

# International Journal of Ophthalmology and Optometry



ISSN Print: 2664-8547  
ISSN Online: 2664-8555  
IJOO 2024; 6(1): 24-29  
[www.opthajournal.com](http://www.opthajournal.com)  
Received: 19-11-2023  
Accepted: 26-12-2023

#### Diaz-Llopis Manuel

<sup>1</sup>University of Valencia, Valencia, Spain

<sup>2</sup>Ophthalmology Unit, Faculty of Medicine, University of Valencia, Valencia, Spain

<sup>3</sup>Molecular Ophthalmology Unit, Faculty of Medicine, University of Valencia, Valencia, Spain

<sup>4</sup>Conselleria de Sanitat (Valencian Regional Health Ministry), Valencian Autonomous Government, Valencia

<sup>5</sup>IOVA - Valencia Ophthalmology Institute, Valencia, Spain

#### Pinazo-Duran Maria Dolores

<sup>1</sup>Ophthalmology Unit, Faculty of Medicine, University of Valencia, Valencia, Spain

<sup>2</sup>Molecular Ophthalmology Unit, Faculty of Medicine, University of Valencia, Valencia, Spain

<sup>3</sup>Doctor Peset University Hospital, Valencia, Spain

<sup>4</sup>Santiago Grisolia Eye Research Laboratory, FISABIO, Valencia, Spain

#### Diaz-Guiñon Loreto

<sup>1</sup>IOVA - Valencia Ophthalmology Institute, Valencia, Spain

<sup>2</sup>Albacete General Hospital, Albacete, Spain

#### Rahhal-Ortuño Miriam

Department of Ophthalmology, La Fe Hospital, Valencia, Spain

#### Gallego-Pinazo Roberto

IMED Hospital, Valencia, Spain

#### Dolz-Marco Rosa

IMED Hospital, Valencia, Spain

#### Diaz-Guiñon Teresa

<sup>1</sup>IOVA - Valencia Ophthalmology Institute, Valencia, Spain.

<sup>2</sup>Health public center, La Roda, Albacete, Spain

#### Diaz-Guiñon Maria

<sup>1</sup>Conselleria de Sanitat (Valencian Regional Health Ministry), Valencian Autonomous Government, Valencia

<sup>2</sup>IOVA - Valencia Ophthalmology Institute, Valencia, Spain

<sup>3</sup>Arnao de Vilanova Hospital, Valencia, Spain

#### Romero Francisco Javier

Conselleria de Sanitat (Valencian Regional Health Ministry), Valencian Autonomous Government, Valencia

#### Corresponding Author:

##### Diaz-Llopis Manuel

<sup>1</sup>University of Valencia, Valencia, Spain

<sup>2</sup>Ophthalmology Unit, Faculty of Medicine, University of Valencia, Valencia, Spain

<sup>3</sup>Molecular Ophthalmology Unit, Faculty of Medicine, University of Valencia, Valencia, Spain

<sup>4</sup>Conselleria de Sanitat (Valencian Regional Health Ministry), Valencian Autonomous Government, Valencia

<sup>5</sup>IOVA - Valencia Ophthalmology Institute, Valencia, Spain

## Efficacy of an isotonic seawater eye wash in patients with mild and moderate allergic vernal keratoconjunctivitis by measuring immunoglobulin E levels in tears: Report 1 clinical trial Gov/USA NCT 04695795

Diaz-Llopis Manuel, Pinazo-Duran Maria Dolores, Diaz-Guiñon Loreto, Rahhal-Ortuño Miriam, Gallego-Pinazo Roberto, Dolz-Marco Rosa, Diaz-Guiñon Teresa, Diaz-Guiñon Maria and Romero Francisco Javier

DOI: <https://doi.org/10.33545/26648547.2024.v6.i1a.28>

### Abstract

**Background:** Vernal keratoconjunctivitis is one of the main and most serious ocular allergic manifestations, with a frequency that has increased alarmingly in recent years. High IgE levels have been correlated with the severity of clinical manifestations of the disease as well as with the development of complications, such as keratoconus and punctate keratitis.

