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Vitreous Humor Rheology After Nd:YAG Laser Photo Disruption

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Abstract This work aimed to consider the hazardous side effect of eye floaters treatment with Q-switched Nd:YAG laser on the protein and viscoelastic properties of the vitreous humor, and evaluate the protective role of vitamin C against laser photo disruption. Five groups of New Zealand rabbits were divided as follows: control group for $(n = 3)$ without any treatment, the second group $(n = 9)$ treated with Q-switched Nd:YAG laser energy of $5 \text{ mJ} \times 100$ pulse delivered to the anterior, middle, and posterior vitreous, respectively $(n = 3$ for each). The third group $(n = 9)$ received a daily dose of 25 mg/kg body weight vitamin C for 2 weeks, and then treated with laser as the previous group. The fourth group $(n = 9)$ treated with 10 mJ \times 50 pulse delivered to the anterior, middle, and posterior vitreous, respectively ($n = 3$ rabbits each). The fifth group ($n = 9$) received a daily dose of 25 mg/kg body weight vitamin C for 2 weeks, and then treated with laser as the previous group. After 2 weeks of laser treatment, the protein content, refractive index (RI), and the rheological properties of vitreous humor, such as consistency, shear stress, and viscosity, were determined. The results showed that, the anterior vitreous group exposed to of 5 mJ \times 100 pulse and/or supplemented with vitamin C, showed no obvious change. Furthermore, all other treated groups especially for mid-vitreous and posterior vitreous humor showed increase in the protein content, RI and the viscosity of vitreous humor. The flow index remained below unity

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indicating the non-Newtonian behavior of the vitreous humor. Application of Q-switched Nd:YAG laser should be restricted to the anterior vitreous humor to prevent the deleterious effect of laser on the gel state of the vitreous humor.

Keywords Consistency · Flow index · Refractive index · Total protein - Viscosity - Vitamin C

Introduction

The vitreous body is filled with a clear highly viscous gel called vitreous humor; it occupies about two-third of the eye's total volume. It provides structural support and serves as a shock absorber. The main constituents of the vitreous humor are water (99 %), collagen (0.2 %), fibrils, hyaluronic acid (0.2 %), and a small amount of soluble proteins. In addition, it contains several substances such as ascorbate, electrolytes, glucose, lactate, urea, and slight amounts of chromophore [[1\]](#page-6-0).

Q-swiched Nd:YAG laser has been frequently used in treatment of eye diseases such as eye floaters, diabetic retinopathy, occlusion of the central retinal vein, and neovascular membranes under the retinal pigment epithelium. It is also used for the treatment of other diseases such as vitreoretinal traction, sickle cell retinopathy, vitreal cyst, cystoid macular edema, macular fibrosis, retinal breaks, peripheral retinal degeneration, choroid melanomas, vitreous humor strands with attached retina, and retinal detachment [\[2](#page-6-0)].

Eye floaters are formed because of age-relateddegeneration of the vitreous humor or caused by inconsistencies in the vitreous humor viscosity. When applying Nd:YAG laser treatment for eye floaters, it is essential to seriously

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consider the possibility and the extent of complication. The most serious side effect consists of lesion in the tissue posterior to the target location [\[3](#page-6-0)]. It has been reported such as damage to the corneal endothelium, the lens, the artificial lens and the retina causing retinal hemorrhage, rupture of retinal vessels, and retinal breaks or retinal detachment [[4\]](#page-6-0).

Many studies reported that high level of vitamin C is considered quite helpful in managing eye floaters, strengthening the connective tissue in the eye, and maintaining the clarity and consistency of the vitreous. In addition, vitamin C accumulates in vitreous humor at concentration several times higher than in plasma [\[5](#page-6-0)]. It was suggested that vitamin C may serve as antioxidant, which reduces the oxidative damage and protects the ocular tissue from free radical attack [[6\]](#page-6-0).

