

# To study the safety and efficacy of topical and intrastromal Amphotericin B in non-responding fungal corneal ulcer patients: A prospective study

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## Abstract

**Purpose:** Corneal ulcer is one of the important ophthalmic condition causing significant morbidity especially in the developing countries. Bacteria and fungi are frequently responsible for suppurative corneal ulcers especially in the developing countries. The present study aimed to know the safety and efficacy of topical and intrastromal injection of Amphotericin B in the management of non-responding fungal corneal ulcer patients.

**Methodology:** It was a hospital based prospective, non-randomized, analytical study. Thirty patients of non-responding fungal corneal ulcer of any age group, of either sex were studied. Cases were studied in terms of clinical examinations, relevant investigations, appropriate treatment and documentation.

**Results:** All 30 patients were given topical Amphotericin B. Out of them, 10 patients (33.33%) responded well and healed. Twenty patients not responded to topical Amphotericin B. Out of them 3 patients did not give consent for intrastromal injection and rest 17 patients were given intrastromal Amphotericin B injection. Post-intrastromal injection, all patients were continued with drops of topical Amphotericin B 0.15%, 1% Atropine eye drop thrice a day, antibiotic eye drop Besifloxacin 0.6% and tablet fluconazole 150mg OD. Out of them 15 patients (88.23%) responded well and healed and remaining 2 were treated with other treatment modalities.

**Conclusion:** Agricultural workers are most commonly affected and vegetative trauma is most common predisposing factor in fungal keratitis. Aspergillus was predominant species in north western Rajasthan. Topical Amphotericin B eye drop was found less effective in management of non-responding fungal corneal ulcer patients, because of poor penetration of the drug topically. Intrastromal injections of Amphotericin B was safe and effective in the treatment of

severe fungal keratitis that was resistant to conventional therapy.

**Keywords:** Topical, intrastromal, amphotericin B, corneal ulcer

## Introduction

Corneal ulcer is one of the important ophthalmic condition causing significant morbidity especially in the developing countries. Scarring of the cornea developed as a result of suppurative corneal ulcer is the second commonest cause of preventable blindness in Asia, Africa and in the Middle East <sup>[1]</sup>. Corneal ulcer can be caused by different microbial agents.

Any organism can invade the corneal stroma, if protective mechanisms of cornea, such as blinking, tear dynamics and epithelial integrity are compromised. Bacteria and fungi are frequently responsible for corneal ulcers especially in the developing countries <sup>[2]</sup>. Breach in the corneal epithelium associated with necrosis and surrounding infiltration, is called corneal ulcer. Fungal keratitis is a suppurative, ulcerative, and sight-threatening infection of the cornea that sometimes leads to loss of the eye. Worldwide and in India the reported incidence of mycotic keratitis is 17%-36% and is 44%-47% respectively <sup>[3-6]</sup>. Trauma is most important predisposing cause, followed by ocular and systemic defects and prolonged use of corticosteroids. History of corneal trauma with vegetable matter or organic matter is reported in 55%–65% of fungal keratitis <sup>[7]</sup>.

Topical corticosteroid therapy has been associated with increased incidence and worsening of fungal keratitis. Additionally, it indirectly promotes fungal replication and corneal invasion by interfering with the host's inflammatory response. The systemic use of corticosteroids may predispose to fungal keratitis by causing immunosuppression. Topical fortified antifungal drops of the commercially available antifungal agents play an important role in the treatment of fungal keratitis <sup>[8]</sup>.

Amphotericin B is one of the broad-spectrum antifungal agents. It is generally effective topically and intrastromally for most cases of fungal keratitis <sup>[9]</sup>. It has an excellent spectrum, being effective against *Candida* species, *Aspergillus* species, *Penicillium marneffei*, *Cryptococcus* species and the causative agents of mucormycosis. It is also effective, to a lesser extent, against the main *Fusarium* species. It has no antibacterial activity <sup>[10]</sup>. Systemic administration of AMB produces little penetration into ocular tissues and does not reach therapeutic levels in the cornea, aqueous or vitreous humor <sup>[11]</sup>. As fungal pathogens tend to deeply penetrate corneal tissues and may reach the anterior chamber. Thus, intrastromal and intracameral injection of antifungal agents, especially polyenes such as Amphotericin B, constitutes the best chance for management of such infections, because these routes result in higher concentrations of the antifungal agents in corneal tissue and aqueous humor than topical use, enhancing the antifungal effects of the drugs <sup>[12, 13]</sup>. Our aim of this study to assess the safety and efficacy of topical and intrastromal Amphotericin B in non-responding fungal corneal ulcer patients.

