

# Intranasal self-administration of high-pressure physiological saline isotonic solution ameliorates dry eye symptoms and tear film composition: a pilot study

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**Abstract. – OBJECTIVE:** High-pressure physiological saline isotonic solution (HPpSIS) delivery into the nasal cavity was found to modulate the local expression of immune cells, increase NGF protein, and enhance the NGF receptors' expression. Since the nasal cavity directly communicates with the eye and as NGF was previously found to ameliorate the symptoms of dry eye when topically delivered, the aim of this study was to establish whether the HPpSIS might ameliorate ocular dryness and tear film composition.

**SUBJECTS AND METHODS:** This is an observational self-controlled case study carried out on 16 patients with dry-eye diagnosis, concerning 3-month self-administration of HPpSIS and two serial assessments of the ocular surface and tear film. OSDI questionnaire was used for ocular symptoms of dryness. BUT and Schirmer tests were used for qualitative and quantitative tear film analysis. The lipid composition was also examined. R-studio was employed for the detection of the difference between the pre- and post-analysis.

**RESULTS:** On the basis of the OSDI questionnaire, the study population was divided into severe (61.1%), moderate (5.5%), and mild (16.6%) dry-eye symptoms. OSDI score was significantly reduced after HPpSIS ( $p < 0.05$ ). BUT and TMH values also ameliorated after HPpSIS ( $p > 0.05$ ), although not significantly. The lipid layer improved statistically ( $p < 0.05$ ) and correlated positively with OSDI grading. The variability of presentation in the numerical distribution before and after therapy suggests poor test sensitivity.

**CONCLUSIONS:** HPpSIS showed a positive effect in reducing OSDI scores and ameliorating tear film quality. The possibility of an endogenous HPpSIS-induced NGF should be taken into account in dry-eye therapy.

## Key Words:

HPpSIS, Dry eye disease, NGF, Evaporation, Lipid thickness, Mucin, Osmolarity, Tear film, Schirmer.

## Introduction

The nasal administration of sterile saline isotonic spray solution (HPpSIS) in healthy patients modulates the innate immune system, enhances the release of NGF, and stimulates the expression of NGF receptors in the nasal cavity<sup>1</sup>. Concomitantly, HPpSIS can enhance the level of NGF and the expression of NGF receptors in human peripheral brain tumoral tissues, without affecting proliferation and differentiation of tumoral cells<sup>2</sup>. The increased NGF expression in the nasal cavity is associated with resident mast cells<sup>3</sup>.

Nerve growth factor (NGF) is a neurotrophin promoting neurons' survival/function<sup>4</sup> and modulating different pleiotropic effects inside the endocrine, immune, and visual systems. NGF effects are mediated by tropomyosin kinase receptor A ( $\text{trkA}^{\text{NGFR}}$ ) and p75 neurotrophin receptor ( $\text{p75}^{\text{NTR}}$ ), either alone or in combination as high-affinity form<sup>5</sup>. Widely expressed in both ocular and nasal mucosa, these receptors work at different levels to guarantee local homeostasis<sup>5</sup>. Due to pleiotropic effects, NGF has been investigated<sup>5,6</sup> for applications in a variety of clinical purposes related to eye diseases, including sensory neuropathies, corneal ulcers, and dry eye. Dry eye syndrome is characterized by ocular surface inflammation, due to an alteration of tear film composition,

and the consequent imbalance of ocular surface homeostasis, with severe impairment in the patient's quality of life<sup>7,8</sup>. The chronicity of disease requires long-term treatment, implying several common causes linked to life-styling (cigarette smoke, allergies, low humidity, pollution, as well as abuse of tablets/computers or antidepressants, antihypertensives, and antihistamines)<sup>9</sup>. Pathological conditions related to dry eye are blepharitis, rosacea, and rhino-conjunctivitis. In 2020<sup>10</sup>, a clinical trial showed both the safety and tolerability of rhNGF eye drop administration in patients with dry eye<sup>6</sup>. Recently, Frampton<sup>11</sup> has demonstrated that the Varenicline solution administered as a nasal spray is a fast-acting, effective, and well-tolerated treatment for DED, offering useful advantages over existing topical ocular therapies in terms of increasing endogenous tear secretion and reducing ophthalmic treatment burden.