Photo disruption is the process in which the laser pulses of nanosecond duration or shorter are used to induce the optical breakdown in tissue. Because of the high-power densities achieved at the focal point, electrons are stripped from their atom with concomitant cavitations (bubbles production) leading to plasma and shock wave formation [\[7](#page-6-0)]. The plasma burst with Nd:YAG laser assumes the shape of a long sphere about 30 μ in diameter. If the target location for the planned photo disruption is close to the retina, coagulation or small plasma explosions may occur in the retinal or the choroidal structure [[8\]](#page-6-0).

The Nd:YAG laser photo disruption is generated by two different physical mechanisms: the thermal effect causing tissue evaporation and the mechanical effect causing shock waves. The shock wave may cause complications such as retinal damage and even retinal detachment. The control of these complications depends on the energy level used and the distance from the target tissue and the neighboring ocular structure. If the energy levels are too high, cavitations' bubbles will be generated. These bubbles move into the vitreous humor cavity at a speed of 100 m/s and may cause microscopic retinal damage [\[9](#page-6-0)]. The present study was designed to investigate the effect of Nd:YAG photo disruption on the vitreous humor viscosity, and to evaluate whether oral supplementation of vitamin C may have a protective role against laser effect.

Thirty-nine New Zealand male rabbits weighing 2–2.5 kg were used in this study. The animals were selected from the

Materials and Methods

Animals and Groups

statement for the use of animals in ophthalmic and vision research. The rabbits were classified into five groups as follows:

Control group (three rabbits) without receiving any treatment.

Group (A) Nine rabbits were divided into three subgroups $(n = 3$ rabbits each) and each eye was received 5 mJ \times 100 pulse of Nd:YAG laser in the anterior, middle, and posterior vitreous humor, respectively.

Group (B) Rabbits $(n = 9)$ were received a daily dose of 25 mg/kg body weight of vitamin C by stomach tube started 2 weeks before laser application. Rabbits were divided into three subgroups $(n = 3$ rabbits each) and treated with 5 mJ \times 100 pulse of Nd:YAG laser in the anterior, middle, and posterior vitreous humor, respectively.

Group (C) Nine rabbits were divided into three subgroups $(n = 3$ rabbits each) and received 10 mJ \times 50 pulse of Nd:YAG laser in the anterior, middle, and posterior vitreous humor, respectively.

Group (D) Nine rabbits received a daily dose of 25 mg/kg body weight of vitamin C by stomach tube started 2 weeks before laser application. Rabbits were divided into three subgroups ($n = 3$ rabbits each) and treated with 10 mJ \times 50 pulse of Nd:YAG laser in the anterior, middle, and posterior vitreous humor, respectively. All the animals were left for 2 weeks after laser treatment.

Clinical Evaluation

Slit lamp biomicroscopic examinations of the eye were performed before vitreous humor photo disruption. All observations were made following papillary dilation with Mydriacyl eye drop 0.5 % (Alcon laboratories, Australia, Pty Ltd.). The results showed no signs of intraocular inflammation and no edema in all the eyes.

Laser Treatment

In accordance with the ARVO resolution on the use of animals in research, rabbits were generally anesthetized by using intramuscular ketamine hydrochloride (ketalar 2.5 mg/kg). Additionally, they received 0.4 % Benoxinate eye drops for local anesthesia. Four groups of rabbits underwent vitreous photo disruption using Q-switched Nd:YAG laser (OPTIMIS II Photodisruptor Laser, Quantel Medical, Inc., USA). The energy levels were 5 and 10 mJ per pulse; the spot size was 10 μ ; the cone angle was 16°; the wavelength was 1,064 nm; and the duration of the pulse was 4 ns. To get better focus of the Nd:YAG laser beam, it is important to use Panfundoscope lens, which offers high magnification and good visualization of the vitreous humor structure to be treated.

Sample Collection and Measurements

Rabbits were decapitated after the demonstrated period and the eyes were enucleated. The vitreous humor samples were collected from the posterior chamber rabbit's eyes with the use of a 21-gauge needle attached to a 10-ml sterile syringe. The needle is inserted carefully through the corneoscleral junction to avoid the lens. Vitreous humor was aspirated and the following measurements were carried out.

