Efficacy of Systemic Vitamin C Supplementation in Reducing Corneal Opacity Resulting from Infectious Keratitis

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Abstract: The objective of this study was to determine the effect of vitamin C supplementation on reducing the size of corneal opacity resulting from infectious keratitis.

The study included 82 patients (82 affected eyes), admitted for infectious keratitis from January 2009 to August 2013, who were followed for more than 3 months. Patients were divided into control, oral vitamin C (3 g/d), and intravenous vitamin C (20 g/d) groups during hospitalization. Corneal opacity sizes were measured using anterior segment photographs and Image J program (version 1.27; National Institutes of Health, Jinju, South Korea) at admission, discharge, and final follow-up. The corneal opacity size used for analysis was the measured opacity size divided by the size of the whole cornea.

The corneal opacity size decreased by 0.03 ± 0.10 in the oral vitamin C group, 0.07 ± 0.22 in the intravenous vitamin C group, and 0.02 ± 0.15 in the control group. Intravenous vitamin C reduced the corneal opacity size more than oral vitamin C ($P = 0.043$). Intravenous vitamin C produced greater reduction in corneal opacity size in younger patients ($P = 0.015$) and those with a hypopyon ($P = 0.036$).

Systemic vitamin C supplementation reduced the size of corneal opacity resulting from infectious keratitis. Intravenous vitamin C was more beneficial than oral supplementation, especially in younger patients and those with hypopyon.

(*Medicine* 93(23):e125)

INTRODUCTION

Infectious keratitis remains a sight-threatening disease despite the development of potent new antibiotic agents and diagnostic techniques. Even with intensive antibiotic treatment, corneal damage can occur as a result of inflammatory processes caused by infection or scarring related to the healing process.[1,2] The scarring that accompanies the resolution of infectious keratitis leaves many eyes visually impaired or blind.[3] Thus, it is logical to employ strategies to reduce or prevent scar formation. Topical corticosteroids may seem like an obvious choice for this purpose, but their use is controversial. Some investigators advocate using topical corticosteroids along with antibiotics to reduce immune-mediated tissue damage and scarring. The Steroids for Corneal Ulcers Trial (SCUT) investigated the safety and efficacy of corticosteroids in the treatment of bacterial corneal ulcers.[4–6] In SCUT, corneal scarring resulting from bacterial keratitis was noted to improve over time with corticosteroid treatment; the density of the scar-related opacity improved, leading to a concurrent improvement in vision.[7] However, corticosteroids may also significantly slow the process of corneal wound healing, prolong infection, and predispose to stromal thinning and perforation.[6–8] Therefore, treatments to reduce corneal scar formation without adverse effects, such as delayed re-epithelialization or perforation, are sought.

Corneal wound healing is a complex process, involving cellular changes and signaling molecules from cells of every layer of the cornea. Furthermore, a major component of the corneal haze in wound healing is because of changes in the composition and configuration of the extracellular matrix (ECM) and corneal neovascularization.[9,10] Experimentally, vitamin C has been shown to play a role in synthesizing parallel arrays of ECM fibrils in cultured human keratocytes.[11] Ascorbic acid is also known to be involved in the suppression of corneal neovascularization via its antioxidant effects and ability to enhance collagen synthesis.[12] Therefore, we speculated that the administration of vitamin C may help to reduce corneal opacity caused by infectious keratitis.

The therapeutic effects of vitamin C were explored by Linus Pauling, who was the first to introduce the concept of high doses of vitamin C for the treatment of various conditions, from common cold to cancer.[13] Since then, high doses of vitamin C have been widely used in the treatment and prevention of diabetes, atherosclerosis, common cold, cataracts, glaucoma, macular degeneration, stroke, heart disease, cancer, and other conditions.[14–17] The purpose of the present study was to determine whether oral (3 g/d) or intravenous (20 g/d) vitamin C supplementation during hospital admission reduces corneal opacity resulting from infectious keratitis.

