The cold eye irrigation BSS solution used during phacoemulsification reduces post-surgery patients discomfort preventing the inflammation

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The cold eye irrigation BSS solution used during phacoemulsification reduces post-surgery patients discomfort preventing the inflammation.

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Abstract:	Purpose: The aim of this study was to assess whether the intraoperative use of the cold eye irrigation balanced salt solution (BSS) could have a protective effect in preventing the anterior chamber flare and conjunctival hyperemia and, thus, reducing patients discomfort after phacoemulsification. Materials and methods: 214 patients were enrolled and randomly divided into: patients whose eye were irrigated with BSS at ~ 20 ° C (Group 1) and patients whose eye were irrigated with BSS at 2.7 ° C (Group 2). Results: In patients of Group 2 the anterior chamber flare, the visual analogue score and the conjunctival hyperemia, used as parameters to evaluated clinical inflammation, at 1 day after surgery were significantly lower than of those in Group 1 who received BSS solution at operating room temperature (p<0.001), while at day 3, 5 and 30 there were not any significant differences. Conclusion: Our study provided evidence supporting the efficacy of the treatment with cold irrigation solution on reduction of anterior chamber flare, pain and conjunctival hyperemia already at 1 day after phacoemulsification suggesting that cooling procedure was fully effective at controlling early post-operative inflammation.

The cold eye irrigation BSS solution used during phacoemulsification reduces post-surgery patients discomfort preventing the inflammation.

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ABSTRACT

Purpose: ~~The protection of endothelial cells and the controlling of post-operative inflammation are always one of the major issues in the cataract procedures, and new surgical alternatives to achieve this goal continue to be sought.~~ The aim of this study was to assess whether the intraoperative use of the cold eye irrigation balanced salt solution (BSS) ~~solution~~ could have a protective effect in preventing the anterior chamber flare and conjunctival hyperemia and, thus, reducing patients discomfort after phacoemulsification.

Materials and methods: 214 patients were enrolled and randomly divided into: patients whose eye were irrigated with BSS at ~ ~~18.0~~ 20 ° C (Group 1) and patients whose eye were irrigated with BSS at 2.7 ° C (Group 2). Anterior chamber flare, visual analogue score and conjunctival hyperemia were evaluated at 1, 3, 5 and 30 day after surgery.

Results: In patients of Group 2 the anterior chamber flare, the visual analogue score and the conjunctival hyperemia, used as ~~all the clinical inflammation scores~~ parameters to evaluated clinical inflammation, at 1 day after surgery were significantly lower than of those in Group 1 who received BSS solution at operating room temperature ($p<0.001$), while at day 3, 5 and 30 there were not any significant differences.

Conclusion: Our study provided evidence supporting the efficacy of the treatment with cold irrigation solution on reduction of anterior chamber flare, pain and conjunctival hyperemia already at 1 day after phacoemulsification~~. The~~ suggesting that cooling procedure was fully effective at controlling early post-operative inflammation.

Keywords: phacoemulsification; cold irrigation balanced salt solution; anterior chamber flare; pain; conjunctival hyperemia

Short title: cold eye irrigation solution prevents post-surgery inflammation

INTRODUCTION

Cataract is a significant cause of visual impairment and blindness worldwide (1) (2). Nowadays, one of the most frequently performed surgeries is cataract and it is considered an ordinary practice in the ophthalmological field (3). Although the safety of the phacoemulsification technique has been markedly improved in terms of refractive results (4) and of decrease in the physical trauma related with the surgical procedure (5) (6) (7), the reduction of both the iatrogenic effects and the complication rates on the eye is still an important issue for all cataract surgeons (1) (8) (9). Indeed the surgical trauma-induced synthesis and release of inflammatory mediators have not been fully eliminated (10). ~~However a~~ Although inflammation is required in tissue healing ~~is usually self-limited (11) for~~ due to the beneficial mediators produced (12) (13) and it is usually self-limited (11), uncontrolled inflammation may cause possible post-operative complications, such as increased intraocular pressure, cystoid macular edema, posterior capsule opacification (14), rarely endophthalmitis, secondary glaucoma (5), triggering discomfort or even severe pain to the patients (15) (16), delayed recovery, and possible suboptimal visual results (17) (18) (19).

