Liquefaction of the Vitreous Humor floaters is a Risk Factor for Lens Opacity and Retinal Dysfunction

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Abstract: Rabbits' eyes were exposed to vitreous humor liquefaction with Q - switched Nd- YAG laser. The biophysical changes in the lens crystallin and the bioelectricity of the retina were investigated. The rabbits were divided into two main groups (n= 12 each). The first group was divided into three subgroups (n=4) and then treated with 500 mJ laser energy delivered to the anterior, middle and posterior vitreous respectively. The second group was received a daily dose of 25 mg/Kg vitamin C for two weeks then divided into three subgroups and treated with laser in the same manner as the first group. Measurements of total protein content, refractive index (RI), sodium dodecyle sulfate poly acrylamide gel electrophoresis (SDS-PAGE) for the lens protein and electroretinogram (ERG) for the retina were studied. The results indicated decease in the soluble lens protein content, increase in the refractive index, pronounced change in the electrophoresis pattern of lens protein and reduction in ERG a- and b- waves amplitude, and latency. Application of Q-switched Nd-YAG laser in vitreous liquefaction induces lens opacity, and retinal dysfunction. Although there were some sort of improvement in lenses and retinas supplemented with vitamin C, it cannot protect them against laser oxidative damage.

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Key Words: Nd-YAG laser; vitamin C; Lens protein; Refractive index; SDS- PAGE; Electroretinogram.

1. Introduction

The vitreous humor is a clear viscous gel, occupies about 90% of the eye's total volume. It provides structural support and serves as a shock absorber. Vitreous floaters are a common complaint in the ophthalmic care setting patients (Sendrowski and Bronstein 2010). Floaters are deposits of different size, shape, consistency, refractive index and motility within the eye's vitreous humor.

Floaters can appear alone or together in the field of vision as spots, threads or fragments, they are not optical illusions but are entopic phenomena (Benhamou *et al*. 1998, Delaney *et al*. 2002, Roth *et al*. 2005). Most commonly, there is no treatment recommended. Vitrectomy may be successful in treating more severe cases (Roth *et al*. 2005) but not warranted in minor symptoms cases due to the potential for complications and severe infection. In laser vitreolysis, usually Nd-YAG laser is focused onto the floater in a series of short bursts to vaporize and lyses the collagen strands of the floaters. Vitreolysis is much less invasive to the eye than vitrectomy with potentially fewer side effects (Delaney *et al*. 2002) that include retinal detachment occurred shortly after Nd- YAG vitreolysis (Benhamou *et al*. 1998). It was reported that, Nd-YAG laser, when focused in mid-vitreous, causes considerable alteration of the molecular structure of the vitreous. These changes may be associated with more serious complications (Krauss *et al*. 1986) such as lesion in the tissue posterior to the target location, damage to the corneal endothelium, the lens, the artificial lens, and the retina causing retinal hemorrhage, rupture of retinal vessels, retinal breaks, or retinal detachment. Another study stated that, when Nd-YAG laser focused in the lens nucleus or vitreous center plane and its pulse energy was 7.1 – 9.3 mJ with a total of 75-100 pulses that lead to injury to the vitreous body with macular edema, retinal detachment, and cataract development (Stepanov *et al*.1990).

Photo disruption is the process in which laser pulses of nanosecond duration or shorter are used to induce optical breakdown in tissue. Because of the high- power densities achieved at the focal point, electrons are stripped from their atom causing cavitations (bubbles production), plasma and shock waves formation (Steinert and Puliafito 1985). The shock waves transient could collapse the vitreous in purely mechanical fashion causing complications such as tissue disruption, retinal damage and even retinal detachment. The cavitations bubbles will move into the vitreous cavity with a rate of 100 m/sec and may cause microscopic retinal damage (Puliafito *et al*. 1984). The control of these complications depends on the energy level used and the distance from the target tissue and the neighboring ocular structure.

Electrorentinogram (ERG) can be used to estimate the extent of functional retina in cases of retinal detachment. In general, ERG b-wave amplitudes correspond to the amount of attached healthy retina, although the detached retina may function for some time (Kolb *et al*. 2011). There are several studies in different animal models have considered the potential for different antioxidant to prevent the development and progression of eye diseases. The most famous one is vitamin C, which plays an important role in lens and retina against oxidative stress (Ropredo *et al*. 2005); slowing down the progression of age - macular degeneration (Oslon *et al*. 2011) and having therapeutic potential for diseases associated with oxidative stress particularly degenerative retinal diseases (Jistanong *et al*. 2011).

The aim of the present work is to study the effect of vitreous liquefaction on the lens and retina and to evaluate if dietary supplementation of vitamin C can help to protect the lens and the retina against the photo oxidative damage of Q-switched Nd –YAG laser vitreolysis.

2. Materials and methods

Groups

Twenty-seven Newzealand male rabbits weighing 2-2.5 kg were used in this study. The animals were selected from the animal house of Research Institute of Ophthalmology, Giza, Egypt and were feed on a balanced diet. All procedures were conducted according to the ARVO statement for the use of animals in ophthalmic and vision research. Three rabbits were used as control, and the rest of rabbits were classified into two main groups (n=12).