MATERIALS AND METHODS

Subjects

This study retrospectively reviewed the medical records of patients who were hospitalized from January 2008 to August 2013 for the treatment of infectious keratitis and followed for >3 months. All enrolled participants provided informed consent for this study. The review of patient charts providing data for this study was approved by the Institutional Review Board of the Gyeongsang National University Hospital (2012-09-003-001). Patients were excluded if they were pregnant or lactating; had a chronic systemic disease such as liver disease, renal disease, uncontrolled diabetes mellitus, or hypertension; had a history of renal calculi or gout; or underwent surgery during the current admission, including creation of a conjunctival flap, amniotic membrane transplantation, or keratoplasty (because of the high risk of impending perforation).

The participants were separated into 3 comparative groups, based on their vitamin C treatment. Group 1 received no vitamin C, group 2 received oral vitamin C (3 g/d), and group 3 received intravenous vitamin C (20 g/d).

Laboratory examinations included a complete blood count, erythrocyte sedimentation rate, C-reactive protein, electrolytes, blood urea nitrogen, creatinine, glucose, liver function tests, arterial blood gas analysis, urinalysis with microscopy, hepatitis B surface antigen, hepatitis B surface antibody, Venereal Disease Research Laboratory test, ABO typing, antibody screening test, prothrombin time, activated prothrombin time, human immunodeficiency virus antigen/antibody, hepatitis C surface antibody, pulmonary function tests, chest x-ray, and electrocardiography. These tests were repeated every 4 days to detect any complications that may occur after vitamin C supplementation.

Main Outcome Measurements

Clinical data were collected at admission, discharge, and last follow-up visit. These data included age, sex, previous medical history including visual acuity, previous ocular history, intraocular pressure, location of corneal ulcer, and culture results from corneal specimens. Best corrected visual acuity was converted into logarithm of the minimal angle of resolution format (counting finger, 2.3; hand movements, 2.6; light perception, 3.0; no light perception 3.6). The size of the corneal lesion was measured using anterior segment photographs; all photographs were taken at the same magnification. The size of the corneal opacity was determined by point to point using the Image J program (version 1.27; National Institutes of Health). The opacity size was divided by the size of the whole cornea to facilitate comparisons (Figure 1).

Vitamin C Supplementation

Participants were classified into control, oral vitamin C treatment, and intravenous vitamin C treatment groups. The latter 2 groups were administered either oral vitamin C (3 g/d) or intravenous vitamin C (20 g/d) throughout their hospital admission. For oral administration, patients were given 1 vitamin C 1000 mg tab (Korea Eundan Co, Ltd, Seoul, South Korea), containing ascorbic acid 1000 mg, 3 times/d. For intravenous administration, patients were given 1 ampoule of Merit C (Huons Co, Ltd, Seoul, South Korea), containing ascorbic acid 10 g/20 mL, 2 times/d. One ampoule of Merit C was added to 200 mL 5% dextrose in water for administration.

Treatment of Infectious Keratitis

On admission, culture specimens were obtained by scraping the base and margins of the corneal ulcer. These specimens were placed on slides for Gram staining and potassium hydroxide (KOH) staining, according to standard protocols. Specimen samples were also inoculated onto blood, chocolate, MacConkey, and Sabouraud agar plate surfaces for culture. Bacterial and fungal isolates were identified using standard laboratory techniques.

All patients were treated with a standard antibiotic regimen immediately after admission and prior to the culture results. This consisted of hourly instillation of topical 0.5% moxifloxacin (Vigamox; Alcon, Fort Worth, TX). If a fungal infection was identified during KOH staining, an antifungal course was initiated, consisting of hourly application of topical 0.2% fluconazole, 0.15% amphotericin B, and 5% natamycin, with oral fluconazole. We changed the therapy as needed, based on the clinical response for each patient. When patients showed evidence of impending perforation, we performed conjunctival flap surgery, amniotic membrane transplantation, or keratoplasty and excluded the patients from the study.