Adverse effects at cellular and subcellular level that can contribute to ocular inflammation during cataract surgery are attributed to mechanical, thermal, and chemical mechanisms. Surgical instruments contact and turbulent fluids which are generated by the phaco tip's jackhammer effect are the causes of mechanical injury (20) (21), while high frequency ultrasound vibration at the tip or ~~by an~~ occlusion of the tip caused by the lens fragmentation during the emulsification are the causes of the thermal injury (22) (21). The imploding of cavitation bubbles that are generated during the procedure causes a chemical damage that is mediated by free radicals formed in the aqueous humor (23) (21).

Notably, we previously demonstrated that the use of an intraoperative cold solution irrigating the eye improves the outcome of this widely ~~practised~~ practiced surgical intervention; in fact, when the ~~as in patients whose~~ eyes were irrigated with balanced salt solution (BSS) at 2.7° C, in these patients the corneal endothelial density was significantly higher than the density measured ~~of those~~ in patients who received BSS solution at operating room temperature, therefore cold irrigation ~~ensuring~~ ensures a less traumatic surgical procedure (24). However, if the cooling procedure is protective against cells loss in patients affected by cataract, the focus of ~~our~~ the previous study has not been ~~paid~~ on pain or on other post-operative ocular symptoms. This aspect is of a relevant importance ~~at the light~~ considering that patients should ~~be provided~~ receive not only appropriate counseling on pain, but also support on pain management as part of routine intraoperative care to reduce afflictions and problems ~~arise~~ arising after the hospital discharge.

The aim of this study was to assess whether the intraoperative use of a cold solution irrigating the eye could be helping patients alleviate the post-operative discomfort due to cataract surgery. For this purpose, in 214 patients which underwent cataract surgery with phacoemulsification, at 1, 3, 5 and 30 days after surgery, we assessed the post-operative effects, on the anterior chamber flare, the pain and conjunctival hyperemia of intraoperative BSS at two different temperature: at ~~~18~~ 20 °C, that is the average temperature of the operating room, and at 2.7 °C.

MATERIAL and METHODS

The study was carried out in accordance with the Declaration of Helsinki for medical research involving human subjects and was authorized by the local Ethical Committee (number 43/19-0006654). Signed, informed and written consent was obtained from all patients accepting to be included in this study.

Two hundred fourteen otherwise healthy patients were randomly enrolled among patients that had to undergo cataract surgery from the central vitreous cavity, with an average age of 65 ± 7.3 (range: from 55 to 75) of both sexes. Different levels of cataracts were recruited, mainly middle degree (2/3) according to the classification of Lens Opacities Classification System III (LOCSIII) (25) (26).

The exclusion criteria from the study were: glaucoma, infections, autoimmune diseases, proliferative diabetic retinopathy, previous corneal diseases or any ocular surgical procedures, and malignant ~~neoplasias~~ neoplasms (24).

Three days prior to the cataract surgery, 1 % sodium hypochlorite 2X/day, 0.3 % and ofloxacin 1 gtt 3X/day ~~and bromfenac (0.9 mg/ml) 1 gtt 2X/day~~ were the medical treatments for all the patients. Topical ~~medicartillary insertion based~~ on tropicamide and phenylephrine (0.28 mg/5.4 mg) (Mydriasert®, Thèa Farma S.p.A., Milan, Italy) were used in all the patients (24). All patients were given benoxinate 0.4% drops 4 times at 3-minute intervals before surgery.

