
Ocular symptoms in nonspecific conjunctival hyperreactivity

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Background: Ocular symptoms can be triggered by nonspecific environmental factors, characterizing conjunctival hyperreactivity (CHR).

Objective: To examine CHR in subjects with ocular symptoms by means of a hyperosmolar conjunctival provocation test (HCPT).

Methods: Sixty-three subjects with ocular complaints (itching, redness, or tearing) were tested for allergy to a common panel of inhalant allergens (Alk Abelló-FDA Allergenic, Brazil). They were considered allergic if tests were positive to at least 1 allergen. HCPT with serial diluted glucose concentrations was positive if it produced conjunctival hyperemia up to a 50% solution. Digital images were analyzed by 2 observers who marked redness in the challenged eyes in red (GIMP 2.6.5 software). The number of red dots of the affected eye was compared with the number of blue dots of the control eye.

Results: HCPT was positive in 6 of 7 grass pollen-sensitive subjects, in 17 of 20 dust mite-sensitive subjects, and in 10 of 11 subjects who were sensitive to both allergens. HCPT was positive in 33 of 38 allergic subjects (87%) and in 4 of 25 (16%) non-allergic subjects; sensitivity was 87%, and specificity was 84%. Significant correlation ($r = 0.96$ Pearson; $P < .0001$) was seen between the number of red dots in 23 digitalized images marked by observers.

Conclusion: HCPT identified CHR in allergic as well as in non-allergic subjects. Allergic subjects exhibited more CHR than did non-allergic subjects. Conjunctival hyperreactivity was present in allergic subjects even when they were asymptomatic. Digital images may be useful for objective evaluation of ocular hyperemia in HCPT.

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INTRODUCTION

Allergic conjunctivitis (AC) is an immunoglobulin E-mediated hypersensitivity reaction of the eye triggered by environmental airborne allergens, mainly house dust mites and grass pollen.¹ Symptoms usually consist of low-grade ocular or peri-ocular itching, tearing, and red eyes that may be present year-round or seasonally. Nasal symptoms are usually associated with ocular symptoms as “rhinoconjunctivitis,” which is the most prevalent clinical form.²

The number of patients with ocular complaints is high, but epidemiologic studies usually address ocular symptoms as rhinoconjunctivitis, which might lead to the underestimation of AC cases³ and consequently the disease’s prevalence.⁴ Approximately 16% of US citizens have allergic rhinitis,⁵ and 40% to 60% of the allergic population present with ocular symptoms combined with other atopic diseases such as

asthma, rhinitis, or atopic dermatitis.⁶ In Brazil, no epidemiologic data are available on AC, but it seems to be high and increasing. Riedi et al⁷ found 13.9% prevalence of allergic rhinoconjunctivitis in 1995 (phase I) and 17.2% in 2001 (phase III) in 13- to 14-year-old students, using the International Study of Asthma and Allergies in Childhood written questionnaire, confirming a rise in prevalence of rhinoconjunctivitis in that interval. In Brazilian public ophthalmology reference centers, the chronic and severe forms of ocular allergy (vernal and atopic keratoconjunctivitis) are more frequent than benign forms such as seasonal or perennial allergic conjunctivitis,⁸ and many patients do not undergo any allergy workup for their symptoms in specialized clinics.⁹

Ocular discomfort has become more frequent. It is clinically manifested by conjunctival redness associated with mild and transient itching, burning, tearing, and abnormal light sensitivity that occur a few minutes after exposure to non-specific stimuli such as wind, light, smoke, or cold or warm air/water. This condition is known as nonspecific conjunctival hyperreactivity,¹⁰ and it may reflect diseases of the ocular surface from different causes.

Nonspecific conjunctival hyperreactivity (CHR) often results from allergic inflammation, but it also may be present in normal subjects without allergy.¹¹ Conjunctival challenges with histamine¹² and hyperosmolar glucose solutions¹³ are used to detect CHR.