Statistical Analysis

All statistical analyses were performed using SPSS for Windows version 15.0 (SPSS Inc, Chicago, IL). Demographic characteristics and clinical factors that may influence corneal ulcer and corneal lesion size were compared using Levene Test for Equality of Variances. Changes in lesion size according to these factors were compared between the intravenous and oral vitamin C treatment groups using repeated measure analysis of variance. P values <0.05 were considered statistically significant.

RESULTS

Eighty two patients were enrolled in this study. Infectious keratitis was present unilaterally in all patients. The patients included 48 men and 34 women; their mean age was 64 ± 17 years. There were no statistically significant differences in baseline characteristics, such as the age, sex, visual acuity, intraocular pressure, previous ocular history, or duration of admission, between the 3 groups (Table 1). Of the 82 patients, 34 (44%) had positive results from culture study of the corneal specimens (Table 2). Positive corneal culture results were noted in 8 (40%) patients in the control group, 15 (41%) patients in the oral vitamin C group, and 11 (44%) patients in the intravenous vitamin C group. Of the risk factors that may affect corneal opacity size, the presence of hypopyon, location of ulcer, initial size of corneal infiltration, clinically suspected pathogen (bacteria, fungus, or other), and pathogen documented by positive corneal culture results were not statistically different between the 3 groups (Table 3). Furthermore, these risk factors were also not statistically different between the oral vitamin C and intravenous vitamin C groups (Table 4). The size of corneal opacity measured using anterior segment photographs at admission, discharge, and the end of the follow-up decreased by 0.03 ± 0.10 after oral vitamin C treatment, 0.07 ± 0.22 after intravenous vitamin C treatment, and 0.02 ± 0.15 in the control group (Figure 2). The decrease in corneal opacity size was significantly greater in the oral and intravenous vitamin C treatment groups than in the control group ($P = 0.043$ and 0.038, respectively; Figure 2). The decrease in corneal opacity size was also significantly greater in the
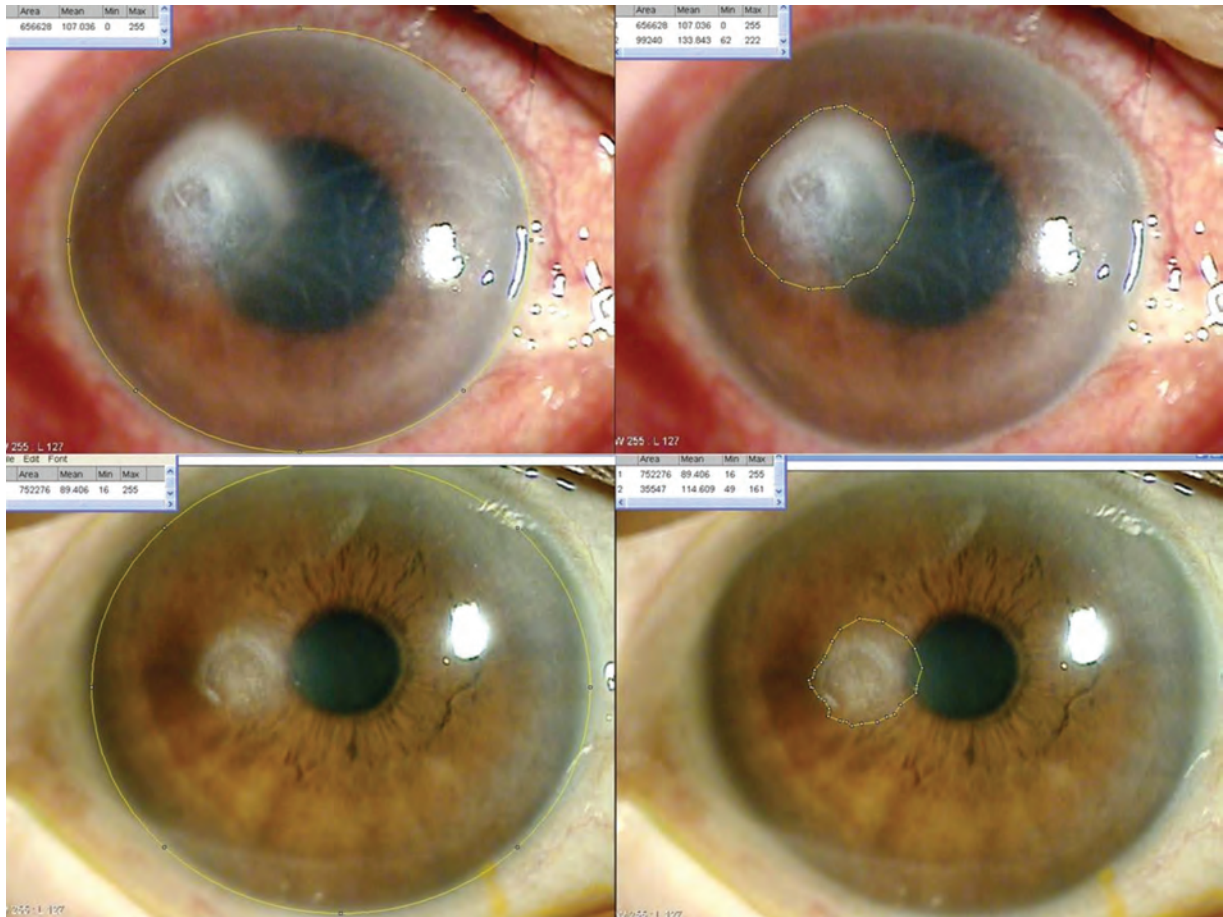
FIGURE 1. Measurement of corneal opacity size with Image J program (National Institutes of Health) (point-to-point check). Representative photographs of the anterior segment in a patient with a corneal ulcer at the initial assessment (top right, top left) and hospital discharge (bottom right, bottom left). Anterior segment photographs were obtained using a slit lamp with the eye in the primary position. The size of the whole cornea (top right, bottom right) and the corneal opacity (top left, bottom left) was determined using the Image J program with point-to-point check (yellow line).

