Original Article

Impression Cytologic Evaluation of the Conjunctiva in Patients Treated with Topical 1% Voriconazole

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Abstract

Objectives: The aim of the present study was to evaluate any conjunctival metaplastic changes by impression cytology in patients who underwent topical 1% voriconazole treatment for severe fungal keratitis.

Materials and Methods: The study was conducted at Ege University Faculty of Medicine, Departments of Ophthalmology and Medical Pathology. Patients who were treated with 1% topical voriconazole for fungal keratitis for at least 3 months were included. The used topical voriconazole treatment was initiated as one drop every hour and was tapered according to clinical improvement in all patients. Treatment was continued 4 times a day for at least 3 months. Impression cytology samples were collected at least 3 months after cessation of topical voriconazole from the affected eyes and from the fellow eyes as a control group. Collected specimens were transferred to the pathology department for evaluation and grading (Nelson's grading system).

Results: The mean age of the patients was 57.68±17.32 years (range, 22-87 years). The impression cytology grade of the inferior bulbar conjunctiva was 1.73±0.77 (range, 0-3) in the study group and 1.19±0.98 (range, 0-3) in the control group (p=0.03). The impression cytology grade of the temporal bulbar conjunctiva was 1.69±0.73 (range, 0-3) in the study group and 1.15±0.88 (range, 0-3) in the control group (p=0.02). The impression cytology grades of the nasal and superior bulbar conjunctiva did not differ statistically (p values 0.13 and 0.17, respectively).

Conclusion: Topical voriconazole is an effective broad-spectrum antifungal drug, but it induces conjunctival squamous metaplasia. Clinicians should be aware of this possible side effect of topical voriconazole and should carefully evaluate the conjunctiva of treated patients at each visit to detect possible metaplastic changes.

Keywords: Fungal keratitis, voriconazole, impression cytology, metaplasia

Introduction

Voriconazole is a broad-spectrum antifungal agent which is derived from fluconazole to improve its potency and spectrum. The mechanism of the drug is to inhibit the cytochrome P450 enzyme lanosterol 14α-demethylase. Inhibition of this enzyme prevents ergosterol synthesis and results in the accumulation of toxic sterols in the cell, leading to membrane disruption. Voriconazole was approved for the treatment of invasive aspergillosis, invasive *Candida* infections, and other fungal infections including *Scedosporium apiospermum* and *Fusarium* spp. It can be used for the treatment of patients who are intolerant or refractory to other treatment options as well. There are also reports of its off-label use for the treatment of histoplasmosis and coccidioidomycosis and for prophylaxis against *Aspergillus* spp. and fungal infections in patients with hematopoietic cell transplant, solid organ transplant, febrile neutropenia, and HIV.[1,2,3,4]

Voriconazole has high oral bioavailability and is available as oral suspensions, tablets, and an intravenous formulation. Almost 98% of voriconazole is metabolized in the liver, primarily by cytochrome P450 enzymes. Its primary metabolite is voriconazole N-oxide (VNO), which accounts for 72% of voriconazole metabolites in the plasma. VNO is not an antifungal metabolite, but it may be responsible for some adverse effects of the drug, such as skin reactions.[5,6]

Voriconazole is effective in the treatment of invasive aspergillosis and refractory fungal infections with other species.

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In ophthalmology practice, it can be used via topical, intrastromal, intracameral, or intravitreal routes for fungal infections. Topical voriconazole reaches therapeutic concentration in the aqueous humor in 24 minutes. However, in some recent articles it was reported to cause conjunctival squamous metaplasia.[6,7,8]

Conjunctival impression cytology is a relatively simple, practical, and non-invasive or minimally invasive technique that allows the collection of one to three layers of cells from the bulbar conjunctival surface. This technique is rapid, convenient, and widely performed to confirm a variety of ocular surface diseases and to monitor changes in the ocular surface.[9,10]

The aim of the present study was to use impression cytology to evaluate any conjunctival metaplastic changes in patients who underwent voriconazole treatment for severe culture-proven fungal keratitis for at least 3 months.

Materials and Methods

The study was conducted at the Ege University Faculty of Medicine, Departments of Ophthalmology and Pathology. It was approved by the Ege University Ethic Committee of the hospital and followed the Declaration of Helsinki ethical principles for medical research involving human subjects (decision no: 22-12T/45, date: 01.12.2022). The study was funded by the Ege University Scientific Research Project Foundation (project number: 18-TIP-005). Written informed consent was obtained from all participants.