Estimation of total protein concentration for vitreous humor by the method of Lowry et al. [\[10](#page-6-0)], and refractive index (RI) using Abbe refractometer attached with temperature control unit at 37 ± 0.02 °C. The rheological properties of the vitreous humor were measured using digital viscometer type ''Brookfield DV-III, Eng. Lab., USA". Shear rate was varied from 7.5 to 375 s^{-1} . By changing the rate of shear, a series of values of the shear stress can be obtained, by plotting these values against one another, the rheological properties of vitreous humor can be defined directly in terms of a shear stress–shear rate diagram and flow curve (viscosity–shear rate diagram). All the measurements were taken using an insulating jacket at 25 C.

Statistical Evaluation

Protein levels and RI were compared between treated and untreated eyes by student t test $[11]$ $[11]$. Where t is the test of significance, differences were considered significant at $P < 0.05$.

Results

Vitreous Humor Protein and RI

Table (1) presents the vitreous humor protein content and the RI for the control and treated groups with and without vitamin C 2 weeks before laser photo disruption. The protein content in control vitreous was 6.98 ± 0.36 mg/ml, but after treatment with Nd:YAG laser, the protein content for the anterior vitreous (5 mJ \times 100 pulse) group showed a non-significant change compared with all other groups. The increase in protein content was more pronounced in mid-vitreous humor $(P < 0.01)$ and posterior vitreous humor $(P < 0.001)$ groups treated with energy of 10 mJ \times 50 pulse ($P < 0.001$) than 5 mJ \times 100 pulse. On

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* % Change with respect to the control group

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** The SD of the refractive index values is in the same sensitivity range of the instrument

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the other hand, the protein level was increased in all groups received vitamin C 2 weeks before laser treatment compared with the control group except for the anterior vitreous one. Furthermore, Table [1](#page-2-0) shows the direct relationship between the RI and the protein content for all groups treated with laser with and without receiving vitamin C.

The Rheological Properties of Vitreous Humor

The measurement of viscosity over a range of shear rates yield a viscosity flow curve of vitreous humor, as shown in Figs. 1, 2, and 3. For all treated groups (anterior, middle, and posterior vitreous humor) with and without receiving vitamin C, vitreous humor flow curve (viscosity against shear rate) shows decrease in the vitreous viscosity as the shear rate increase. It is characterized by two regions, the low-shear rate up to 225 s^{-1} , and high-shear rate region up to 375 s^{-1} at which no further reduction in viscosity is obtained. Low-shear rate region can be characterized by the consistency (low shear viscosity) and flow index, which

Fig. 1 Flow curve for control and anterior vitreous humor treated groups with Q-switched Nd:YAG laser

Fig. 2 Flow curve for control and mid-vitreous humor treated groups with Q-switched Nd:YAG laser

Fig. 3 Flow curve for control and posterior vitreous humor treated groups with Q-switched Nd:YAG laser

can be calculated from the power fitting of this range of the flow curve and calculated from the equation:

 $F = mS^n$

where F and S are the shear rate (s^{-1}) and shear stress (dyne/cm²), respectively, m is the measure of consistency in centi poise (cp) of the fluid, and the exponent n is the fluid flow index. The value of the flow index is less than unity for all treated groups indicating the non-Newtonian behavior of vitreous humor as shown in Table [2](#page-4-0).

High-shear rate at 375 s^{-1} can be characterized by apparent viscosity (η) . The viscosity of vitreous humor was increased for all treated groups with Nd:YAG laser and it was more pronounced in mid-vitreous humor and posterior vitreous humor (Table [2\)](#page-4-0). Moreover, the data indicated that the groups received vitamin C before Nd:YAG laser photo disruption showed obvious increase in viscosity than control, but the degree of increment was lower than the previous groups.

Figures [4,](#page-4-0) [5](#page-4-0), and [6](#page-4-0) show the power fitting for the shear rate–shear stress curves for the control and the treated groups. The data obtained from the figures indicated that there were detectable changes between the pattern of the control vitreous humor and those treated by Nd:YAG laser without and with vitamin C. Moreover, the pattern of shear rate–shear stress indicating that the vitreous humor for control and all treated groups is shear-thinning fluid (pseudo plastic).