214 patients were enrolled and therefore randomly divided into: patients whose eye were irrigated with BSS maintained at room temperature in the operating room (about 20 °C) (Group 1) (*n* = 110 eyes of 110 patients) and patients whose eye irrigated with BSS at 2.7 ° C (Group 2) (*n* = 104 eyes of 104 patients). Cataract surgeries were carried out with different intensity setups on the basis of the cataract degree (24) by the same surgeon using a traditional OPMI Lumera 700 microscope (Carl Zeiss Vision Italia, S.p.A., Varese, Italy) and a SIGNATURE® Phacoemulsification System (Johnson & Johnson Vision Medical SpA, Pomezia - Roma, Italy) was used (24). A sample of patients was evaluated for anterior chamber temperature; measurements were obtained using the FLIR T440 (FLIR Systems AB, Wilsonville, USA).

In the post-operative period, each patient was treated with a combination of cortisone and antibiotics (betamethasone - chloramphenicol) 4X/day for 1 month, artificial tears (Ialuvit®) (Alfa Intes Industria Terapeutica Splendore S.r.l., Naples, Italy) 4X/day for 1 month and bromfenac (0.9 mg/ml) 3X/day for a month.

All patients underwent an ophthalmologic assessment including anterior chamber flare, pain, and conjunctival hyperemia evaluation on days 1, 3, 5 and 30 after cataract surgery. Neither the patients nor the same examiner was informed about the temperature of BSS used during the cataract surgery.

Clinical grade of anterior chamber flare

Immediately after clinical assessment, patients had flare readings measured by an experienced technician using the slit lamp, according to the manufacturer's guidelines (27). ~~All patients were assessed by the same technician.~~ Based on SUN, the standardization of uveitis nomenclature (28) (29) (30), the aqueous flare was graded as follows: in the absence of any notable flare 0, for faint flare 1+, for moderate flare (iris and lens details are clear) 2+, for marked flare (iris and lens details are hazy) 3+, and for intense flare (fibrin in the aqueous humor) 4+ (31).

Clinical grade of pain

The average pain intensity was evaluated in each case using the Visual Analogue Score (VAS) (32) (33). A modified VAS scale 100 cm in length (equivalent to 100 degrees) was used, with its numbers (degrees) being visible only on the side of the examiner (34). The examiner explained to the patient that the 0 point represented no pain and that the 100 point represented the most intense pain he or she felt throughout the surgical procedure (34). Each patient was encouraged to pass the marker along the scale and to point out to the number.

Clinical grade of conjunctival hyperemia

Clinical grade of conjunctival hyperemia occurred at the temporal bulbar conjunctiva was evaluated in each case on the basis of the number of dilated vessels the day post surgery and at 30 ± 2 (SD) days (35). The palpebral conjunctiva is not evaluated. The clinical grade scores were: none, no hyperemia of the bulbar conjunctiva 0; mild, the dilation of a few conjunctival blood vessels (1), moderate, the dilation of some conjunctival blood vessels (2) and severe, the dilation of many conjunctival blood vessels (3), based on Japanese guidelines for allergic conjunctival disease (36) (35). Clinical grades were evaluated by three medical ophthalmic physicians, using the photographs taken at each of the 6 time points (35). The most frequent grade value generated by the three physicians has been selected and, when the scores differed among the technicians, the maximum value has been ~~choosen~~ chosen. The mean score was used for the subsequent analysis. The degree of agreement among the three observers regarding the conjunctival hyperemia scores was also evaluated (37).

Statistical analysis

Based on the results of Shapiro-Wilk test of normality a parametric analysis was carried out. Student's paired and unpaired t-test was applied to compare pre- and post-surgery data for each group (intra-groups analysis) and pre- and post-surgery data between the two groups data (inter-group analysis), respectively. Analysis were performed using an open source R3.0 software package. Significance level was set at p= 0.05. A power calculation was done using StatSoft software. The power of the study, given a 1.5 fold change in flare, conjunctival hyperemia and VAS scores as a significant difference between the groups, was calculated to be 85% with an α= 0.05 and 100 randomly patients for each group enrolled.