intravenous vitamin C treatment group than in the oral vitamin C group ($P = 0.026$, Figure 2). From admission to discharge, the size of the corneal epithelial defect decreased by 0.04 in the control group, 0.05 in the oral vitamin C group, and 0.10 in the intravenous vitamin C group (Figure 3). The differences between the control and oral vitamin C groups and the control and intravenous vitamin C groups were statistically significant ($P = 0.045$ and 0.044, respectively) (Figure 3). The change in epithelial defect size was also significantly different between the oral and intravenous vitamin C groups ($P = 0.026$) (Figure 3).

Table 5 presents the results of univariate analysis of clinical risk factors that may affect the size of the corneal opacity for the 2 vitamin C groups. Comparing the intravenous vitamin C group with the oral vitamin C group demonstrated a significant reduction in corneal opacity size for infectious keratitis accompanied by hypopyon ($P = 0.036$) and patients <60 years ($P = 0.015$). Other clinical factors, such as age, previous ocular history, sex, duration of hospitalization, and pathogen (bacteria vs fungi), did not affect the decrease in corneal opacity size after vitamin C treatment.

DISCUSSION

The main finding of the current study is that systemic supplementation of vitamin C (oral or intravenous) effectively decreased the size of corneal opacities resulting from infectious keratitis. Furthermore, intravenous vitamin C was more effective than oral vitamin C in decreasing the corneal opacity size. Also, the reduction in corneal opacity size because of vitamin C treatment was greater when hypopyon was present and the patient was <60 years.