**Purpose and Aim:** The objective of this study was to establish the efficacy of eye wash spray monotherapy on the levels of immunoglobulin E (IgE) in the tears of patients with vernal conjunctivitis. **Materials and Methods:** Registered prospective study (Clinical Trial Gov/USA NCT 04695795). IgE levels in tears were measured with Lacrytest® before and after treatment.

**Study design:** A total of 35 patients (70 eyes) with mild-moderate vernal conjunctivitis were treated exclusively with isotonic seawater solution spray 5 times a day for 21 days.

**Results:** 82.9% of patients were able to finish the 3 weeks of treatment with a good clinical response without needing any other support therapy such as antihistamines or corticosteroids; this was necessary for the other 17.1%. Before treatment, initial IgE levels in tears were positive in 92.9% of cases. This reduced to 37.1% of cases after treatment, a 55.8% statistically significant ( $p < 0.01$ ) reduction; becoming negative afterwards.

**Conclusion:** Washing with seawater was very effective in drastically reducing IgE levels in the tears of patients with mild and moderate vernal keratoconjunctivitis.

**Keywords:** Vernal keratoconjunctivitis, allergic conjunctivitis, seawater ophthalmic solution, IgE tear level, lacrytest®

### Introduction

Vernal keratoconjunctivitis (VKC) is a chronic, severe inflammation, usually bilateral, of the conjunctiva and secondarily of the cornea, and normally recurs for years. It more frequently affects males in childhood or adolescence from hot, dry climates. The main manifestations are intense itching and photophobia, associated with excessive mucus and cobblestone papillae; as well as complications with corneal infiltrates, keratitis, corneal ulcers and leukoma [1-3].

Previous studies have shown the efficacy of washing with seawater in other allergic clinical conditions, such as rhinitis and dermatitis [4-7], although a search in PubMed revealed the efficacy of seawater on allergic conjunctivitis of any kind had not been tested. Previous studies also showed that washing with seawater is effective in controlling the symptoms of dry eyes and in reducing the levels of inflammatory substances in tears [8-9].

The total level of IgE in tears plays an important role in allergic conjunctivitis and especially vernal ones; with levels correlating with the degree of activity and severity. Previous studies have shown that the commercial test Lacrytest® is capable of detecting IgE levels with a high sensitivity of 91.5% and a high specificity of 98.7% [10-11].

The aim of this study was to establish the efficacy of seawater eye washes in reducing IgE levels in the tears of patients with mild and moderate vernal conjunctivitis.

## Materials and Methods

### Study Design

Registered prospective study in Clinical Trial Gov/USA NCT 04695795. The test was performed in the spring of 2022, from March to June, which is the period of greatest activity for vernal conjunctivitis. This multicenter study was carried out in different medical and ophthalmological centers that recruited cases in Valencia, a Spanish city adjacent to the Mediterranean Sea, with a high prevalence of

cases due to its climate and vegetation. The study protocol was approved by the University of Valencia ethics committee and was subject to the Declaration of Helsinki principles at all times. All patients signed specific, detailed informed consent forms before starting the study. The legal guardians of minors under 18 years of age provided legal authorization for the procedure.

### Subjects: inclusion and exclusion criteria

Inclusion criteria for patients were having active vernal allergic conjunctivitis, mild (grade I) or moderate (grade II) in both eyes; not including severe (grade III), very severe or blinding (grade IV), according to Table 1.