Fleiss' κ factor using Microsoft Excel® XLSTAT (Redmond, WA, USA) (37) (38) was used to assess the degree of agreement among the three observers for the anterior chamber flare and conjunctival hyperemia scores.

RESULTS

The clinical study investigated the short (at 1 and 5 day after surgery) and long-term (at 30 day after surgery) the effects of the use using during phacoemulsification of two different temperatures of the BSS irrigating solutions on anterior chamber flare, pain and conjunctival hyperemia, which are the most common post-operative negative consequences after cataract surgeries.

Patients of Group 1, including 52 women and 58 men, received BSS solution at ~ 20 °C and patients of Group 2, including 49 women and 55 men, received BSS solution at 2.7 °C. The two groups were comparable with respect to age, sex and education grade ($p > 0.05$) (Table 1). Preoperative variables between the two groups were not significantly different and no surgical complications, such as capsule rupture or zonular dialysis in any eye, occurred. Particularly, in the group receiving cold BSS we did not observed any macular edema, intraocular pressure spike or posterior capsule opacification occurrence after surgery. None of the patients experienced intraoperative pain or immediate postoperative pain.

At day 1 after surgery Group 2 presented a significant lower mean flare than in Group 1 ($p= 0.04$). At day 3, 5, and 30 after treatment a statistical significant reduction of the mean flare evaluated at day 1 were observed in both groups; these measurements were comparable between Group 1 and 2 ($p>0.05$) (Table 1 2).

Moreover, t The mean pain score in Group 2 was significantly decreased if compared to Group 1 at day 1 after the surgery (Table 1 2). On the contrary, the mean pain score between Group 1 and Group 2 at day 3, 5, and 30 after the surgery was not significantly different (Table 2).

Considering the recorded scores, eighty two (78 %) of 104 patients in Group 2 considered the procedure less painful than patients in Group 1 in which seventy nine

~~(72 %) of 110 said surgery in the eye without cold intraocular irrigation was more painful.~~ Moreover, eighty-two patients (78%) in Group 2 reported a VAS score smaller than those recorded in Group 1 while seventy-nine patients (72%) in Group 1 reported a VAS score higher than those recorded in Group 2.

~~On the contrary, the mean pain score between Group 1 and Group 2 at day 30 after the surgery (Table 1) was not significantly different.~~

Furthermore, all patients of Group 2 showed a lower conjunctival hyperemia score at 1 day after the surgery, whereas at 3, 5 and 30 days the conjunctival hyperemia mean score of patients of Group 2 was equal to that of patients of Group 1. The κ coefficient (Fleiss' κ for the three observers) for the conjunctival hyperemia score grading in the right eye was moderate: 0.467 (95% confidence interval, 0.423–0.512). ~~At long term~~ In the long run, for each of the inflammation scores there ~~is~~ were not significant differences between the two groups at 30 days after surgery.

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	Patient whose eye were irrigated with BSS at ~20° C (Group 1) n = 110	Patient whose eye were irrigated with BSS at 2.7° C (Group 2) n = 104	
Gender			n.s.
Male	52	49	
Female	58	55	
Age (years)	69.02 ± 6	68.65 ± 7	n.s.
Nationality	italian	italian	n.s.
Education	0	0	n.s.
Primary	9	11	
Secondary	73	68	
Higher	28	25	

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214 **Table 1.** A descriptive table of variables by groups. p>0.05 (n.s.) Group 2 vs Group 1 before and
215 after treatments.