Anatomically, the eye and nose are closely connected by the nasolacrimal apparatus. Although only a part of topically administered ocular formulations may combine with tears and drain into the nose, it is not yet clear whether nasally administered drugs can reach the ocular surface via a direct route<sup>12</sup>. In fact, several obstacles may impair the route of spray drugs from nose to eye, such as gravity, nasal cavity anatomy, and the presence of mucous/cilia<sup>12</sup>.

In this study, we examined the effects of 3-month self-administration of high-pressure isotonic solution on some clinical features of the ocular surface and the tear film stability in subjects suffering from dry eye.

## Subjects and Methods

### Study Population

This observational self-controlled case study was conducted retrospectively in 16 patients (9 males, 7 females) suffering from dry eye. Diagnosis was made by complete ocular observation and tear film evaluation. The age of the study population ranged from 40 to 65 years. Exclusion criteria were: age <18 years old, patients undergoing dry eye therapies, or patients with exacerbating factor continuous exposure (video terminal work, contact lens wearers, cold room work). Patients having rosacea, Sjogren's Disease, herpetic keratitis, mucous pemphigoid, congenital corneal damage, or undergoing previous eye surgery were also excluded from the study. After adhering to the study and signing the informed consent, patients were

asked to self-administer HPpSIS (high-pressure physiological isotonic solution) for three consecutive months, according to a previous protocol<sup>13</sup>.

### Clinical Examination and Bioinstrumental Analysis

All patients underwent a complete ophthalmological examination for diagnosis of dry eye, and they repeated the right eye tests after 3 months from recruitment. Participants were asked to fill in the Ocular Surface Disease Index (OSDI; Allergan Inc, Irvine, California) for symptoms of ocular dryness<sup>14</sup>. Tests for tear film analysis included the Schirmer test, the non-invasive tear film break time (NIBUT), the Lacrimal Meniscus Height (LMH), and the Lipid Layer (LL) thickness, as described below in detail.

- OSDI: This is a 12-item patient-reported outcome questionnaire designed to quantify ocular disability due to dry eye disease. The questionnaire classifies the disorder as "severe dry eye condition" (75-100 points), "moderate dry eye condition" (50-75 points), and "mild dry eye condition" (25-50 points), with respect to "normal ocular surface" (0-25 points), as previously described. OSDI questionnaire was administered to patients to evaluate symptoms of ocular dryness before and after treatment. Of note, the test evaluates the perception of the subject's symptoms during the last week before the questionnaire.
- Schirmer test: This diagnostic test was used to measure tear film by placing a membrane strip in the conjunctival fornix and waiting for 5 min. The portion of the moistened strip was then registered. Test cut-off is usually 15 mm for youths and 10 mm for elders<sup>15</sup>.
- NIBUT: This routine test was used to assess the tear film stability above the ocular surface in a higher sensitivity and specificity. This is a non-invasive alternative to the classical BUT using fluorescein dye. The first tear film break was evaluated by automatic detection. The test cut-off was 12 sec<sup>16</sup>.
- LMH: The test was used to estimate the amount of water produced by the lacrimal gland, evaluating the tear deposit on the lower eyelid. The human eye should have a tear deposit of about 0.22 mm<sup>17</sup>.
- LL thickness: The test of Interferometry was used to measure the thickness of the lipid layer and to consequently measure the secretion of the Meibomian glands. The cut-off value was 80 nm<sup>18</sup>.

### Statistical Analysis

Prism 9.1 (GraphPad Software Inc., San Diego, CA, USA) software and R-studio scripts (open-source programming language for statistical computing and graphics) were used for statistical comparisons and graphical representations. Row data (mean $\pm$ SD) were analyzed for adherence to the nominal of continuous variables. The normality of data sample distribution was assessed by the Shapiro-Wilk and K-W tests. To assess differences between baseline (pre) and follow-up (post), comparisons were performed by the Wilcoxon Signed-Rank test for paired data. The statistical significance was set as  $p<0.05$ .