## Material and Methods

It was a hospital based prospective, non-randomized study conducted in the Department of Ophthalmology, S.P. Medical College, Bikaner. Cases were studied in terms of clinical examinations, relevant investigations, appropriate treatment and documentation. A minimum of 30 patients of any age group, of either sex were included.

## Inclusion criteria

1. Consenting patients with corneal ulcer.
2. Proven presence of fungal organism on smear and/or culture.

Patients who refused for regular follow up, scleral involvement, impending or frank corneal perforation are excluded from our study.

### Methods of evaluation

After obtaining informed consent from the patient, detailed history and ocular examination was carried out. The examination protocol were included:

1. Collection of demographic data.
2. Appropriate history.
3. Complete anterior segment examination with Slit lamp.
4. Visual Activity Testing using Snellen's chart, Jaeger's chart.
5. Posterior segment examination with Direct Ophthalmoscope/indirect ophthalmoscope whenever possible.
6. Microbial cultures.
7. Laboratory investigations such as HIV, HBsAg, VDRL, HCV, Blood sugar, Urine complete and microscopy, Renal function test, Liver function test.
8. Parameters for assessment of ulcer-size, shape, depth, margin, surrounding area, location, size of the infiltrate, height of the hypopyon if present, corneal vascularization and corneal sensation.

**Topical Amphotericin B:** Concentration of 0.15%.

**Method of preparation:** 10 ml distilled water added to 50mg of Amphotericin B injection powder, 3ml of this solution was taken and 7ml of artificial tear was added.

**Stored in refrigerator at 4 °C**

In patients of fungal keratitis, which were not responded to other topical antifungal agents, topical Amphotericin B 0.15% was started 5 times a day with debridement of corneal epithelium for better penetration along with drops of 1% atropine thrice a day, antibiotic eye drop Besifloxacin 0.6% and tablet fluconazole 150mg OD. Follow up was done after 3 and 7 day. If no objectively demonstrable response to therapy was seen in 7 days or if infection shows the signs of worsening, Amphotericin B was injected intrastromally around the fungal abscess. Post intrastromal injection, all patients were continued with drops of topical Amphotericin B 0.15%, 1% Atropine eye drop thrice a day, antibiotic eye drop Besifloxacin 0.6% and tablet fluconazole 150mg OD. Intrastromal Amphotericin B used in concentration of 5-10 microgram/0.1ml. To reconstitute 10microgram/0.1ml, 10ml distilled water was added to 50mg of Amphotericin B injection powder. 0.2ml solution was taken and 0.8ml BSS or sterile water added. Now 0.1 ml of this solution was taken and 0.9ml BSS/sterile water added to create 0.1mg/ml equivalent to 10microgram/0.1ml used immediately.