Corneal wound healing is a complex process of sequential synthesis and secretion of ECM components. For corneal injury because of infectious keratitis, the corneal epithelium initially heals by 3 sequential processes: migration, mitosis, and differentiation.[18] If something goes awry in these healing processes, injured epithelial cells secrete specific growth factors or cytokines, such as tumor necrosis factor-α (TNF-α) and transforming growth factor-β1 (TGF-β1), depending on the degree of damage.[18–20] These substances can affect adjacent epithelial or stromal cells. All layers of the cornea must heal to maintain original corneal transparency, but the layer that is most crucial to the development of corneal haze in response to injury is the corneal stroma.[9] Once the corneal

TABLE 1. Baseline Subject Characteristics

	Control	Oral Vitamin C	Intravenous Vitamin C	*P* Value*	Total
Number of patients	20	37	25		82
Age, y (mean ± SD)	62.3 ± 12	62 ± 17	62.5 ± 18.6	0.531	64 ± 17
Sex (men/women)	10/10	23/14	15/10	0.743	48/34
BCVA** (mean ± SD)	1.39 ± 0.15	1.22 ± 0.14	1.09 ± 0.17	0.920	3.09 ± 1.32
IOP (mean ± SD)	14.30 ± 3.58	13.6 ± 4.28	15.25 ± 3.61	0.089	14.93 ± 3.82
Previous ocular disease, n	2	3	3	0.743	8
Prior ocular surgery	4	7	3	0.510	14
Duration of admission, d (mean ± SD)	8.53 ± 4.27	8.86 ± 4.54	8.36 ± 4.59	0.780	8.66 ± 4.53
Causative material					
Vegetative matter	6	6	9	0.074	21
Soil/sand/stone	4	6	4	0.982	14
Miscellaneous	10	25	12	0.123	47

BCVA = best, IOP = intraocular pressure, SD = standard deviation.
*Levene test for equality of variances.
**Logarithm of the minimal angle of resolution (counting fingers, 2.3; hand movements, 2.6; light perception, 3.0; no light perception, 3.6).

stroma is injured, keratocytes adjacent to the wound are activated and undergo myofibroblast transformation to repair the damaged stroma; contraction of the myofibroblasts results in an irregular contour of the healing scar, which may contribute to corneal haze.[21] Moreover, several matrix metalloproteinases (MMPs) function in corneal wound healing and contribute to the initial tissue destruction after an injury.[19] It is widely believed that the corneal opacity resulting from infectious keratitis is somewhat more serious than the postoperative corneal opacity after photorefractive keratectomy and Laser in situ keratomileusis surgery.[22,23]

Many investigations evaluating strategies to decrease corneal scar formation after treatment of infectious keratitis are currently in progress; SCUT is representative of these efforts.[4–7,24,25] According to recent reports, the primary outcome of this trial revealed no benefit of adjunctive corticosteroids at 3 months after enrollment. Despite a possible delay in epithelial healing, no harm was observed with the use of corticosteroids.[6] In the SCUT 12-month clinical trial, adjunctive topical corticosteroid therapy was shown to be associated with further improvement in long-term clinical outcomes in 5 patients with bacterial corneal ulcers not caused by *Nocardia* species.[7] However, the use of topical corticosteroids in the treatment of bacterial keratitis continues to be controversial because of concerns about the potential for corticosteroids to exacerbate or prolong infection and/or delay corneal epithelial healing.[6] Many investigators and clinicians have focused on treatments to decrease corneal opacity without these potential adverse effects. In the current study, our results suggest that vitamin C is a powerful candidate for reducing corneal opacity.