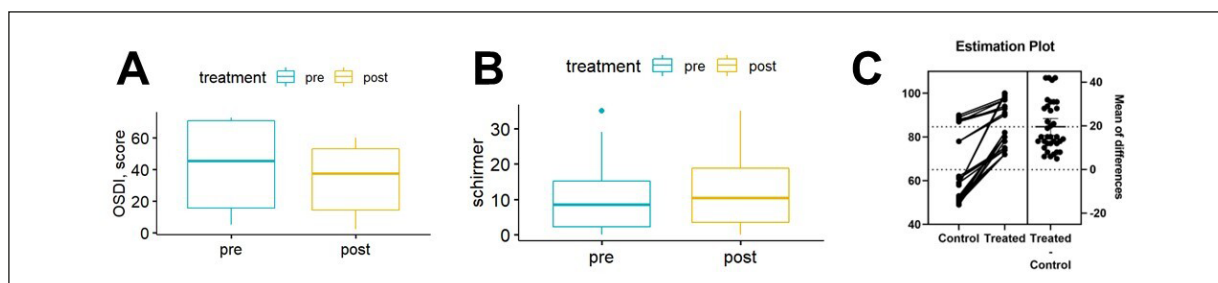
### Results

OSDI questionnaire administered before treatment identified severe, moderate, and mild dry eye subjects in respectively 61.1%, 5.5%, and 16.6%. Another group of three patients (16.6%) described poor symptoms at the beginning. They described random periods with more frequent eye disorders, so OSDI resulted in the norm. Except for these, the other patients were categorized as pathological. After treatment, OSDI mean values decreased significantly from  $44.40\pm 26.90$  to  $31.80\pm 19.40$  ( $p<0.05$ ). Since OSDI identified a large range of severe dry eye from 33 to 100 points, only two patients passed from the severe to the moderate stage after the therapy (Figure 1A). Schirmer test analysis did not show any significant improvement (Figure 1B). In two out of five tests, OSDI and lipid layer thickness (LL) scores improved with a statistically significant difference ( $p<0.05$ ). The difference was significant despite the poor sample population (Figure 1C). As shown in Figure 2A, LL thickness improved

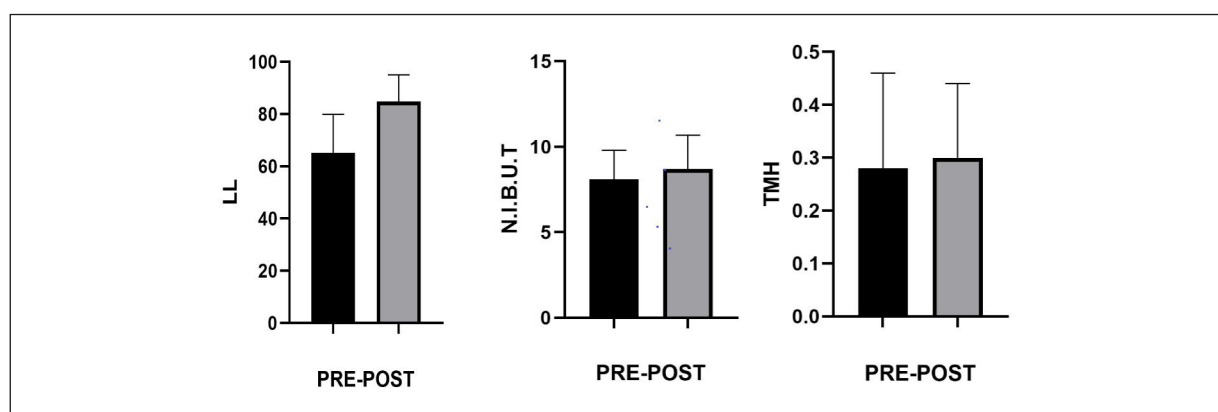
from  $65.00\pm 14.70$  to  $84.80\pm 10.20$  upon HPpSIS treatment (cut-off 80 nm). In the other two scales (NIBUT and TMH), the average values showed an improvement in the post-treatment, although differences were not significant (Figure 2). In fact, NIBUT ( $8.10\pm 1.70$  vs.  $8.70\pm 1.90$ ; cut-off 10 sec; Figure 2B) and TMH ( $0.28\pm 0.18$  vs.  $0.30\pm 0.10$ ; cut-off 0.22 mm; Figure 2C) values before and after treatment were quite similar. The variability of numerical distribution pre/post therapy suggests poor test sensitivity.