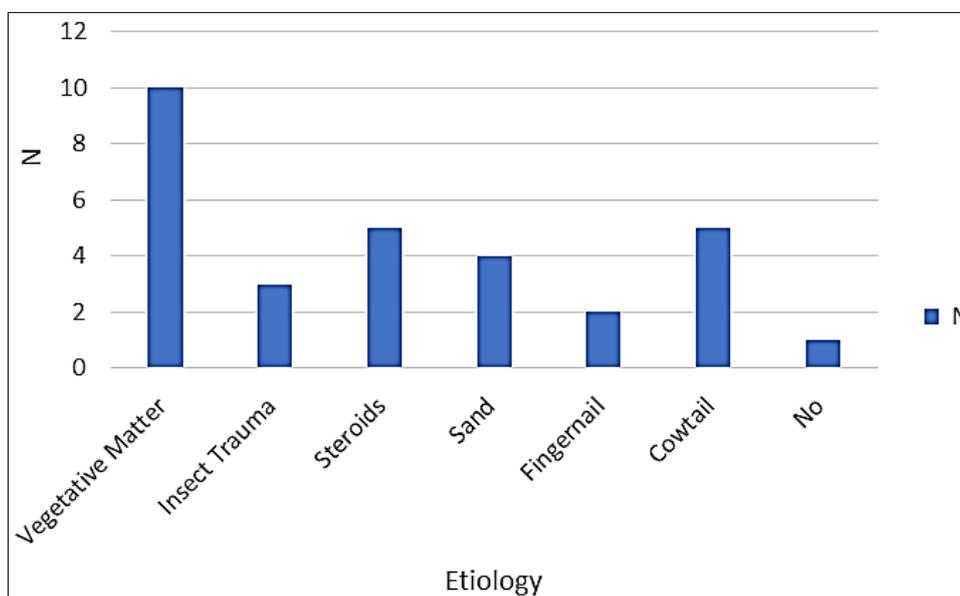
### Result

This study was carried out on 30 patients of non-responding fungal corneal ulcer patients. Patients were examined for clinical profile, safety and efficacy of topical and intrastromal Amphotericin B in management. Age range of study population was 27-71 year with 63.33% patient's being male. Agricultural workers were most commonly affected group both in males (30%) and females (16.66%), followed by labour (20%) in males and house wives (10%) in females. According to size of ulcer, 76.67% of patients were categorised under severe group and 23.33% were under moderate group. We found that depth of ulcer majority of cases were categorised under moderate (66.67%) followed by severe (33.33%).

In most cases corneal injury was an important predisposing factor. Most common cause of injury was vegetative matter (33.33%) followed by cow tail trauma (16.67%), sand trauma (13.33%), insect (10%) and fingernail trauma (6.67%). Other major predisposing factor was chronic use of corticosteroids (16.67%) [Table-1, Graph-1]. We found corneal injury was an important predisposing factor. In table 2, we found Aspergillus (43.33%) was the most common species followed by Candida (23.33%). [Table-2, Graph-2]The size of ulcer varied from 3mm to 8.5mm and the size of infiltrate varied in 0.5 to 3.2mm area around the ulcer. Based on this observation 76.67% of patients were categorised under severe group and 23.33% were under moderate group. In majority of cases (66.67%), the ulcer depth was 1/4 to 2/3 of cornea i.e. moderate followed by severe (33.33%).

**Table 1:** Distribution of patients according to etiology

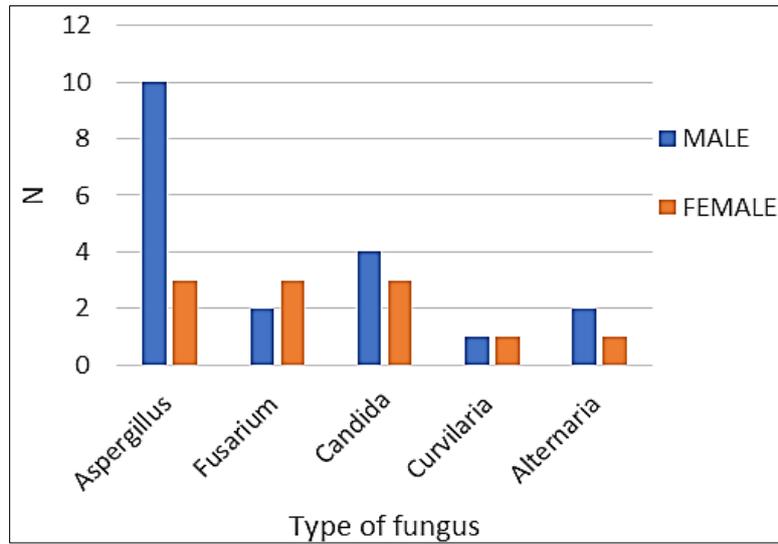
<b>Etiology</b>	<b>N (%)</b>
Vegetative Matter	10 (33.33%)
Insect Trauma	3 (10%)
Steroids	5 (16.67%)
Sand	4 (13.33%)
Fingernail	2 (6.67%)
Cow tail	5 (16.67%)
No	1 (3.33%)
<b>Total</b>	<b>30 (100%)</b>