Discussion

The vitreous humor is a very important intraocular fluid because of its optical function and its significant roles in the pathogenesis and treatment of the whole eye. Vitreous humor, present in the posterior cavity, often becomes dysfunctional due to floaters, which takes place during aging leading to separation of the vitreous humor and its

Table 2 Apparent viscosity, consistency, and flow index for control and treated groups with Nd:YAG laser

Group	Vitreous humor			Vitreous humor with vitamin C		
	Apparent viscosity (cp)	Consistency (cp)	Flow index	Apparent viscosity (cp)	Consistency $\left(\text{cp}\right)$	Flow index
Control	1.03 ± 0.02	40.37 ± 2.3	0.614 ± 0.02	1.03 ± 0.02	40.37 ± 2.3	0.614 ± 0.02
Anterior vitreous (5 mJ \times 100 pulse)	0.98 ± 0.01	30.61 ± 1.1	0.416 ± 0.03	0.98 ± 0.05	31.72 ± 1.01	0.585 ± 0.01
Anterior vitreous (10 mJ \times 50 pulse)	1.19 ± 0.02	36.36 ± 2.1	0.547 ± 0.02	1.08 ± 0.06	23 ± 1.2	0.520 ± 0.02
Med-vitreous $(5 \text{ mJ} \times 100 \text{ pulse})$	1.39 ± 0.03	52.11 ± 3.2	0.590 ± 0.04	1.26 ± 0.05	29.22 ± 1.9	0.808 ± 0.03
Med-vitreous (10 mJ \times 50 pulse)	1.92 ± 0.04	46.49 ± 2.9	0.508 ± 0.05	1.62 ± 0.04	26.84 ± 0.7	0.668 ± 0.02
Posterior vitreous $(5 \text{ mJ} \times 100 \text{ pulse})$	1.88 ± 0.04	33.31 ± 2.03	0.475 ± 0.04	1.63 ± 0.02	26.69 ± 0.6	0.450 ± 0.01
Posterior vitreous (10 mJ \times 50 pulse)	1.89 ± 0.03	30.87 ± 2.1	0.461 ± 0.02	1.64 ± 0.02	29.56 ± 0.9	0.666 ± 0.03

Fig. 4 The power fitting for the shear rate–shear stress curves for control and anterior vitreous humor treated groups with Q-switched Nd:YAG laser

Fig. 5 The power fitting for the shear rate–shear stress curves for control and mid-vitreous humor treated groups with Q-switched Nd:YAG laser

detachment from the retina, physical collapse, opacification, and vision loss [[12\]](#page-6-0). In addition, the destruction of vitreous humor can also occur by mechanical, chemical, and thermal trauma. This may result in collapse of vitreous, which has a great tendency to detach from the retina [\[13](#page-6-0)].

Fig. 6 The power fitting for the shear rate–shear stress curves for control and posterior vitreous humor treated groups with Q-switched Nd:YAG laser

In the present study, the characterization of vitreous humor protein after treatment with Q-switched Nd:YAG laser (5 mJ \times 100 pulse and 10 mJ \times 50 pulse) was evaluated with and without intake of vitamin C (Table [1](#page-2-0)). The results indicated elevated levels of vitreous humor protein percentage after laser for all treated groups except for the anterior vitreous. Previous report evidenced this increase of protein content in rabbit's eyes following retinal exposure to Nd:YAG laser. A transitory 50 % elevation above baseline in vitreous humor protein levels was observed during the second week after exposure [[14\]](#page-6-0). It was suggested that laser exposure was associated with an enhanced vitreous humor prostaglandins concentration, leading to disruption of the blood–retinal barrier by the long-term exposure to mildly elevated vitreous prostaglandins levels that was accountable for protein leakage. In addition, Nasisse et al. [[15\]](#page-6-0) reported that vascular injury due to Nd:YAG photo disruption results into the escape of erythrocytes from the vascular compartment indicated by the number of erythrocytes found in aqueous humor and extravascular uveal tissues. Consequently, the refractive indices were significantly increased, this may lead to impairment of vision due to light scattering. Obviously, the

present data show direct relation between the protein concentration and the RI.