The direct mechanism whereby vitamin C reduces corneal opacity after infectious keratitis has not been established. To the best of our knowledge, this is the first report showing that systemic supplementation of vitamin C (oral or intravenous) effectively reduces the size of corneal opacity resulting from infectious keratitis. However, several reports have elucidated mechanisms by which vitamin C may affect corneal wound healing. First, vitamin C has been shown to accelerate the proliferation of corneal epithelial cells and the healing of epithelial defects.[26] Impaired corneal epithelial cells around an infected wound release various inflammatory cytokines, such as TNF-α and TGF-β1, and these cytokines can worsen tissue necrosis.[19,20] In our study, when the size of epithelial defects was compared between hospital admission and discharge, the differences between the control and oral vitamin C groups and the control and intravenous vitamin C groups were significant. Moreover, the change in epithelial defect size was significantly

TABLE 2. Organisms Isolated from Corneal Cultures

	Control, n	Oral Vitamin C, n	Intravenous Vitamin C, n
Bacteria			
Gram-positive bacteria			
Staphylococcus epidermidis	2	2	
Staphylococcus aureus	1		3
Other			2
Gram-negative bacteria		3	3
Fungus			
Fusarium species		5	
Unidentified filamentous fungus	3	3	2
Candida albicans		1	
Other	2	1	1
Total number of isolates	8	15	11

TABLE 3. Factors Associated With Corneal Opacity Size in All Groups

	Control	Oral Vitamin C	Intravenous Vitamin C	*P* Value*
Presence of hypopyon	4	10	5	0.063
Location of ulcer				0.089
Central	15	25	20	
Paracentral	3	9	3	
Peripheral	2	3	2	
Size of corneal opacity** at initial visit (mean ± SD)	0.25 ± 0.11	0.23 ± 0.13	0.28 ± 0.12	0.095
Clinically suspected pathogen				0.650
Bacteria	7	10	8	
Fungus	10	12	13	
Other	3	15	4	
Positive result on corneal culture	8	15	11	0.823

SD = standard deviation.
*Levene test for equality of variances.
**Measured opacity size divided by the size of the whole cornea.

different between the oral vitamin C and intravenous vitamin C groups. One previous report described the protective effect of topically administered vitamin C on rabbit corneas after excimer laser keratectomy,[27] and another report on rabbits noted that topical ascorbate significantly reduced the incidence of corneal ulceration and perforation after an alkali burn.[28] Increasing the vitamin C concentration in the anterior chamber by systemic supplementation is expected to increase the vitamin C concentration in the corneal epithelium,[29] thereby, promoting acceleration of corneal epithelial healing and reduction of corneal opacity. Another mechanism whereby vitamin C may reduce corneal opacity size is by its effects on collagen synthesis. Vitamin C is an important modulator of collagen production, acting as a cofactor for the hydroxylation of proline and lysine residues in procollagen. It enhances the production of several types of collagen in cultured rabbit keratocytes. Vitamin C is

also known to suppress angiogenic factors, including vascular endothelial growth factor and MMPs, thereby inhibiting corneal neovascularization.[30–33] Consequently, vitamin C may act as a cofactor of ECM synthesis and help damaged corneal stromal tissues recover in a normal manner. Finally, vitamin C exerts antioxidant and protective effects on the inflammatory response of the eye by scavenging reactive oxygen radicals and metabolites, such as myeloperoxidase, that are released by infiltrating inflammatory cells.[34–36] Because vitamin C neutralizes oxygen-free radicals, it is expected to inhibit damage to neighboring epithelial and stromal tissues around a wound.[37] Thus, vitamin C has a protective effect on both layers of the cornea and a preventive effect on the formation of corneal haze after infectious keratitis.

In our study, we compared the effectiveness of intravenously injected vitamin C with orally administered vitamin C. Intravenous vitamin C decreased the corneal opacity more effectively than oral vitamin C. The reduction in opacity size was particularly greater when a hypopyon was present and the patient was <60 years. Since the presence of hypopyon represents severe inflammation, this suggests that vitamin C is particularly effective in more inflamed infectious keratitis, likely because of its anti-inflammatory and antioxidant effects.[12,37] Elderly patients are known to have several systemic or ocular features that increase their vulnerability to inflammation, such as immunologic senescence, comorbidities (eg, lid abnormalities or conjunctivochalasis), and reduced corneal sensitivity. These features are risk factors of poor visual outcomes after infectious keratitis. Indeed, all 7 perforations (3 in the oral vitamin C group and 4 in the intravenous vitamin C group [data not shown]) occurred in patients >60 years of age, and the effect of vitamin C in older patients is limited because of age-related decreases in wound-healing activity. Therefore, the present study shows that vitamin C is more effective in reducing the size of corneal opacities in younger patients.