**Table 1:** Grades of vernal keratoconjunctivitis (adapted from the Bonini-Gokhale scale) (12, 13)

	<b>I Mild</b>	<b>II Moderate</b>	<b>III Severe</b>	<b>IV Blinding</b>
Bulbar conjunctiva	congestion	congestion	trantas dots	granuloma
Tarsal conjunctiva	micropapillae	macropapillae 1-3 mm	giant papillae > 3 mm	cobblestone
Corneal staining		microstaining	macrostaining	shield ulcer
Limbal involvement		< 180 degrees	> 180 degrees	insufficiency
				limbus/
				pannus/
				vascularization
				corneal

### Exclusion criteria were

- Any other type of conjunctivitis, especially bacterial or viral.
- Other types of acute seasonal allergic conjunctivitis (SAC) such as perennial-chronic (PAC), as ruled out by the airborne allergens skin prick test (SPT).
- Blepharitis of any type and any other eyelid pathology, especially meibomian gland dysfunction syndrome (MGS) and styes/chalazions.
- Dry eye disease, for which patients were required to have a Schirmer's test > 10 mm without anesthesia in both eyes and TBUT > 10 in both eyes.
- Any type of topical ocular anti-allergy treatment, including any type of lubricant or eye wash, e.g. antihistamines, macrophage stabilizer, corticosteroids, ocular decongestant and/or vasoconstrictor, cyclosporine, tacrolimus, alpha interferon and mitomycin, at least 3 months before being assessed for inclusion in the study. The priority of the study was to include all cases of patients without any previous ocular treatment. This included all types of associated nasal drops or spray treatment.
- Any previous eye surgery, especially including refractive corneal surgery.
- Use of contact lenses.
- Any previous ocular pathology, such as ocular hypertension-glaucoma with or without treatment with eye drops, and especially pathologies of the ocular surfaces, such as pterygium or ocular pemphigoid.
- Treatment with oral or subcutaneous allergy vaccines any time prior to the start of the study, even if years earlier.
- Oral or subcutaneous treatment, such as non-steroidal anti-inflammatory drugs, antihistamines, antileukotrienes (montelukast), corticosteroids, immunosuppressants and biological drugs.
- Associated rhinitis and dermatitis of any nature (e.g. atypical), as well as asthma or any other type of bronchopathy.

- Children under 9 years old.

### Subjects: Preliminary study

Before beginning the trial, a complete study of each patient was done at the time of inclusion in the trial. This included general data (age, sex), pathologies and general disease treatments with a special search for allergic manifestations in other areas (e.g. asthma, rhinitis, dermatitis); a complete slit-lamp examination of the anterior segment of both eyes, tonometry and retinography was also done. Schirmer's test, TBUT and fluorescein staining to accurately delineate secondary corneal involvement. To perform the tear film break-up time (TBUT) test, a fluorescein strip (Ful-Glo®) was placed on the lower conjunctiva moistened with a saline solution. The TBUT was quantified by counting the breakup time after 3 consecutive blinks. The lighting was carried out with a Topcon lamp with maximum blue light width. 3 values were measured and the average of the two closest values was taken. A TBUT of less than 10 seconds was considered pathological. The Schirmer test was performed after instilling a drop of double anesthetic eye drops (Colircusi®, Laboratorio Alcon, Barcelona, Spain) and placing a strip (Tear strips, Aivimed GmbH, USA) in the external third of the lower eyelid, allowing for natural blinking. Values less than 10 mm at 5 minutes were considered pathological.

### Treatment protocol

All patients received a single treatment of cold microfiltered isotonic seawater solution 5 times a day for 3 weeks. The solution was applied as a spray a few centimeters from the patient's eye with the eye slightly open. Quinton® seawater solution (Quinton® Eye Health supplied by Laboratoires Quinton, Alicante, Spain) (Figure 1) is a 0.22 micron, filtered solution in a cold and clean room, from an area of the Atlantic Ocean rich in plankton blooms (Bay of Biscay, Spanish coast). The solution is rich in electrolytes and organic nutrients, such as Na<sup>+</sup>, Cl<sup>-</sup>, Mg<sup>2+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup>, Be<sup>2+</sup>, D-Biotin, Riboflavin, Nicotinamide and

Cyanocobalamin. Spring water was added to achieve an isotonicity and salinity similar to human tears.