Two major degenerative changes in vitreous humor can be distinguished: liquefaction (synchysis) and collapse (syneresis) [[16\]](#page-6-0). The former is characterized by specific biochemical degradation of the collagen or hyaluronic acid portions of the vitreous while the latter involves dispersion of liquid from the collagen network, usually as a result of collagen contraction. When either process occurs in individuals above the age of 50 years, it is generally considered to be physiological [[17\]](#page-6-0). Since, however, the collagen of a partly liquefied vitreous gel and syneresis of the gel mass may lead to rapid detachment of the vitreous humor from the retina; it is probably desirable to avoid inducing such changes in normal vitreous humor. Alternatively, it has been proposed that deliberate liquefaction of the vitreous humor may aid in reducing the vitreous traction on the retina [\[18](#page-6-0)] and speed resorption of hemorrhages [\[19](#page-6-0)]. Clearly, any changes that Q-switched Nd:YAG photo disruption may cause in vitreous humor are of considerable clinical significance. Generally, 6-month follow-up of patients, who had undergone Q-switched Nd:YAG laser, demonstrated an incidence of retinal detachment of only 0.4 % [[20\]](#page-6-0).

The rheological properties of vitreous humor deal with the relation between the viscosity at different shear rates. Viscosity measurement of the vitreous humor is complicated by the separated gel and liquid portion. When a relatively uniform gel portion of vitreous humor was inserted into the plate of the viscometer, the mechanical disruption caused by the rotation of the spindle induced a non-uniform syneresis of the sample. For example, viscosity measurements would be artificially low, if the remaining gel fraction had been displaced to the periphery of the plate. Even with these impediments, it is possible to make some qualitative and approximate quantitative assessments about the viscoelastic properties of the vitreous humor samples [\[9](#page-6-0)].

There are several types of non-Newtonian flow behavior, characterized by the changes of fluid viscosity in response to variation in shear rate. The most common types of non-Newtonian fluid are the shear thinning or called ''pseudo plastic'' (the viscosity decrease as the shear rate increase) and shear thickening or called ''dilatants'' (the viscosity increase as the shear rate increase) [\[21](#page-7-0)]. Under laminar flow conditions, a shear stress–shear rate relationship is used to define the fluidity of liquids and this relationship reflects the viscosity of a fluid [[22,](#page-7-0) [23\]](#page-7-0).

In the present work, the viscosity ranged from 5.2 to 17.5 cp at a shear rate of 7.5 s^{-1} to 0.96–1.92 cp at a shear rate of 375 s^{-1} . The nonlinear change in viscosity of vitreous with the changing shear rate is very clear that is characteristic of such non-Newtonian fluids. Moreover, vitreous humor proved to be thixotropic, that is, for each shear rate, there is a gradual exponential-like decrease in viscosity and the pattern for all treated groups are differed from the control one (Figs. [1,](#page-3-0) 2, [3\)](#page-3-0). Furthermore, there are detectable changes in the consistencies and the flow indices for all treated groups (Table [2\)](#page-4-0) and vitreous humor, like most biologic fluids are non-Newtonian shear-thinning fluid characterized by a marked nonlinearity of the shear stress to the shear rate ratio as indicated in Figures [4](#page-4-0), [5,](#page-4-0) and [6](#page-4-0). These results contradict the results of Krauss et al. [\[8](#page-6-0)], who reported no significant difference between the shear stress–shear rate pattern of irradiated vitreous (4 mJ \times 10 pulse and 10 mJ \times 100 pulse) and that of control.