The chronic and recurrent pattern of CHR symptoms is similar to those of allergic conjunctivitis, likely to be labeled as

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allergy. Approximately 60% to 70% of chronic diseases attributed to allergy may originate from structural defects or other non-allergenic factors.¹⁵ Thus, allergy must not be presumed only on signs and symptoms, because both allergic and non-allergic conditions may have similar clinical presentation.

The aim of this study was to detect CHR in subjects with ocular symptoms.

METHODS

Sixty-three subjects (12–50 years old) with ocular or nasal complaints were selected for the study from the outpatient Allergy Clinic, Hospital de Clínicas, University of Paraná (Brazil). Inclusion criteria were history of nasal and ocular symptoms such as itching, tearing, red eyes, nasal congestion, aqueous rhinorrhea, and sneezing initiated for more than 1 year. Exclusion criteria were pregnant women, current active conjunctivitis or rhinitis, past or current history of other ophthalmic diseases, active eczema, dermatographism or skin lesions in the areas of the skin tests, and unstable asthma.

An index of ocular discomfort (IOD) was calculated by adding the number of 8 environmental agents (wind, light, cold air, warm air, water, smoke, dust, and odors) reported by subjects when asked whether one of these agents could trigger any ocular symptom, particularly red eyes. The IOD scores were stratified into 4 classes (0, 1–2, 3–4, 5–8) ranging from none to a maximum of 8 reported nonspecific triggers.

Subjects with ocular symptoms were grouped according to allergic sensitization to dust mites or grass pollen. A third group of nonsensitized subjects without ocular complaints served for comparison.

Conjunctival provocation test with hyperosmolar glucose solutions were conducted in all subjects enrolled in the study. At the time the tests were performed, subjects should be asymptomatic and not using topical or systemic H1 antihistamines or corticosteroids and tricyclic antidepressants within 15 days. Any kind of eyedrops or contact lens should be discontinued 3 days before the tests. All tests were performed out of the pollen season.

Skin Prick Tests and Serum-Specific Immunoglobulin E Levels

Skin prick tests (SPT) were performed in all subjects to establish their allergic sensitization status to a common panel of inhalant allergens. The antigens tested (ALK Abelló) were provided by FDA Allergenic (Rio de Janeiro, Brazil). Histamine base (5 mg/mL) was used as a positive control and diluent (50% glycerin) as a negative control. Reactions were graded 15 minutes later and were considered positive if the mean wheal diameter was equal to or greater than 3 mm measured by an analog paquimeter device. Serum-specific immunoglobulin E to *D. pteronyssinus*, *B. tropicalis*, or *L. perenne* were determined by enzymatic immunofluorescence (ImmunoCAP, Phadia).

Hyperosmolar Conjunctival Provocation Test

The hyperosmolar conjunctival provocation test (HCPT) was carried out using serial glucose solutions from a stock solution of 50% glucose in distilled water (10%, 20%, 30%, 40%, and 50%) in 0.5% sodium carboxymethylcellulose eyedrops (Fresh tears, Allergan/Brazil) that were newly prepared at the time of each conjunctival challenge. Forty microliters of glucose solution were instilled using a pipette into the lower conjunctival sac in the subject's eye, starting with the lowest concentration (10%) and moving up every 10 minutes.¹¹ HCPT was considered positive and was halted when it elicited conjunctival redness, and no additional glucose solutions were challenged to the eye. The time when a positive reaction appeared was recorded. The contralateral eye was used as control and challenged with the same volume of diluent.

Analysis of Conjunctival Hyperemia by Digital Photography

Digital images were taken before and after each HCPT with a digital camera (Sony, model DSC-T20 8.1 megapixels; Sony Corp, Tokyo, Japan). Two observers, a physician and a technician, marked in the same image of each subject what they considered redness in the challenged eye in red by using a standard brush tool (GIMP 2.6.5, available at <http://www.gimp.org>).¹⁶ Brush size and RGB values for both red and blue colors were the default ones provided by the tool. The observers were blinded to each other, marking the images in different moments and places. The total area of the control eye was marked in blue. In each image, the number of red dots of the affected eye was compared with the number of blue dots of the control eye.