The findings of this study should be interpreted in the context of the following limitations. First, this study did not measure the concentration of vitamin C in the anterior chamber or serum. However, quantification of vitamin C may not be necessary because uptake of vitamin C by the active transporter of corneal epithelial cells has been reported to occur in a linear, concentration-dependent manner.[29]

TABLE 4. Factors Associated With Corneal Opacity Size in the Vitamin C Groups

	Oral Vitamin C	Intravenous Vitamin C	*P* Value*
Presence of hypopyon	10	5	0.074
Location of ulcer			0.150
Central	25	20	
Paracentral	9	3	
Peripheral	3	2	
Size of corneal opacity** at initial visit (mean ± SD)	0.23 ± 0.13	0.28 ± 0.12	0.461
Clinically suspected pathogen			0.249
Bacteria	10	8	
Fungus	12	13	
Others	15	4	
Positive result of corneal culture	15	11	0.375

SD = standard deviation.
*Levene test for equality of variances.
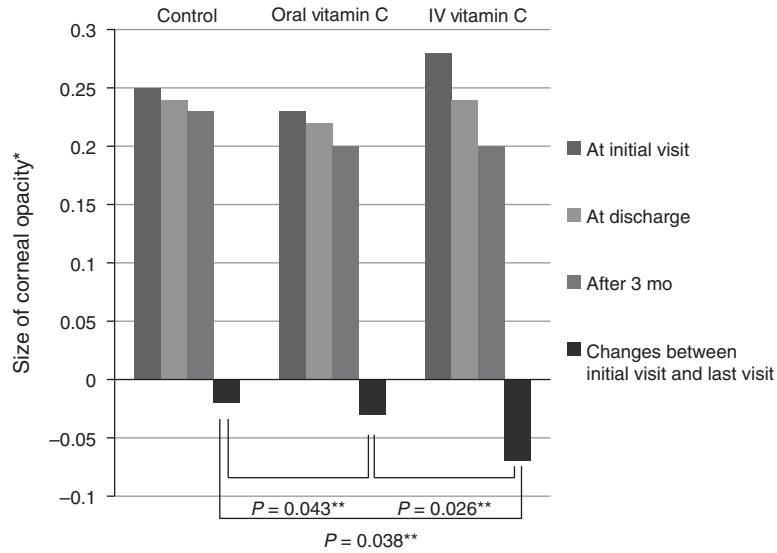**Measured opacity size divided by the size of the whole cornea.

FIGURE 2. Comparison of the size of corneal opacity. This histogram shows that the size of the corneal opacity in all groups decreased 3 months after discharge compared with the size at the initial visit. The reductions were significantly greater in the oral and intravenous vitamin C treatment groups than in the control group. The reduction in corneal opacity size was significantly greater in the intravenous vitamin C treatment group than in the oral vitamin C group. IV = intravenous. *Measured opacity size divided by the size of the whole cornea. **Repeated measures analysis of variance.