### Biochemical tear analysis

The commercial test, LacryTest® (ADIATEC SA, Nantes, France) (Figure 2) was used to measure Immunoglobulin E levels. These were measured before and after 21 days of treatment. The post-treatment levels were measured 100-120 minutes after ocular application of the treatment in all cases. All measurements were analyzed by the same 3 researchers (DM, PD & DL) simultaneously. To ensure the greatest objectivity for the results, the researchers were blinded to the clinical status and situation of patients and to whether they were pre- or post-treatment samples. An average result from the three researchers was rounded up to the next highest level.

Lacrytest® (Adiatec, Nantes, France) is a non-invasive rapid immunoassay that measures total IgE levels in tears. The overall sensitivity was 91.5% and specificity was 98.7%, according to the manufacturer. The strip was placed on the lower fornix in the lateral third for 5 minutes. The strip was then placed in a tube with demineralized water for 10 minutes, which produced a reading that appeared as a purple band. No line appeared for negative values (Grade 0) below 2.5 kU/L. Positive results were divided into 3 different subgroups on a semi-quantitative scale: Grade 1-LOW: IgE levels 2.5-10 kU/L, a line less intense than the control; Grade 2-MEDIUM: IgE levels 10-40 kU/L, a line similar in intensity to the control; Grade 3-HIGH: IgE > 40 kU/L, a line more intense than the control [14, 15].

### Statistical analysis

A preliminary study was carried out to calculate the number of patients needed to be included in the study (XISTAT life science software and STATISTICA version 8 software).

A descriptive analysis of the demographic data and clinical features of the patients was performed. The SPSS 18 program (SPSS, Chicago, Illinois, USA) and CIA software were used to calculate the 95% confidence interval. The statistical significance level was established at  $p < 0.05$ . For the comparative statistical assessment of IgE levels before and after treatment, the Mann-Whitney U test, Kruskal-Wallis test and Wilcoxon test were used, as the variables were semi-quantitative.

### Results

35 patients (22 males, 13 females) were included. Mean age 13.5 (SD  $\pm$  4.3). The main data from the IgE results before and after treatment with Quinton® seawater solution are summarized in Table 2.

Patients completed the study if their response to clinical symptoms was good and sufficient and they did not need to resort to other supportive treatments (antihistamines or corticosteroids orally or topically). The latter occurred in 6 patients (17.1% of recruited cases) who abandoned the seawater monotherapy and were excluded from the study.

Of the patients who completed the 21-day treatment, 7.1% had negative IgE values in tears before starting the study, compared with 92.9% who were positive. After the seawater treatment, 62.9% of cases had negative IgE levels in tears compared to 37.1% who were positive; a statistically significant difference ( $p < 0.01$ ). In addition, in the semi-quantitative intensity assessment, High positives went from 10% to 1.4%, Medium positives from 38.6% to 8.6%, and

Low positives from 44.3% to 27.1%; the reduction was greater the higher the initial levels of IgE in the tears.

**Table 2:** IgE level in tears & Quinton® seawater treatment

	Before treatment		After treatment	
	N	%	N	%
Negative	5	7,1	44	62,9
Low positive	31	44,3	19	27,1
Medium positive	27	38,6	6	8,6
High positive	7	10	1	1,4
Mann Whitney $p < 0,05$	Kruskall-Vallis $p < 0,05$		Wilcoxon $p < 0,05$	

Qualitative Lacrytest® (IgE) results in patients with vernal conjunctivitis before and after treatment with Quinton® seawater isotonic saline solution. The total number of eyes (n=70) of the patients with vernal conjunctivitis (n=35) is shown in each column section.

There were no side effects attributable to the treatment in any case, nor did any patient abandon treatment due to intolerance.