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~~**Variables**~~	~~**Group 1**~~ ~~(patients who received BSS solution at ~ 18°C) n = 110~~	~~**Group 2**~~ ~~(patients who received BSS solution at 2.7°C) n = 104~~	~~p value~~
~~**Flare at 1 day after surgery (photons/milliseconds)**~~	~~2.83 ± 0.41~~	~~1.68 ± 0.18~~	~~0.043~~
~~**Flare at 30 days after surgery (photons/milliseconds)**~~	~~0.51 ± 0.29~~	~~0.46 ± 0.31~~	~~0.293~~
~~p value~~	~~<0.0001~~	~~<0.0001~~	
~~**VAS at 1 day after surgery (units)**~~	~~6.72 ± 1.98~~	~~3.35 ± 2.12~~	~~<0.001~~
~~**VAS at 30 days after surgery (units)**~~	~~1.02 ± 0.77~~	~~0.95 ± 0.5~~	~~0.426~~
~~p value~~	~~<0.0001~~	~~<0.0001~~	
~~**conjunctival hyperemia at 1 day after surgery (units)**~~	~~2.2 ± 0.77~~	~~1.5 ± 0.5~~	~~<0.0001~~
~~**conjunctival hyperemia at 30 days after surgery (units)**~~	~~0.6 ± 0.5~~	~~0.5 ± 0.5~~	~~0.1387~~
~~p value~~	~~< 0.0001~~	~~< 0.0001~~	

Variables	Group 1 (patients who received BSS solution at with BSS at ~20° C) n = 110	Group 2 (patients who received BSS solution at 2.7°C) n = 104	p value
FLARE (photons/milliseconds)			
at 1 day after surgery	2.83 ± 0.41	1.68 ± 0.18	0.043
at 3 day after surgery	1.91 ± 0.61 [a]	1.18 ± 0.24 [b]	0.172
at 5 day after surgery	0.55 ± 0.31 [c]	0.53 ± 0.27 [c]	0.451
at 30 days after surgery	0.51 ± 0.29 [d]	0.46 ± 0.31 [d]	0.293
VAS (units)			
at 1 day after surgery	6.72 ± 1.98	3.35 ± 2.12	<0.001
at 3 day after surgery	3.64 ± 1.39 [a]	2.85 ± 1.55 [a]	0.273
at 5 day after surgery	1.82 ± 0.95 [b]	1.65 ± 1.12 [b]	0.390
at 30 days after surgery	1.02 ± 0.77 [c]	0.95 ± 0.5 [c]	0.426
CONJUNCTIVAL HYPEREMIA (units)			
at 1 day after surgery	2.2 ± 0.77	1.5 ± 0.5	<0.0001

at 3 day after surgery	1.1 ± 0.51 [a]	1.1 ± 0.49 [b]	0.250
at 5 day after surgery	0.8 ± 0.62 [c]	0.7 ± 0.62 [c]	0.110
at 30 days after surgery	0.6 ± 0.5 [d]	0.5 ± 0.5 [d]	0.139

Table ~~1~~ 2. Comparison of inflammation scores (anterior chamber flare, VAS, visual analogue score and conjunctival hyperemia) between the two groups. p value reported in table refers to intergroup comparison, and p values for intragroup comparison at different times after surgery are as follows: [a] $p = 0.03$ (1 day vs 3 day); [b] $p = 0.04$ (1 day vs 3 day); [c] $p < 0.0001$ (1 day vs 5 day); [d] $p < 0.0001$ (1 day vs 30 day) for flare; [a] $p < 0.001$ (1 day vs 3 day); [b] $p < 0.0001$ (1 day vs 5 day); [c] $p < 0.0001$ (1 day vs 30 day) for VAS; [a] $p = 0.015$ (1 day vs 3 day); [b] $p = 0.01$ (1 day vs 3 day); [c] $p < 0.0001$ (1 day vs 5 day); [d] $p < 0.0001$ (1 day vs 30 day) for conjuctival hyperemia.

DISCUSSION

The use of ultrasounds during phacoemulsification can lead to endothelial cell damage due to mechanical trauma (39) and also to the onset of an intraocular inflammatory status (40). Stănilă et al. demonstrated that the excessive amount of ultrasound energy during phacoemulsification increases the temperature (9) leading to a reduction of about 20 % of the human corneal endothelial cells (41).