**Graph 1:** Distribution of patients according to Etiology

**Table 2:** Distribution of Patients with type of Fungus

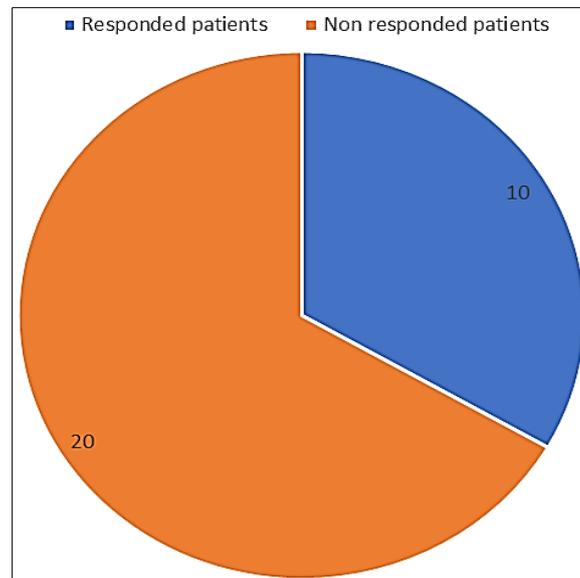
<b>Type of fungus</b>	<b>Male N (%)</b>	<b>Female (N %)</b>	<b>Total</b>
Aspergillus	10(33.33%)	3 (10%)	13(43.33%)
Fusarium	2 (6.67%)	3 (10%)	5 (16.67%)
Candida	4 (13.33%)	3 (10%)	7 (23.33%)
Curvilaria	1 (3.33%)	1 (3.33%)	2 (6.67%)
Alternaria	2 (6.67%)	1 (3.33%)	3 (10%)
<b>Total</b>	<b>19(63.33%)</b>	<b>11 (36.67%)</b>	<b>30 (100%)</b>



**Graph 2:** Distribution of Patients with type of Fungus

**Table 3:** Distribution of patients according to response with topical Amphotericin B (N=30)

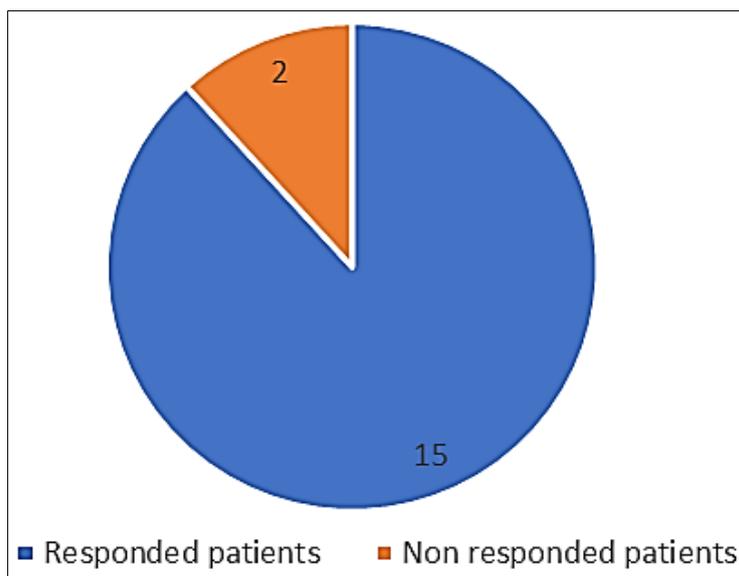
	Responded patients N (%)	Non-responded patients N (%)	Total patients N (%)
Topical Amphotericin B	10 (33.33%)	20 (66.67%)	30 (100%)



**Graph 3:** Distribution of patients according to response with topical Amphotericin B (N=30)

**Table 4:** Distribution of patients according to response with intrastromal amphotericin B (N=17)

	Responded patients N (%)	Non-responded patients N (%)	Total patients N (%)
Intrastromal Amphotericin	15 (88.23%)	2 (13.33%)	17 (100%)



**Graph 4:** Distribution of patients according to response with intrastromal amphotericin B (N=17)

We found Topical Amphotericin B was given to 30 patients, out of them 33.33% patients responded and healed and remaining 66.67% did not show any improvement [table-3, Graph-3]. Intrastromal injection of Amphotericin B was given to 17 patients. Out of them 15 patients (88.23%) responded and 13.33% patients did not show any improvement [Table-4, Graph-4]. Out of 10 patients who responded to topical Amphotericin B, 8 patients presented with hypopyon. Hypopyon disappeared in 1st week in 62.5% patients and second week in rest of cases. 15 patients who responded with intrastromal Amphotericin B, 73.33% cases resolved in 1st week and 26.66% resolved in 2nd week. Mean duration of healing was 5 weeks after treatment with topical Amphotericin B and 6 weeks after intrastromal Amphotericin B injection.