Statistical Analysis

A χ^2 test with continuity correction was applied to compare the proportion of ocular symptoms in the groups and to evaluate the presence of nonspecific triggers and type of allergic sensitization to HCPT. Pearson correlation coefficient was calculated to measure the degree of association between the number of red dots marked in digital images by the observers. STATISTICA 6.0 (StatSoft Inc, Tulsa, Oklahoma) was the statistical software used. $P < .05$ were considered statistically significant.

The study protocol was approved by the Committee of Ethics of Hospital de Clínicas–Federal University of Paraná, Curitiba, Brasil. Physical examination and procedures were taken after participants signed a written informed consent.

RESULTS

Of the 63 subjects, 38 were sensitized to common inhalant allergens (mean age, 31.4 years), and 25 (mean age, 34.4 years) were not sensitized. One nonsensitized subject was excluded from the study for showing intense conjunctival reaction to both the diluent and the glucose solution. In the sensitized group, the proportion of females to males was 60.5%:39.5%, and in the nonsensitized group, it was 84%:16%. Clinical diagnosis of rhinoconjunctivitis was more frequent in sensitized subjects (71%) than in nonsensitized

Table 1. Demographic and Clinical Characteristics of the Study Population

Subjects	Allergic	Nonallergic	Total
Number	38	25	63
Age (years) Mean $\pm$ SD	31.4 $\pm$ 10.34	34.4 $\pm$ 10.62	32.9
Range	(12–49)	(18–50)	(12–50)
Sex (n) F/M	23/15	21/4	44/19
Rhinoconjunctivitis (%)	27 (71)	2 (8)	29 (46)
Asthma (%)	8 (21)	3 (12)	11 (17)
Asymptomatic (%)	0	8 (32)	8 (13)

(8%). Demographic characteristics of the study population are shown in Table 1.

In the allergic group, ocular itching was reported by 84% of the subjects, conjunctival redness by 47.4%, and tearing by 31.5%. Tearing was the most frequent symptom reported by nonallergic subjects (36%), followed by conjunctival redness (24%) and ocular itching (24%). Pruritus was the only ocular symptom that correlated with the clinical diagnosis of allergic conjunctivitis ($P < .0001$).

Conjunctival provocation test with a hyperosmolar glucose solution induced conjunctival redness within 2 to 4 minutes, associated with mild itching or stinging in 59% (37/63) of all subjects; 87% (33/38) were allergic, and 16% (4/25) were nonallergic. Reactions peaked in 4.38 ± 1.89 minutes and gradually and spontaneously dissipated within 20 minutes. The sensitivity of HCPT was 87%, and the specificity was 84%. Ocular itching was associated with 82% (27/33) of positive HCPT but was also reported by all allergic subjects with negative HCPT ($P < .57$). Only 1 allergic subject complained of mild headache after a positive test that lasted for 2 hours.

Seventy-three percent of all subjects (46/63) reported ocular symptoms after exposure to nonspecific triggers; 76% were allergic (35/46), and 24% (11/46) were nonallergic. The allergic group had mean IOD of 3.1 (range, 0–6), and in the nonallergic group, the mean IOD was 2.3 (range, 0–5). The

Table 2. Nonspecific Triggers and HCPT in Allergic and Nonallergic Subjects (N = 63)

IOD	Positive HCPT (n = 37)		Negative HCPT (n = 26)		
	+ SPT	– SPT	+ SPT	– SPT	
0	1	1	1	14	$P < .00001$
1–2	12	1	2	4	NS
3–4	15	1	2	3	NS
5–8	5	1	—	—	$P < .03$

Abbreviations: HCPT, hyperosmolar conjunctival provocation test; SPT, skin prick test; NS, not significant.

IOD: An index of ocular discomfort was calculated by adding the number of 8 nonspecific triggers (wind, light, cold air, warm air, water, smoke, dust, and odors) reported by the subjects when they were asked whether 1 of these agents triggered any ocular symptom, particularly red eyes. Range, 0–8.