### Discussion

The multifactorial dry eye is one of the most common pathologies found in ophthalmological practice, and various treatment options are available mainly for relieving ocular symptoms<sup>7,19,20</sup>. Early therapies include the use of artificial tears for lubricating the impaired ocular epithelial layer and reducing friction<sup>10</sup>. Several medications have been developed with the aim of reducing ocular surface inflammation (immunosuppressive drugs and/or steroids), with poor ability to restore impaired tear film<sup>21</sup>. Since natural tears are better than artificial once, since they contain more than just aqueous and a mixture of lipids, proteins, mucins, and electrolytes, the possibility of finding alternative ways to ameliorate dryness at the ocular surface cannot be excluded<sup>21</sup>. More recently, the possibility to restore the tear film composition has already been prospected in literature by alternative therapies, rather than by topical application. An example of this is the varenicline nasal spray solution, a cholinergic agonist thought to pharmacologically activate the trigeminal parasympathetic pathway receptors in the treatment of the dry eye<sup>11</sup>.



**Figure 1.** OSDI, Schirmer, and Lipid thickness pre/post-therapy. Ophthalmological examination and bioinstrumental analysis of tear film composition between pre- and post-therapy. Subjects were asked to fill in the OSDI questionnaire and thereafter subjected to Schirmer and LL thickness evaluation. The mean values of OSDI score (A), Schirmer quantification (B) and LL thickness (C) were compared between baseline and post therapy.



**Figure 2.** Nasal HPpSIS treatment improved the tear film properties. Ophthalmological examination and bioinstrumental analysis of tear film composition between pre and post-therapy. **A**, Lipid layer thickness: note the improvement of LL after treatment (LL, cut-off 80 nm). **B**, Non-invasive Tear break-up time (TBUT): 10 sec is usually suspicious and may indicate tear film instability (NIBUT, cut-off 10 sec); **C**, TMH (cut-off 0.22 mm). Real values are mean±SEM. Comparison between the subgroups was assessed by Student *t*-test with a *p*-value set at 5% confidence. \*indicates *p*<0.05.

Herein, the HPpSIS treatment significantly reduced the OSDI score and significantly improved the LL thickness in dry-eye subjects undergoing a continuous 3-month treatment. In clinical practice, the assessment of a patient's perception remains a fundamental parameter in the diagnosis of dry eye<sup>14</sup>. Herein, the OSDI questionnaire allowed a better categorization of the study population in severe, moderate, and mild symptoms, and the decreased OSDI score would imply a relief mechanism, most probably HPpSIS-driven. Since the tear film composition includes lipids, proteins, glycoproteins (mucins), and electrolytes, all contributing to the integrity of the tear film, some bioinstrumental analyses were carried out. While LL thickness was significantly enhanced, only a slight correction was observed for the Schirmer test, BUT, and TMH parameters.

A probable explanation for HPpSIS effect might be the local release/accumulation of mediators able to improve tear film quality and restore ocular surface homeostasis. A potential candidate in this challenge might be NGF, as this pleiotropic factor was found to improve mucin quality and restore innervation if i) topically applied in experimental dry eye and ii) used as rhNGF in a clinical trial<sup>10,22</sup>. Related to this study, HPpSIS was found to stimulate the release of NGF in the nasal cavity and was also detected inside the brain<sup>2</sup>. The pleiotropic effects played by NGF in eye homeostasis have been extensively described in literature. Pharmacodynamics studies<sup>23</sup> have shown that NGF topically administered on the ocular surface not only reaches the ocular surface