## Discussion

Fungal keratitis is a fungal infection of the cornea that causes ulceration, necrosis and inflammation, usually following vegetative matter trauma or prolong use of steroids. Although mycology has undergone remarkable changes by taking full advantages of spectacular developments in molecular biology, and chemistry to improve the understanding of phenotypic and genotypic characteristics of fungi, fungal keratitis remains a diagnostic and therapeutic challenge to ophthalmologist.

The present study aimed to know the safety and efficacy of topical Amphotericin B and intrastromal injection of Amphotericin B in the management of non-responding fungal corneal ulcer patients. This prospective and single centre study was carried out in the Department of Ophthalmology in S.P. Medical College & Associated group of hospitals, Bikaner.

We included 30 patients in our study. Out of 30 patients, 10 patients responded when treated with topical Amphotericin B. Twenty patients did not respond to topical therapy after 7 days. Out of them 3 patients did not give consent to intrastromal injection and rest 17 patients were given intrastromal Amphotericin B injection. Out of 17 patients, 15 patients responded and were successfully treated with intrastromal Amphotericin B.

Fungi are a part of normal microbial environment but in the absence of a precipitating event, they rarely cause keratitis. Various ocular factors (i.e. include trauma, contact lens wear, medications and prior ocular surgery) may predispose to the occurrence of fungal keratitis. In our study vegetative matter trauma (33.33%) was most common predisposing factor followed

by cow tail trauma (16.67%) and chronic use of corticosteroids (16.67%). M Jayahar Bharathi *et al.* [14] also identified vegetative trauma (61.28%) as the predominant predisposing factor for fungal corneal ulcer. Our study showed that as per gillus species (43.33%) were most common followed by *Candida* (23.33%) and *Fusarium* (16.67%). Jagdish Chander *et al.* [15] reported that *Aspergillus* (41.18%), was the commonest fungal species followed by *Fusarium* species (23.53%).

In our study, Amphotericin B eye drops were started in patients of fungal corneal ulcer, who were nonresponsive to other topical antifungal agents. Out of 30 patients, 10 patients (33.33%) were responded well and healed with topical Amphotericin B. Our results correlated with a study done by SH EL-Sayed [16]. In this study 15 patients who responded with intrastromal Amphotericin B, out of them 40% patients were healed by 5th week, followed by 6th week (26.66%), 7th (20%) and 8th week (13.33%). Our results correlated with a study done by J Hu *et al.* [17], where mean duration of healing was  $39.22 \pm 10.45$  days in patients treated with intrastromal and intra cameral Amphotericin B injection.

In our study there was no evidence of either local or systemic toxic side effects with topical and intrastromal Amphotericin B, like a study done by J Hu *et al.* [17]. Out of 10 patients who responded with topical Amphotericin B, 6 patients (60%) healed with macular corneal opacity followed by leucomatous in 30% and nebular in 10% patients. Out of 15 patients who responded with intrastromal Amphotericin B, 7 patients (46.67%) healed with leucomatous corneal opacity, followed by macular (33.33%) and adherent leucoma (20%).

Our results correlated with a study done by J Hu *et al.* [17], where mostly patients healed with leucomatous corneal opacity. At the time of presentation 70% patients presented with visual acuity between perception of light to  $<1/60$ , which after completion of treatment with topical Amphotericin B mainly improved upto visual acuity  $1/60-6/60$ . Fifteen patients treated with intrastromal Amphotericin B, out of them 86.67% presented with visual acuity between perception of light to  $<1/60$ , after completion of treatment 26.66% patients improved upto visual acuity  $1/60-6/60$  and 20% patients improved to  $6/60-6/18$ .

## Conclusion

Keratomycosis is emerging as a major cause of vision loss and morbidity and can be life-threatening. After close analysis of observations we have come to the following conclusion. *Aspergillus* was predominant species in north western Rajasthan. Topical Amphotericin B eye drop was found less effective in management of non-responding fungal corneal ulcer patients, because of poor penetration of the drug topically. Intrastromal injections of Amphotericin B was safe and effective in the treatment of severe fungal keratitis that was resistant to conventional therapy.