~~Even today the optimal temperature of solutions for intraocular surgery, especially phacoemulsification, is controversial and the benefit of hypothermia during cataract surgery remains questionable (42).~~ ~~As our~~ Our previous study has demonstrated that the use of a cold irrigation solution has a fundamental role in decreasing the damage of corneal endothelial cells during phacoemulsification (24). Indeed, in post-operative we found a significant reduction of the loss of corneal endothelial cell density in patients treated with BSS at 2.7 °C compared to those were treated with BSS at ~ ~~18~~ 20 °C (24). Most strikingly, we observed that in patients affected by softer cataract, who require a percentage of ultrasounds less than 10 % of the maximum power, the corneal endothelial cell density was not significantly different in pre- and post-surgery when BSS was applied at cold temperature. Even in patients affected by medium and hard cataract and treated with higher ultrasound power (from 10 to 29 %) we ~~assisted to~~ detected a significant corneal endothelial cell ~~density saving~~ survival when BSS 2.7°C was used (24).

Although we ~~well~~ clearly established that the cooling of the irrigation solution during phacoemulsification prevents, almost in part, the endothelial damage, we had not ~~any evidence of~~ investigated the beneficial effects of a cold irrigating solution on the post-operative discomfort. Indeed, it is well known that changes in the integrity of the endothelium might result in edema (34) and, subsequently, in corneal opacity with associated visual loss (21) accompanied by a painful, debilitating foreign body sensation (42).

~~Praveen et al. reported that the use of moderately cooled BSS Plus does not affect post-operative corneal parameters and inflammation showing no detectable effect and benefit on the outcome of phacoemulsification (43). This could be ascribed to the composition of the BSS Plus; Even if ocular tissue is highly sensitive to depletion of cellular glutathione that can result in inflammation and cell apoptosis, the possible beneficial effect of glutathione supplementation has not been proven. as antioxidant molecule maintains the junctional complexes of corneal endothelial cells and protects the blood-aqueous barrier integrity acting as inflammatory response modulator. Indeed ocular tissue is highly sensitive to depletion of cellular glutathione that can result in inflammation and cell apoptosis.~~

In this study we demonstrated that the use of BSS at 2.7 °C during phacoemulsification is able to reduce the onset of inflammatory reaction after cataract surgery, as demonstrated by the reduction of flare, pain and conjunctival hyperemia 1 day after treatment. Our findings demonstrated the advantage of cold treatment in reducing the immediate negative impact of intervention on eye tissues, whereas at 3, 5, and 30 day after cataract surgery no significant difference between the two groups occurred, suggesting that cold BSS could reduce the local inflammation in the early post-surgical period. The early phase of recovery is the most critical, representing a moment in which the defenses of the tissues from inflammation could fail in some patients, hence the application of the cooling procedure investigated in this study could be important.

Taken together our *in vivo* data highlight the advantage of cold irrigating eye solution usage in preventing ~~patients discomfort~~ post-surgical ocular damage and thus lowering the clinical scores of inflammation already after 1 day. This is ascribable, at least in part, to the reduction of the temperature within phacoemulsification procedure as cold irrigation decreases the ultrasound thermal rise at the tip of the phacoemulsifier, which ~~that~~ contributes to cellular damage and inflammation. In fact, the anterior chamber temperature variation, measured with a thermal camera during phacoemulsification in presence of cold solution, was about of 6-8 degrees less than ~~that~~ the variation measured in presence of ultrasounds and room temperature solution (data not shown). However the authors do not rule out that high voltage ultrasounds generated by phacoemulsifier could cause the cellular damage by additional mechanisms other than heat generation. Indeed, using the cold BSS the cellular damage has not been completely prevented (24) suggesting that by the cooling procedure it is possible to reduce part of the harm attributable to a thermic effect of the phacoemulsification, but that the cellular damage could also be due to other temperature-independent mechanisms, such as cavitation (43).