Patients who were treated with 1% topical voriconazole for fungal keratitis for at least 3 months were included. Topical voriconazole treatment was initiated as one drop every hour and was tapered according to clinical improvement in all patients. The treatment was maintained as one drop 4 times a day for at least 3 months and maximum 8 months. Patients with any previously known ocular diseases including keratoconjunctivitis sicca, Stevens-Johnson syndrome, ocular cicatricial pemphigoid, pterygium, pinguecula, and glaucoma were excluded from the study. Also, patients who wore contact lenses or were taking any type of ocular medications were excluded. Samples for impression cytology were collected at least 3 months after cessation of topical voriconazole from the affected eye (study group) and from the contralateral eye (control group).

All eyes underwent a routine ophthalmological examination including best corrected visual acuity, intraocular pressure measurement (Tonopen AVIA, Reichert Technologies, Depew, NY), and anterior and posterior segment evaluations.

Impression cytology was performed at least 2 hours after ophthalmologic evaluation in order to prevent any interference. Cellulose acetate filter paper trimmed into four equal pieces was used to collect samples. After instilling one drop of local anesthetic into the inferior fornix, the impression cytology papers were applied to the superior, inferior, nasal, and temporal bulbar quadrants of the conjunctiva for approximately 5-10 seconds while the patient was looking in the opposite direction. Afterwards, the cellulose acetate filter paper was transferred to the fixative medium containing acetic acid, formaldehyde, and 70% ethyl alcohol in a 1:1:20 volume ratio. After staining with periodic acid-Schiff, the samples were examined under a light microscope. Conjunctival specimens were graded according to Nelson's[11] grading system (Table 1).

Statistical Analysis

Statistical analyses were performed using SPSS software (version 20; IBM Corp., Armonk, NY) and a significance level of 5% (95% confidence interval) was accepted. A p value 0.05 was accepted as statistically significant. Results were given as mean ± standard deviation. Data were compared with t-test for parametric variables and Mann-Whitney U test for non-parametric variables.

Results

After obtaining ethical approval, 26 patients with culture-proven keratitis were included in the study. The median visual acuity of the patients was 1/10 (range, hand motion to 6/10) with Snellen chart at the time of sampling. The median intraocular pressure was 11 mmHg (range, 4-26 mmHg). The mean age of the patients was 57.68±17.32 years (range, 22-87 years). Seventeen patients were male (65%) and 9 patients were female (35%). After scraping the cornea for culture, topical voriconazole treatment was started and continued at least 3 months. The maximum duration, which was not limited at the beginning of the study, was 8 months. The mean duration of topical voriconazole use was 124.57±34.12 days (93-198 days). The mean impression cytology grade of the inferior bulbar conjunctiva was 1.73±0.77 (range, 0-3) in the study eyes and 1.19±0.98 (range, 0-3) in the control eyes (p=0.03). The impression cytology grade of the temporal bulbar conjunctiva was 1.69±073 (range, 0-3) in the study eyes and 1.15±0.88

Table 1. Nelson classification of squamous dysplasia

	Grade 0	Grade 1	Grade 2	Grade 3
Cell size	Small	Small	Large	Large
Nucleus	Large	Small	Small	Pyknotic
Cytoplasm	Eosinophilic	Eosinophilic	Variable	Basophilic
Nucleus/cytoplasm	1:2	1:3	1:4-1:5	1:6
Goblet cell	>500	350-500	100-350	<100
Goblet cell cytoplasm (periodic acid-Schiff staining)	+++	+++	++	-

+++: High, ++: Moderate, -: None

(range, 0-3) in the control eyes (p=0.02). The impression cytology grades in the nasal and superior bulbar conjunctiva did not show statistically significant differences between the groups (p values 0.13 and 0.172, respectively) (Table 2). There was no relationship between duration of drug use and grade (p=0.11).

Discussion

This study evaluated the relationship between topical 1% voriconazole use and conjunctival metaplastic changes as demonstrated with impression cytology in patients with severe fungal keratitis. To the best of our knowledge, this the first study to demonstrate these metaplastic changes by impression cytology after topical voriconazole use.