## References

1. B Harathi MJ, Ramakrishnan R, Vasu S, Meenakshi R. Aetiological Diagnosis of Microbial Keratitis In South India. *Indian J Med Microbiol.* 2002;20:19-20.
2. P Rosant Grag MS. Corneal Ulcer Diagnosis and Management. *Community Eye Health.* 1997;12:30.
3. Dunlop AA, Wright ED, Howlader SA, Nazrul I, Husain R, McClellan K, *et al.* Suppurative corneal ulceration in Bangladesh. A study of 142 cases examining the microbiological diagnosis, clinical diagnosis and epidemiological features of bacterial and fungal keratitis. *Aust. NZJ Ophthalmol.* 1994;22:105-110.
4. Polack FM, Kaufman HE, Newmark E. Keratomycosis. Medical and surgical treatment. *Arch Ophthalmol.* 1971;85:410-416.
5. Srinivasan M, Gonzales CA, George C, Cevallos V, Mascarenhas JM, Asokan B, *et al.*

- Epidemiology and aetiological diagnosis of corneal ulceration in Madurai, South India. *Br J Ophthalmol.* 1997;81:965-971.
6. Srinivasan M, Gonzales CA, George C, Cevallos V, Mascarenhas JM, Asokan B, *et al.* Epidemiology and aetiological diagnosis of corneal ulceration in Madurai, South India. *Br J Ophthalmol.* 1997;81:965-971.
  7. Thomas PA. Mycotic keratitis-an underestimated mycosis. *J Med Vet Mycol.* 1994;32:235-256.
  8. Bharathi MJ, Ramakrishnan R, Vasu S, Meenakshi R, Palaniappan R. Aetiological diagnosis of microbial keratitis in South India. *Indian J Med Microbiol.* 2002;20:19-24.
  9. Morand K, Bartoletti AC, Bochot A, Barratt G, Brandely ML, Chast F. Liposomal amphotericin B eye drops to treat fungal keratitis: physico chemical and formulation stability. *Int J Pharm* 2007;344(1-2):150-153.
  10. Qu L, Li L, Xie H. Corneal and aqueous humor concentrations of amphotericin B using three different routes of administration in a rabbit model. *Ophthalmic Res.* 2010;43(3):153-158.
  11. Shirley SF, Little JR. Immunopotentiating effects of amphotericin B. I. Enhanced contact sensitivity in mice. *J Immunol.* 1979;123(6):2878-82.
  12. Bennett J. Agentes antimicrobianos, agentes antifúngicos. In: Brunton L, editor. *Goodman & Gilman: as bases farmacológicas da terapêutica.* 11a ed. Rio de Janeiro: McGraw-Hill, c2007, 1103-17.
  13. Qu L, Li L, Xie H. Corneal and aqueous humor concentrations of amphotericin B using three different routes of administration in a rabbit model. *Ophthalmic Res.* 2010;43(3):153-158.
  14. Garcia-Valenzuela E, Song CD. Intracorneal injection of amphotericin B for recurrent fungal keratitis and endophthalmitis. *Arch Ophthalmol.* 2005;123(12):1721-1723.
  15. M. Jayahar Bharathi, R. Ramakrishnan, Samala Vasu, R. Meenakshi, R. Palaniappan. Epidemiological characteristics and laboratory diagnosis of fungal keratitis. A three-year study. *Indian J Ophthalmol,* 2003;51:315-321
  16. Chander J, Singla N, Agnihotri N, Arya SK, Deep A. Keratomycosis in and around Chandigarh: A five-year study from a north Indian tertiary care hospital. *Indian J Pathol Microbiol* 2008;51:304-6.
  17. Saber H El-Sayed, Fareed M Wagdi, Ahmed A El-Hagaa, Eman F Mottawea. Topical amphotericin B versus subconjunctival fluconazole injection in the management of fungal keratitis. DOI: 10.4103/1110 2098.198726.
  18. Jianzhang Hu, Jiangjin Zhang, Yanling Li, Xmoli Han, Weidong Zheng, Juan Yang, Guoxing Xu. A Combination of Intrastromal and Intracameral Injections of Amphotericin B in the Treatment of Severe Fungal Keratitis. *J Ophthalmol.,* 2016, 343-6415.