but also the retina and the optic nerve. In dry-eye suffering patients, the initial damage was associated with an increase of tear NGF, most probably due to the role of this factor at the beginning of the inflammatory process<sup>6</sup>. It has been hypothesized<sup>6,24</sup> that NGF increase might not enhance the disease pathogenesis but, as a consequence of damage to the ocular surface, NGF might be released in a compensatory mechanism and promote protection/homeostasis. A clinical trial by Sacchetti et al<sup>10</sup> showed that recombinant human NGF, administered as eye drops in patients with dry eye, can significantly ameliorate disease, providing promising effects for therapy. In particular, NGF seems to stimulate the proliferation and differentiation of conjunctival and corneal in vitro cultured epithelial cells and induce the mucin release by goblet cells, helping the correct tear film homeostasis<sup>6,22,25</sup>.

A further aspect is the ability of NGF, like other neuromediators, to influence the lacrimal glands via parasympathetic stimulation, creating a feedback mechanism during the inflammation process<sup>26</sup>. Chronic inflammation indeed can cause neurovascular dysregulation, inducing atrophy of peripheral innervation and acinar glands<sup>27</sup>. Studies<sup>4,28,29</sup> on the peripheral nervous system have revealed a pleiotropic impact of NGF in the visual pathway, as in experimental monocular sensory deprivation studies. Whereas, NGF-receptor knock-out mice showed a reduced density of corneal fibers causing a decrease in sensitivity.

In addition to the concept that NGF “moves” through the neuronal sheaths, it is reasonable



to hypothesize that some molecules can travel from the nose to the eye via the trigeminal fibers, reaching the lacrimal glands via the vaso-ciliary nerve, including NGF. Since 1995, non-invasive intranasal delivery has been found<sup>30-32</sup> successful in bypassing the blood-brain barrier and reaching the brain, along the olfactory neural pathway and the trigeminal neural pathway.

Finally, the increase in ocular sensitivity and patient-perceived irritability correlates with an increase in NGF in tears, as a reflection of microscopic damage. In studies<sup>33,34</sup> on animal models, the administration of the above-mentioned laser-induced corneal lesion was able to increase the density of the sensitive fibers, with potential improvement of the dry eye.

### Limitations

A limitation of this study is the absence of tear NGF quantification between pre/post therapy, i.e., by using the Schirmer test collection and specific analysis. As illustrated in the graphical representation, the complete ophthalmological visit included the slit-lamp examination (A, normal; B, dry eye) and some bioinstrumental analyses (Schirmer, NI-BUT, LMH, LL). The biochemical analysis of tear composition carried out by Schirmers' strips is still under investigation.

Herein, the HPpSIS reduced OSDI score and ameliorated BUT and MHY (tear film quality). The possibility that NGF endogenous nasal stimulation could be helpful in dry eye pathology is supported by previous studies in literature showing the beneficial effects of NGF application and the potential necessity of endogenous molecules naturally expressed in the nasal cavity, following high-pressure nasal stimulation.

Taken together, the possibility of an endogenous HPpSIS-induced NGF should be taken into consideration, although further studies are required to confirm the presence of NGF and other neuromediators in tears.

### Conclusions

NGF endogenous nasal stimulation (with high-pressure isotonic solution) could be helpful in dry eye pathology. The beneficial role of NGF has already been studied at the eye level, using a recombinant molecule, while the authors, for the first time in the literature, support the use of endogenous molecules naturally expressed by the nose, following high-pressure nasal stimulation.

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### Conflicts of Interest

The authors declare no conflict of interest.

### Informed Consent

All patients signed an informed consent before being enrolled in the study.

### Funding

None.

### Authors' Contributions

All authors contributed to the study conception and design. The first conceptualization were performed by Fabrizio Salvinelli and Luca Gualdi. Material preparation, data collection and analysis were performed by Bijorn Balzamino, Alessandra Micera. The first draft of the manuscript was written by Francesca Bonifacio, Valeria Frari, Federica Gualdi. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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