~~It is well known that temperature is an~~ In addition to the reduction of damaging heating, temperature cooling could exert a beneficial metabolic effect. In fact, an important parameter of tissue metabolism is the temperature. Mitochondria are the primary source of cellular energy and their activity is central to the determination of metabolic rate and, consequently, the generation of metabolic heat (44). The environmental temperature can modulate the mitochondrial energy metabolism (45). Indeed, the cold temperature influenced the metabolism of human keratinocytes by enhancing the oxidative metabolism of mitochondria and not the glycolysis (45). This increment of mitochondrial activity supporting homeostatic thermogenesis was accompanied by a metabolic switch toward a catabolic quiescent phenotype and was triggered by ~~33°C~~ a drop of only 4 degrees compared to basal temperature. ~~Pamenter et al. demonstrated that mitochondrial respiration is more tightly coupled to the H^+ gradient in the response to cold, indicating that mitochondrial activity is more efficient at the 28°C rather than at 37°C. The authors conclude that the enhancement of mitochondrial function at colder temperature contributes to energy conservation and increases cellular viability in hypoxic murine brain demonstrating the therapeutic effect of hypothermia in a neurological disorder.~~ Based on these considerations it is plausible that the use of refrigerated irrigation solution during phacoemulsification, ~~mimicking a condition of hypothermia~~ lowering the anterior chamber temperature, could enhance the efficiency of mitochondrial respiration in order to generate heat and maintain the physiological intracellular temperature; the consequent increased energy production would ameliorate the adaptability of corneal endothelial mitochondria to tissue stress due to surgery ~~and could increase cell viability~~. As a consequence of a reduced damage to the cells, the cell death inducing a local acute inflammation is ~~lowered~~ decreasead. Indeed, patients belonging to Group 2 reported a ~~decreased~~ lower degree of anterior chamber flare, pain and conjunctival hyperemia, used as signs of inflammation (17).

~~Indeed,~~ The chemical effects of ultrasound in aqueous solution are attributed to acoustic cavitation, which refers to the formation, growth and collapse of small gas bubbles in liquids (46). The high temperature and pressure resulting from a collapsing gas bubble leads to thermal dissociation of water and a reactive oxygen species (ROS) overproduction (46), whereas in case of ultrasonic intensity below cavitation threshold ROS is not generated (47). Indeed, the generation of free radicals, through the phenomenon of sonolysis ($H_2O \rightarrow \cdot OH + \cdot H$) (48), and thus the consequent oxidative stress, are additional harmful factors for corneal endothelium ~~can be damaged~~ during phacoemulsification ~~as another harmful factor is oxidative stress which is due to the generation of free radicals through the phenomenon of sonolysis ($H_2O \rightarrow \cdot OH + \cdot H$) (46).~~

An attempt to prevent ROS formation has been proposed by Praveen et al. using a moderately cooled BSS Plus (supplemented with glutathione) without any detectable effect on post-operative corneal parameters and on inflammation (49). Therefore the possible impact of ROS generation remains to be investigated. ~~This could be ascribed to the composition of the BSS Plus;~~ ~~Even if ocular tissue is highly sensitive to depletion of cellular glutathione that can result in inflammation and cell apoptosis, the possible beneficial effect of glutathione supplementation in the BSS solution has not been proven suggesting that . as antioxidant molecule maintains the junctional complexes of corneal endothelial cells and protects the blood-aqueous barrier integrity acting as inflammatory response modulator. Indeed ocular tissue is highly sensitive to depletion of cellular glutathione that can result in inflammation and cell apoptosis.~~

The temperature abatement of the anterior chamber that in our study significantly decreased the damage of corneal cells (24) could possibly decrease ROS production thus reducing tissue oxidative stress and, subsequently, the ROS-mediated inflammation. ~~Moreover, as reported for the reduced cleaning efficiency of ultrasonic cleaning solutions among which acids and alkaline salts (49) (50), the cavitation intensity in presence of BSS solution, that is a balanced salt solution, could be different from the intensity cavitation of water.~~ As consequence, the ROS generation could be atypical in presence of a cold BSS solution as well as the types of ROS produced, their kinetic/homeostasis (50) and diffusion (51). Also in this case the reduced amount of ROS leads to a decrease damage of the tissue and ~~less~~ to a lighter inflammatory response. Moreover, the benefic effects of refrigerated intraocular irrigation solution could be due to the vasoconstriction, induced by cold, which could reduce the release of the pro-inflammatory mediators (34) during cataract surgery, similarly to the nonsteroidal antiinflammatory drugs effects (52).

