrimazole	favorable	6	Healing, improved V.A.
12	Aspergillus	Non-severe	Amphotercin- B	favorable	Clotrimazole	favorable	4	Healing, improved V.A.
13	Fusarium	Severe	Amphotercin- B	failure	Clotrimazole	failure	10	Perforation Anterior staphyloma
14	Aspergillus	Severe	Amphotercin- B	favorable	Clotrimazole	favorable	7	Healing, improved V.A.
15	Alternaria	Severe	Amphotercin- B	favorable	Clotrimazole	favorable	9	Healing, unimproved V.A.
16	Fusarium	Severe	Amphotercin- B	favorable	Clotrimazole	favorable	10	Healing, unimproved V.A.
17	Fusarium	Severe	Amphotercin- B	failure	Clotrimazole	failure	12	evisceration
18	Aspergillus	Non-severe	Amphotercin- B	favorable	Clotrimazole	favorable	4	Healing ,improved V.A.
19	Aspergillus	Severe	Amphotercin- B	favorable	Clotrimazole	favorable	8	Healing, unimproved V.A.
20	Aspergillus	Non-severe	Amphotercin- B	favorable	Clotrimazole	favorable	4	Healing, improved V.A.
21	Pencilliu m	Severe	Amphotercin- B	favorable	Clotrimazole	favorable	10	Healing, unimproved V.A.
22	Aspergillus	Severe	Amphotercin- B	favorable	Clotrimazole	favorable	8	Healing, unimproved V.A.
23	Curvularia	Non-severe	Amphotercin- B	favorable	Clotrimazole	favorable	4	Healing, improved V.A.
24	Fusarium	Severe	Amphotercin- B	failure	Clotrimazole	favorable	10	Healing, unimproved V.A.

Table 6: Comparison between control study (patients treated with amphotercin-B as monotherapy) and current study (patients treated sequentially with amphotercin-B followed by clotrimazole suspension)

	Control Group Amphotercin-B (monotherapy)	Amphotercin-B and Clotrimazole (Sequential therapy)
Number of patients	22	**24**
Age (mean)	4-65(36 year)	**16-65(39 year)**
Gender: Male/Female	12/10	**13/11**
Severity: Severe/Non-severe	13/9	**15/9**
Fungi isolated :		
Aspergillus sp.	10	**11**
Fusarium sp.	8	**5**
Pencillium sp.	1	**1**
Curvularia sp.	0	**1**
Alternaria sp.	0	**1**
Scopulariopsis sp.	2	**0**
Candida sp.	1	**0**
Response on treatment: (favorable/failure)		
Severe cases	10/3	**11/4**
Non-severe cases	9/0	**9/0**
Mean time of therapy (weeks)		
Severe cases	12	**9.5**
Non-severe cases	4	**4.1**
Final visual outcome		
Severe cases		
Healing with improved V.A.	**0**	**2**
Healing without improved V.A .	**10**	**9**
Staphyloma, evisceration	**3**	**4**
Non-severe cases		
Healing with improved V.A.	**9**	**9**
Healing without improved V.A.	**0**	**0**

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