Voriconazole is a broad-spectrum antifungal agent and is effective against fungi that are resistant to other antifungal drugs. It is commercially available in oral and intravenous forms. It is also effective in refractory fungal keratitis when applied topically, which can be prepared by diluting intravenous 200 mg voriconazole lyophilized powder to a concentration of 1% as described previously.[12] Vemulakonda et al.[13] stated that topically administered voriconazole reached the minimum concentration to inhibit 90% of isolates for pathogens in the aqueous and vitreous. Edwar et al.[14] investigated topical 1% voriconazole combined with single-dose intrastromal 0.05% voriconazole versus topical 5% natamycin monotherapy in an experimental *Fusarium* keratitis model in rabbits. They concluded that topical 1% voriconazole combined with single-dose intrastromal 0.05% voriconazole was as effective as topical 5% natamycin monotherapy for the treatment of *Fusarium* keratitis. The efficacy of the topical voriconazole was also determined in other studies.[12,15]

Voriconazole has adverse effects including visual disturbances, skin rashes, photosensitivity, and squamous cell neoplasia.[16,17,18] VNO absorbs ultraviolet (UV)-A and -B radiation, causing skin photosensitivity and resulting in severe sunburns. Skin squamous cell neoplasia may arise from these sunburned areas. In 2015, Palamar et al.[7] reported ocular surface neoplastic changes demonstrated with confocal microscopy in a patient who received topical 1% voriconazole treatment for 4 months. This was the first report in the literature regarding a possible effect of topical voriconazole to induce conjunctival squamous cell neoplasia, which was subsequently investigated in two animal studies. Arikan et al.[19] observed histological changes in rabbits with topical 1% voriconazole application. Degirmenci et al.[20] also detected immunohistochemical changes in rats with 2% voriconazole eye drops. The most striking finding from the latter study was that rats exposed to sunlight had more prominent conjunctival metaplastic changes, demonstrating the additive effect of sunlight exposure.

Table 2. The impression cytology grades of the patients (n=26)

	Treated eyes Mean ± SD (range)	Fellow eyes Mean ± SD (range)	p value
Inferior	1.73±0.77 (0-3)	1.19±0.98 (0-3)	0.03
Temporal	1.69±073 (0-3)	1.15±0.88 (0-3)	0.02
Nasal	1.26±0.87 (0-3)	0.96±0.72 (0-2)	0.13
Superior	1.19±0.63 (0-2)	0.92±0.62 (0-2)	0.17

SD: Standard deviation

In the current study, the inferior and temporal quadrants of the bulbar conjunctiva were the areas most affected according to impression cytology findings. The temporal bulbar conjunctiva was probably the quadrant most exposed to direct sunlight, which enhanced metaplastic changes. Although the nasal bulbar conjunctiva also is exposed to sunlight, it is more likely to be indirect reflection from the nose.[21] The superior bulbar conjunctiva is covered by the upper eyelid and thus not exposed to enough sunlight to induce metaplastic changes. As mentioned earlier, VNO absorbs UV-A and UV-B but does not emit UV-B. This leads to accumulation of free oxygen radicals and causes oxidative stress.[22,23] Waste products of the ocular surface are eliminated after accumulating in the inferior fornix. Kojima et al.[24] detected conjunctival epithelial alterations from normal epithelium to metaplastic epithelia by increased oxidative stress. The current study revealed that the most affected area was the inferior bulbar conjunctiva, which has the most interaction with the tear film and its contents. Although the inferior bulbar conjunctiva is not exposed to direct sunlight, all waste products accumulate in the inferior fornix and are in contact with inferior bulbar conjunctiva. Moreover, the inferior conjunctiva has the most exposure to topical voriconazole. These factors may explain the greater conjunctival metaplastic changes in the inferior conjunctival quadrant.

Study Limitations

The main limitation of the study is the small sample size. Moreover, as these were resistant keratitis cases, the possibility of multiple topical agent use affecting the impression cytology results cannot be ruled out. It would be better to set up a prospective study to exclude the possible effects of multidrug use.

Conclusion

Although voriconazole is an indispensable agent in fungal infections including fungal keratitis, it may trigger conjunctival metaplastic changes that can lead to conjunctival squamous cell carcinoma. Clinicians should be aware of this serious adverse effect and be cautious even in topical use. Further studies correlating this side effect with the duration of the topical voriconazole use are needed.

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Ethics

Ethics Committee Approval: It was approved by the Ege University Ethic Committee of the hospital and followed the Declaration of Helsinki ethical principles for medical research involving human subjects (decision no: 22-12T/45, date: 01.12.2022).

Informed Consent: Obtained.

Authorship Contributions

Surgical and Medical Practices: C.D., Concept: C.D., M.P., Ö.B.S., Design: M.P., Ö.B.S., Data Collection or Processing: C.D., Z.E., Analysis or Interpretation: M.P., A.V., A.Y., Literature Search: C.D., Z.E., Writing: C.D., M.P.

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