CONCLUSIONS

~~One of the major issues in the cataract procedures is the protection of endothelial cells; thus, new surgical alternatives to achieve this goal continue to be sought. In light of~~ Supported by our previous encouraging results that have demonstrated the protective role of the cold irrigating solution in reducing endothelial cells damage, and based on the results of the present study we ~~now could affirm~~ believe that the use of this new technique could be definitely recommended in cataracts as it also contributes to reduce patients discomfort preventing a local inflammation in the early post-surgical period.

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Conflict of interest statement

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	Patient whose eye were irrigated with BSS at ~20° C (Group 1) n = 110	Patient whose eye were irrigated with BSS at 2.7° C (Group 2) n = 104	
Gender			n.s.
Male	52	49	
Female	58	55	
Age (years)	69.02 ± 6	68.65 ± 7	n.s.
Nationality	italian	italian	n.s.
Education	0	0	n.s.
Primary	9	11	
Secondary	73	68	
Higher	28	25	

Table 1. A descriptive table of variables by groups. $p>0.05$ (n.s.) Group 2 vs Group 1 before and after treatments.

Variables	**Group 1** (patients who received BSS solution at with BSS at ~20° C) n = 110	**Group 2** (patients who received BSS solution at 2.7°C) n = 104	p value
FLARE (photons/milliseconds)			
at 1 day after surgery	2.83 ± 0.41	1.68 ± 0.18	0.043
at 3 day after surgery	1.91 ± 0.61 [a]	1.18 ± 0.24 [b]	0.172
at 5 day after surgery	0.55 ± 0.31 [c]	0.53 ± 0.27 [c]	0.451
at 30 days after surgery	0.51 ± 0.29 [d]	0.46 ± 0.31 [d]	0.293
VAS (units)			
at 1 day after surgery	6.72 ± 1.98	3.35 ± 2.12	<0.001
at 3 day after surgery	3.64 ± 1.39 [a]	2.85 ± 1.55 [a]	0.273
at 5 day after surgery	1.82 ± 0.95 [b]	1.65 ± 1.12 [b]	0.390
at 30 days after surgery	1.02 ± 0.77 [c]	0.95 ± 0.5 [c]	0.426
CONJUNCTIVAL HYPEREMIA (units)			
at 1 day after surgery	2.2 ± 0.77	1.5 ± 0.5	<0.0001

at 3 day after surgery	1.1 ± 0.51 [a]	1.1 ± 0.49 [b]	0.250
at 5 day after surgery	0.8 ± 0.62 [c]	0.7 ± 0.62 [c]	0.110
at 30 days after surgery	0.6 ± 0.5 [d]	0.5 ± 0.5 [d]	0.139

Table 2. Comparison of inflammation scores (anterior chamber flare, VAS, visual analogue score and conjunctival hyperemia) between the two groups. p value reported in table refers to intergroup comparison, and p values for intragroup comparison at different times after surgery are as follows: [a] $p = 0.03$ (1 day vs 3 day); [b] $p = 0.04$ (1 day vs 3 day); [c] $p< 0.0001$ (1 day vs 5 day); [d] $p< 0.0001$ (1 day vs 30 day) for flare; [a] $p < 0.001$ (1 day vs 3 day); [b] $p < 0.0001$ (1 day vs 5 day); [c] $p < 0.0001$ (1 day vs 30 day) for VAS; [a] $p= 0.015$ (1 day vs 3 day); [b] $p= 0.01$ (1 day vs 3 day); [c] $p< 0.0001$ (1 day vs 5 day); [d] $p< 0.0001$ (1 day vs 30 day) for conjuctival hyperemia.