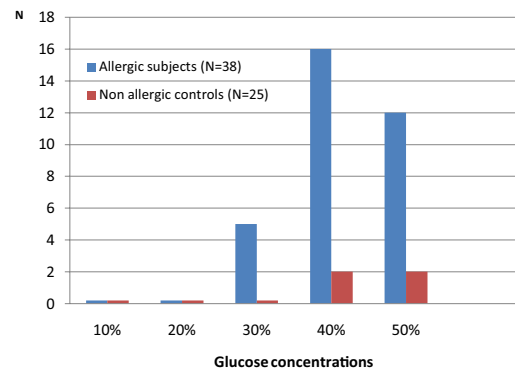


Figure 1. Hyperosmolar conjunctival provocation test with serial glucose concentrations in allergic and nonallergic subjects.

HCPT resulted positive in 35 (95%) subjects with positive IOD (32 allergic and 3 nonallergic) and in 2 (5%) subjects with negative IOD. In general, subjects with high IOD (5–8) had a greater chance of presenting positive HCPT ($P < .04$), whereas subjects with negative IOD had a greater chance of not reacting to HCPT ($P < .00001$). Eight subjects in the nonallergic control group did not refer to any ocular complaints triggered by either allergens or nonspecific agents. One of these subjects reacted positively to HCPT despite a negative IOD (Table 2).

Two thirds of the allergic subjects presented ocular hyperemia on HCPT to a 40% glucose solution or less (21/33), whereas 2 of 4 nonallergic subjects reacted to the same concentration and the remaining two subjects to a higher glucose concentration. The remaining 21 (84%) nonallergic subjects did not react at all (Figs 1 and 2). No positive reactions to 10% and 20% glucose solutions were found in any group. The HCPT for each glucose solution to 30%, 40%, and 50% had sensitivity of 13%, 42%, and 32% and specificity of 100%, 92%, and 92%, respectively. The 40% glucose solution was more sensitive and specific in predicting a positive HCPT. Two nonallergic subjects showed intense

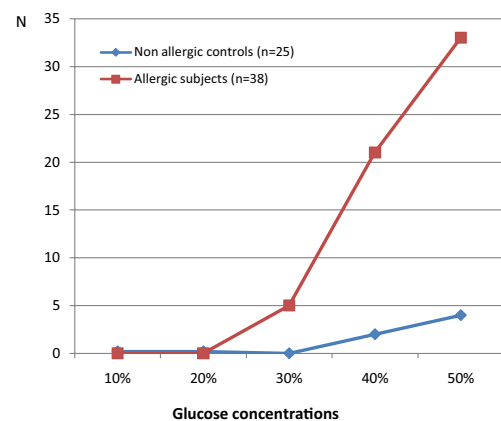


Figure 2. Cumulative frequency of positive HCPT in allergic and nonallergic subjects.



Figure 3. Digital images of positive HCPT. (A) Technician's analysis: number of red dots, 2521; number of blue dots, 8717. (B) Technician's analysis: number of red dots, 2521; number of blue dots, 8717.

conjunctival hyperemia, lacrimation, and chemosis with 40% glucose solution.

Twenty subjects were sensitized to dust mites (20/38), 7 were sensitized to grass pollen, and 11 to both allergens. The HCPT was positive in 6 of 7 (86%) grass pollen-sensitive subjects, in 17 of 20 (85%) dust mite-sensitive, and in 10 of 11 (91%) allergic to both. The type of allergic sensitization did not affect the results of HCPT ($P = .89$).

Serum-specific immunoglobulin E to the allergens tested was detected in 87% (33/38) of the allergic group with positive SPT, was negative in 3 subjects, and was not done in the other 2. The HCPT was positive in 85% (28/33) and negative in 15% (5/33).

Twenty-three digital images of positive HCPT were eligible for analysis. Significant correlation ($r = 0.96$ Pearson; $P < .0001$) was seen between the number of red dots marked by observers in these images (Figs 3 and 4).