### Discussion

Vernal keratoconjunctivitis (VKC) is a chronic and severe form of ocular allergy that can affect the conjunctiva and cornea bilaterally, and generally asymmetrically. It is found mainly in male children and young adults in the first prepubertal decades of life; especially in certain areas of the world, such as the Mediterranean, Japan, India, Central Africa and South America. There was a personal or family history of atopy (asthma, rhinitis or skin eczema) in 5-20% of cases; but these were excluded from the study to clarify and homogenize the study sample. It is the most severe of all the clinical forms of ocular allergy, giving rise to frequent corneal complications, such as corneal shield ulcers, corneal leukoma and keratoconus and even blindness in extreme cases. In addition, it must not be forgotten that this pathology is often associated with chronic treatment with antihistamines, corticosteroids and the immunosuppressants tacrolimus and cyclosporine, via topical ocular route and oral route, with their consequent serious side effects (e.g. cataracts and glaucoma). Our study was limited to mild-moderate cases of VKC who were less likely to abandon the study than cases requiring more intense treatments [16, 17].

Although hormonal levels [18] to type I and type 4 hypersensitivity reactions are implicated in its pathogenesis, as well as multiple cells (mast cells, eosinophils, CD4/Th1/Th2 T lymphocytes) and other substances (metalloproteinases, interleukins IL3/IL4 /IL5, TGF/FGF/EGF growth factors), the central role played by IgE levels at the tear level in the intensity and chronicity of the clinical symptoms is accepted, with a direct relationship between the levels and the clinical intensity of the disease (19,20,21,22). In addition, the positive IgE levels in tears is much higher in VKC than for other ocular allergy conditions (89% vs 20%) [10]. These were the reasons for selecting this ocular allergy condition and for measuring IgE in the study. In addition, Lacrytest® is a safe, reliable and simple test to measure IgE in tears. This makes it much easier for multicenter studies to collect samples in large population groups and from very young people who reject, out of fear, other more dangerous types of tear collection with glass pipettes for more sophisticated biochemical studies.

Lacrytest® is capable of detecting IgE levels with a high sensitivity of 91.5% and a high specificity of 98.7%. [23].

There are many studies of the efficacy of seawater in other allergic pathologies (e.g. rhinitis and dermatitis;) [24-26], but not in ocular allergies. There are studies of the efficacy of seawater in dry eye syndrome and its ability to drastically reduce the levels of pro-inflammatory molecules in tears (IL1, IL6, MMP9) [8-9]. Therefore, we decided to quantitatively establish the ability to make IgE levels in tears negative by exclusively applying seawater. A reduction in positivity from 92.9% before treatment to only 37.1% after treatment was found, with an attendant reduction in the degree of intensity of test positivity from 10% to 1.4% in cases classified as High positive. Also, only 17.1% of the cases had to resort to new associated therapies (mainly corticosteroids and antihistamines) during this monotherapy treatment with seawater. This allowed an indirect conclusion, without having specifically quantified clinical manifestations, that 86.8% required no other short-term treatment. This efficacy supports the inclusion of seawater in the first steps for gold standard ocular allergy treatment, especially for non-serious cases. Future studies should investigate whether it can reduce corticosteroid and immunosuppressive dependence in severe and blinding cases.

The ability of seawater to inhibit inflammatory mechanisms, such as phospholipases and arachidonic acid, and thus improve allergic inflammatory processes, especially rhinitis, has already been demonstrated. However, this study is the first to demonstrate its efficacy in allergic conjunctivitis; especially in the most severe, chronic cases, such as VKC. There are two possible mechanisms to explain its efficacy, which should be investigated in future studies: (a) the dragging and washing effect of the ocular surface, physically removing debris, cells, electrolytes and proinflammatory molecules; and (b) cicatricial repair of the integrity of damaged corneal and conjunctival mucosa through an increase in epithelial growth factor (EGF), given that the seawater used (Quinton®) is an isotonic solution of alkaline pH with high levels of bicarbonate, rich in electrolytes and organic nutrients, such as Na<sup>+</sup>, Cl<sup>-</sup>, Mg<sup>2+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup>, Be<sup>2+</sup>, D-Biotin, Riboflavin, Nicotinamide and Cyanocobalamin, for example; as well as high levels of magnesium and potassium reducing the levels of inflammatory cascade mediated by TNF, IL1, IL and IL8 (27,28,29,30,31,32,33).