Second, vitamin C treatment was only administered during the admission of the patients and did not continue after discharge from the hospital. Thus, we do not know whether the effectiveness of vitamin C treatment could have been further increased if it had been given for a prolonged period after discharge. Third, size measurements using the Image J program (National Institutes of Health) can be somewhat subjective, so interobserver variability may have occurred. In an attempt to compensate for this, we calculated the opacity

size as a proportion of size of the whole cornea, and we conducted all measurements in a careful manner. Fourth, we measured the opacity size with slit lamp photographs; this method degrades the accuracy in measuring the size of the corneal opacity. So, future studies may use more accurate and precise ways to measure the size of corneal opacity, such as ConfoScan or cornea optical coherence tomography. Last, this study included a small number of subjects, and it was retrospective. Hence, further prospective studies with
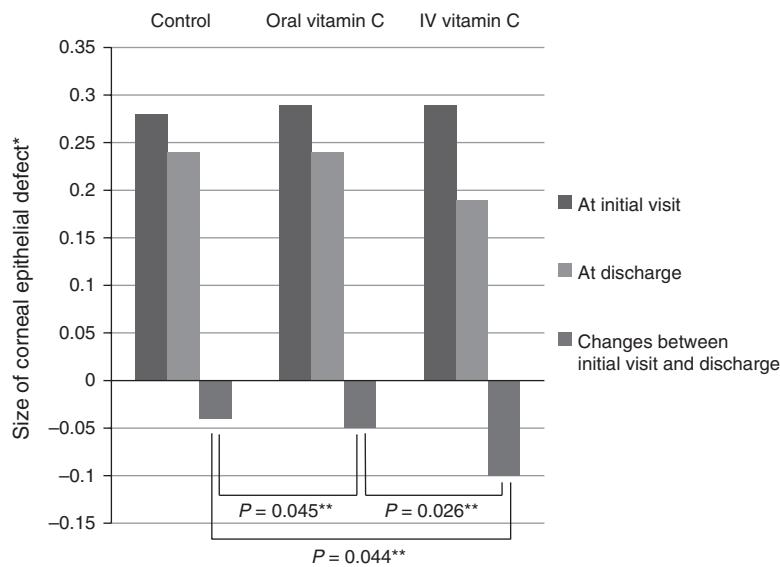

FIGURE 3. Comparison of size of corneal epithelial defect. This histogram reveals that the size of the epithelial defect decreased in all groups at hospital discharge compared with the size at the initial visit. The changes in epithelial defect size were significantly greater in the oral and intravenous vitamin C groups than in the control group. The reduction in the epithelial defect size was also significantly greater in the intravenous vitamin C group than in the oral vitamin C group. IV = intravenous. *Measured epithelial defect size divided by the size of the whole cornea. **Repeated measures analysis of variance.

TABLE 5. Factors Affecting the Change in Corneal Opacity Size* for the Vitamin C Groups

Variables	Oral Vitamin C (mean)	Intravenous Vitamin C (mean)	P Value**
Age, y			
>60	0.01	0.03	0.015
≤60	0.08	0.18	0.145
Sex			
Men	0.01	0.08	0.110
Women	0.01	0.08	0.126
Duration of admission, d			
≥8	0.03	0.03	0.122
<8	0.04	0.11	0.231
Previous ocular disease	0.02	0.09	0.086
Presence of hypopyon	0.08	0.09	0.036
Causative pathogen			
Bacteria	0.02	0.16	0.070
Fungus	0.04	0.04	0.067
Positive result on corneal culture	0.03	0.06	0.059

*Change in the size of corneal opacity (measured corneal opacity size divided by the size of the whole cornea) between the initial and last visits.

**Repeated measures of analysis of variance.

larger samples are required to clarify whether vitamin C reduces the size of corneal opacities resulting from infectious keratitis.

In conclusion, the results of this study showed that when used as an adjunct to antibiotic therapy, systemic (oral or intravenous) vitamin C supplementation has a beneficial effect on the healing process of infected corneas and reduces the size of corneal opacity resulting from infectious keratitis. Furthermore, intravenous vitamin C treatment decreased the corneal opacity size more effectively than oral vitamin C. In future, long-term clinical studies are warranted to establish the optimal dose, route of administration, duration of treatment, and frequency of administration of vitamin C for infectious keratitis.

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