DISCUSSION

Nonspecific CHR is characterized by mild ocular redness, itching, tearing, and burning after the ocular surface is exposed to nonspecific triggers such as wind, light, cold or warm air, smoke, water, dust, and other irritants.¹⁷ HCPT with increasing serial glucose concentrations has been used to detect CHR in both allergic and nonallergic subjects, because it reproduces the signs and symptoms of CHR. Although

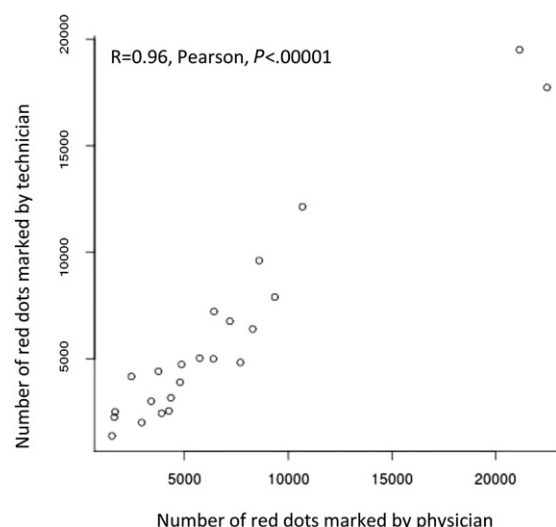


Figure 4. Number of red dots recorded in digital images of positive HCPTs by observers.

CHR is commonly associated with allergic conjunctivitis, it has also been shown in other conditions.¹⁸ We identified CHR by hyperosmolar conjunctival provocation in house dust mite- and grass pollen-allergic subjects as well as in nonallergic subjects. Conjunctival hyperreactivity was more frequently detected in the allergic group (87%) than in the nonallergic group (16%). Only one allergic subject complained of mild headache for 2 hours after a positive test. No severe reactions and no long-lasting side effects were seen, indicating that HCPT was a safe test.

Conjunctival provocation with histamine and hyperosmolar solutions reproduces the signs and symptoms of AC. Histamine causes conjunctival vasodilation without inducing inflammation in the eye. Histamine ocular challenge discriminates between asymptomatic patients with vernal keratoconjunctivitis requiring lower doses of histamine for a positive ocular test from healthy individuals.¹⁴ Conversely, an immediate inflammatory response with clinical signs and symptoms, inflammatory cell infiltrates, and CD54 expression on the conjunctival epithelium of allergic subjects and healthy volunteers occurs with hyperosmolar stimulus.¹³

Ciprandi et al¹⁹ demonstrated that patients allergic to dust mites exhibited conjunctival hyperreactivity as a result of minimal persistent inflammation caused by frequent exposure of the conjunctival mucosa to the allergen present all year round. Those patients were also found to react with lower glucose doses than healthy controls or exclusively pollen-sensitive patients.

In our study, the type of sensitizing allergen did not interfere with the result of HCPT in allergic subjects. Similar numbers of positive reactions were seen in subjects sensitized either to dust mites or to grass pollen or to both allergens ($P > .05$). Minimal persistent inflammation has also been demonstrated in pollen-sensitive patients, which might explain the presence of conjunctival hyperreactivity in those patients out of the pollen season when they were asymptomatic.²⁰

Itching, burning, tearing, and red eyes are common ocular symptoms that may or may not reflect an allergic disease. Ocular itching is strikingly characteristic of allergic conjunctivitis. Ocular itching is said to imply allergy until proven otherwise.²¹ In our study, 84% of allergic subjects had itching as the main ocular symptom associated with the clinical diagnosis of allergic conjunctivitis. Most allergic subjects who reported ocular itching had positive HCPT (82%); however, ocular itching was not predictive of CHR in this group, because all allergic subjects with negative HCPT also reported the symptom. Itching is subjective and frequently described as stinging and burning eyes. This misinterpretation could explain why 24% of nonallergic subjects in our study without AC or aeroallergen sensitization have complained of that symptom.

Allergic and nonallergic subjects were shown by Sacchetti et al¹¹ to react to HCPT at lower glucose doses if they had a history of ocular discomfort unlike those with no such history. Positive HCPT was associated with a larger number of nonspecific triggers of ocular symptoms reported by subjects.