We believe the present study has shown that seawater should be included in the gold standard of VKC treatment in its basic, initial steps; having the potential ability to reduce antihistamine treatment and the potentially dangerous chronic corticosteroid dependence common in these patients.

### Study limitations

1. To measure IgE levels in tears, a highly sensitive, specific and semiquantitative test (Lacrytest®) was used: giving negative, weak positive or strong positive as a result. We believe the present study should be validated with future studies measuring the exact levels of IgE in tears and in larger case series.
2. The study was limited to mild and moderate vernal keratoconjunctivitis (VKC) cases. It would be interesting to establish its efficacy in serious, severe and

blinding cases, as well as in other allergic conjunctivitis conditions such as SAC or PAC.

3. Although the study was prospective, it should be expanded by conducting new studies with.
  - a) An untreated control group: although this is difficult to perform in cases which are not very mild.
  - b) A control group with sterile distilled water: to distinguish between the dragging effect of spray washing and the extent to which seawater is effective due to its composition.
  - c) A control group treated with antihistamines and corticosteroids - independently and individually - as well as with seawater treatment, to establish the proportionality of seawater efficacy compared to these classic treatments; as well as its possible synergistic effect to establish the extent to which the use of this natural product can reduce corticoid-dependent side effects in these patients.
  - d) a study of the degree of response of the clinical manifestations studied individually (e.g. itching, secretions, redness, papillae and keratitis), which was not evaluated in this study.

### Conclusion

Washing with seawater is very effective in significantly reducing IgE levels in the tears of patients with vernal keratoconjunctivitis, and can reduce its positivity from 92.9% to 37.1% of cases.



Fig 1: Quinton ® isotonic seawater spray.

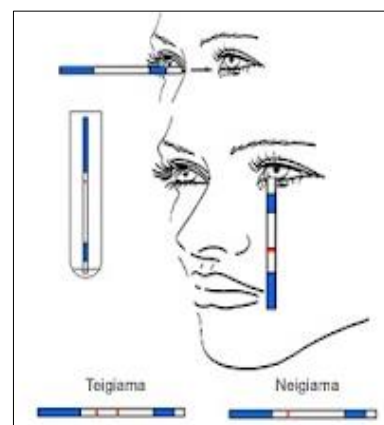


Fig 2: Lacrytest ® test. Level of IgE Tear in a semiquantitative scale

**Correspondence author**

Professor Manuel Diaz-llopis, IOVA Ophthalmological institute & Molecular Ophthalmology Unit, Faculty of Medicine University of Valencia, Calle San vicente martir 63, 46002 Valencia

Email: manuel.diaz@uv.es Phone/Whatsapp: +34 666530085

**Author contributions**

All authors conceived of and designed the study protocol; PD, DL, DT, DM, DR & RF supervised, selected and recruitment the patients for the study; DM, PD & DL supervised clinical study and Lacrytest® reading; DR statistical analysis; DL, RM, DT & DM performed the literature search and assessed study details; RJ & GR wrote the first draft of the paper, which was critically revised by all the other authors. All authors have read and agreed to the published version of the manuscript.

**Patient consent**

Specific written informed consent was collected from each of the patients or their legal guardians for minors under 18 years of age.

**Sponsorship and financial support**

Nil. This research received no external private or company funding.

**Conflict of interest**

The authors declare no conflicts of interest.

**Abbreviations**

IgE Immunoglobulin E.

PAC Perennial allergic conjunctivitis.

SAC Seasonal allergic conjunctivitis.

VKC Vernal keratoconjunctivitis.

SPT Skin prick test.

TBUT Tear break-up time.

IL Interleukin.

**Acknowledgment**

To the University of Valencia, which provided its facilities - Molecular Ophthalmology Unit, Faculty of Medicine - free of charge to carry out the study.

This study was supported by the Thematic Network for Cooperative Research in Health (RETICS), Carlos III Institute, Spanish Ministry of Health, Madrid, Spain

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