The apparent viscosity (η) of a fluid has been defined by Newton as the ratio of shear stress to the shear rate of the fluid. The results indicate detectable increase in the apparent viscosity (at $375~\text{s}^{-1}$) of vitreous humor especially for midvitreous and posterior vitreous groups (Table [2](#page-4-0)). Moreover, the results were compatible with the increase in protein contents in which, as the protein concentration of a fluid increase its viscosity also increase. In addition, the results indicate pronounced increase in vitreous humor viscosity of the groups treated with 10 mJ \times 50 pulse and received a daily dose of vitamin C 2 weeks before laser (Table [2](#page-4-0)). By comparing the energy level of laser, the present data are in disagreement with Lerman et al. [[24\]](#page-7-0). He reported that the Nd:YAG laser (4 mJ \times 10 pulse), whether focused on the posterior lens capsule or mid-vitreous of the rabbits eyes, caused considerable alteration of the molecular structure of the vitreous humor, manifested by an average decrease of 44 % in the viscosity associated with retinal hole formation and retinal detachment. The present work hypothesized that when the vitreous humor floaters were treated with the Nd:YAG laser, it induces optical breakdown. Because of the short-pulsed nature and the highly localized site of this laser, the temperature reaches several thousand degrees Kelvin, but the extremely short duration of the energy increase makes widespread thermal effects unlikely and produces a shock wave. The shock wave transient could conceivably collapse the vitreous humor in a purely mechanical fashion, resulting in increase in its viscosity.

There is a relationship between vitreous humor photo disruption and vitamin C concentration in the eye. The function of vitamin C is to scavenge free radicals of the eyes and protect against oxidative or photo-oxidative damage [[25,](#page-7-0) [26](#page-7-0)]. It was concluded that light stimulated the reaction of vitamin C with oxygen to produce dehydroascorbic acid and hydrogen peroxide [[27–29\]](#page-7-0). Vitamin C accumulates in ocular tissues several times higher than in plasma, and furthermore, is at a higher concentrations than other watersoluble antioxidants in the ocular tissue. Vitamin C levels are critical to the overall antioxidant protection of the eye. It has been shown that the amount of radiation delivered by a visible laser was directly proportional to the amount of ascorbic acid oxidized to dehydro-L-ascorbic acid [[30\]](#page-7-0). This implies

that vitamin C is one of the first antioxidants used to quench light-induced free radicals. This would be expected since ascorbic acid is very effective at quenching hydrogen peroxide radicals, one of the major secondary free radicals formed during the quenching of superoxide by SOD (superoxide is formed directly from light energy). Vitamin C is also able to protect alpha tocopherol (Vitamin E) from oxidation within the rod outer segments [[31\]](#page-7-0), a function that is enhanced by both glutathione and lipoic acid. The interrelationship between Vitamin C and glutathione is an interesting and important one in the regeneration of ocular antioxidants and retarding disease potential. One to two grams per day of vitamin C (as ascorbic acid) should provide more than adequate levels of this water-soluble antioxidant in the ocular tissues [\[32](#page-7-0)]).

Changes in the gel state structure of the vitreous humor, because of laser photo disruption may lead to decrease of antioxidant, which normally protect the vitreous humor from free radical. This would lead to loss of vitreous function to scavenge free radicals. Consequently, vitamin C becomes less efficient than that in the normal eye. This result is in agreement with a previous study, which concluded that the supplementation of vitamin C 2 weeks before Nd:YAG laser did not improve or protect against the effect of laser photo disruption [\[33](#page-7-0)].

Some precautions must be taken during the treatment of floaters to protect the vitreous humor against laser effects. Laser must be focused anterior to the treatment area because the propagation of the laser damage is posterior, and energy values close to threshold must be used, keeping a safe distance between a target tissue and the neighboring tissue as the retina and the lens [18]. The present work may provide a model to compare the relative effect of different treatment parameters to help in establishing the least adverse effects. The result proved that vitreous humor is a non-Newtonian and shear-thinning fluid. Laser tissue interaction after photo disruption with the two energy protocols (5 mJ \times 100 pulse and 10 mJ \times 50 pulse) of Q-switched Nd:YAG laser-induced optical breakdown. Therefore, this laser effect could conceivably collapse the vitreous in a purely mechanical fashion changing its protein content, RI, viscosity, and consistency. The effect of laser on vitreous humor is site and energy dependent. When it is applied near the retina, the effect will be more pronounced. Oral supplementation of vitamin C 2 weeks before Q-switched Nd:YAG laser may have inefficient protective effect against laser complications and it should be given for 1–2 weeks after laser treatment.

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