We found 95% of positive HCPT (35/37) in allergic and nonallergic subjects who reported symptoms triggered by nonspecific agents. However, significant correlation was solely observed in subjects who complained of 5 or more nonspecific triggers ($P < .03$). HCPT was more frequently negative in those without history of ocular discomfort by nonspecific triggers ($P < .00001$). Thus, the greater the number of nonspecific triggers involved, the greater the chance of CHR to be present. The absence of symptoms by nonspecific triggers usually indicates no CHR. The 10-minute interval to increase the glucose concentration appeared to be appropriate because ocular hyperemia occurred in a mean time of 4.38 ± 1.89 minutes. Residual symptoms were not recorded and unlikely influenced additional glucose concentrations.

A 40% glucose solution was the optimal dose to show CHR in allergic subjects (16/33) with better sensitivity (42%) and specificity (92%) than other concentrations. However, a large number of allergic subjects in our study reacted to a 50% glucose solution (12/33). One possible reason for this discrepancy could be the use of carboxymethylcellulose as vehicle. Inert intranasal cellulose powder has been shown to alleviate symptoms of hay fever in adults²² and to significantly reduce nasal symptoms in specific nasal provocations with *Der p 1* and *Der f 1*.²³ Tear substitutes directly help to remove and to dilute allergens that may come in contact with the ocular surface,²⁴ which could be useful to reduce allergic inflammation in the eyes. Carboxymethylcellulose is a water-soluble polymer that enhances the viscosity of the tear film and increases the drug's bioavailability for extending the residence time of the drug with the ocular surface.²⁵ Saline albumin solution was the vehicle in previous reports,^{11,13} and CHR was detected in both allergic and nonallergic patients, even though allergic patients exhibited greater CHR than nonallergic patients. Carboxymethylcellulose is a common type of over-the-counter eyedrops. It is nonallergenic and nontoxic for the cornea,²⁶ and it presents as an alternative for sterile saline albumin solution not readily available in our region. Carboxymethylcellulose could have contributed to shift the curve to a greater number of subjects reacting to higher glucose concentrations. Despite a potential soothing effect of this viscous artificial tears substitute, carboxymethylcellulose as a diluent did not compromise the overall HCPT outcome and corroborated the clinical observation that allergic subjects complained of CHR to a greater number of nonspecific stimuli.

Conjunctival provocation tests are well established human models for studying allergic conjunctivitis.^{27,28} However, reactions elicited by allergens are typically evaluated in a subjective way. Redness is usually graded by investigators' direct observation, and itching is graded by subjects when questioned about the symptom. Few methods have been developed over the years to objectively measure each sign and symptom of the ocular allergic reaction.^{29,30} Chun et al.³¹ measured the variation in conjunctival blood vessel diameters after histamine challenge. Vasodilation affected conjunctiva

within the first 3 minutes, whereas symptoms lasted up to 20 minutes, suggesting that subjective symptoms last longer than objective symptoms. Digital images of our subjects during HCPT provided objective evidence of ocular redness when present. A limitation was that our images were not obtained using a well-calibrated system, which may have created slightly different angles, brightness, orientation, eye sizes, and resolutions.³² Different resolution of each image, for instance, did not allow precisely counting the number of pixels in images of the same subject taken before and after challenge and comparing them, which would lead to a wide difference and poor quality of images in many cases. Visible surface area of challenged and control eyes may differ as a result of slight upper lid ptosis that occurs as a secondary reflex to any ocular irritation. To avoid misinterpretation of the number of red dots, different observers marked the same visible area of each image of positive reactions. Abstracting the subjectivity of the conjunctival provocation tests might help physicians to better understand the ocular reaction and to develop more efficient medications to treat nonspecific as well as allergen-specific conjunctival reactivity.

In conclusion, HCPT was shown to be a safe and specific test to detect conjunctival reactivity to nonspecific triggers in both allergic and nonallergic subjects with ocular symptoms. Allergic subjects exhibited more CHR than nonallergic subjects, even when asymptomatic.

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