Group (I) : divided into three subgroup (four rabbits each) and received 500 mJ of Q- switched Nd-YAG laser in the anterior, middle and posterior vitreous humor respectively, and the animals were left for two weeks.

Group (II): received a daily dose of 25 mg/ kg body weight of vitamin C by stomach tube. This dose was started two weeks before laser application. The rabbits were treated with laser in the same manner as the previous group, and the animals were left for two weeks.

Clinical examination

Before vitreous liquefaction, papillary mydriasis was induced in rabbits' eyes using "Mydriacyl" eye drop 0.5% (Alcon laboratories, Australia, Pty Ltd.) followed by slit lamp biomicroscopic examinations. The results showed no signs of edema or intraocular inflammation in all eyes.

Laser treatment

Animals were generally anesthetized using intramuscular ketamine hydrochloride (ketalar 2.5mg/ kg) and 0.4% Benoxinate eye drops was used for local anesthesia. Rabbits underwent vitreous liquefaction using Q- switched Nd-YAG laser. The energy was 5 mJ x100 pulse, the spot size was 10 microns, the cone angle was 16 degree, the wavelength was 1064 nm, and the duration of the pulse was 4 n sec. A Panfundoscope lens was used to enhance a better focusing of the Nd-YAG laser beam, a high magnification and a good visualization of the vitreous structure.

Electroretinogram

After the demonstrated periods, the rabbits were dark adapted for 2 hours before ERG recording and anesthetized by intramuscular injection. The ERG was recorded by using three Ag –AgCl electrodes placed on rabbit skin. The active electrode was placed near the margin of the lower eyelid, the reference electrode was placed on the forehead, and the earth electrode was clipped to the earlobe. Fifty flashes were used with a flash energy of 0.2 joule and a frequency of 1 HZ i.e. one flash per second and background intensity of zero. The flashes were derived from a computerized system (EREV 99 apparatus, lace Eletronica Co-, Italy).

Lens protein separation

Rabbits were decapitated, and the eyes were enucleated. The lenses were removed from the anterior chamber, weighted and homogenized in bi-distilled water and then centrifuged at 8,000 (rpm) for 30 minutes. The supernatant were separated for the determination of soluble lens protein according to the method of Lowry *et al*. (1951), refractive index (RI) using Abbe refractometer attached with temperature control unit at 37°C ± 0.02 and Sodium dodecyl sulfate- polyacrylamide gel electrophoresis (SDS-PAGE) analysis according to Laemmli (1970) using 4% stacking gel and 10% separating gel.

Statistical analysis

Statistical comparison was performed between treated and untreated eyes using the Student's t- test. The results were presented as the mean ± SD and studies were repeated at least four times independently. Differences were considered significant at $P< 0.05$.

3. Results

Protein content

The protein content for normal rabbits' lens was 95.2±1.2 mg/ g tissue wet weight (Figure1). After treatment of the anterior, Middle and posterior vitreous with energy of 500 mJ delivered from Q-switched Nd- YAG laser, there were very high significant decreases ($P< 0.001$) in soluble lens protein concentration, with respect to the control group (40.2±0.9, 48.2±0.5 and 53.4±0.4 mg/g respectively). The groups supplemented daily with

25 mg/kg of vitamin C for two weeks before laser treatment also showed very high significant decreases (P< 0.001) with respect to the control group (56.9 ±0.7, 62.2±1.1 and 63.9±1.3 mg/ g respectively). In addition, the data indicate that, the decreasing of protein concentration in the treated groups without receiving vitamin C is higher than the groups supplemented with vitamin C.

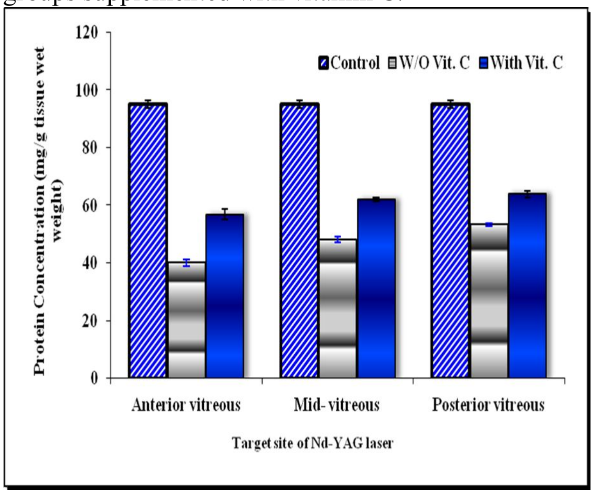

Figure1. Lens protein concentration for treated rabbits' vitreous humor with 500 mJ delivered from Q- switched Nd- YAG laser W/O and with vitamin C supplementation.

Refractive index measurements

Figure (2) showed that, the refractive index for normal rabbits' lens was 1.3405. After treatment of the anterior, Middle and posterior vitreous with Q-switched Nd- YAG laser, there were very high significant increases (P< 0.001) in refractive indices values, with respect to the control group (1.3490, 1.3455 and 1.3426 respectively). Moreover, the groups daily supplemented with 25 mg/kg of vitamin C for two weeks before laser treatment, showed very high significant increases (P< 0.001) with respect to the control group (1.3469, 1.3444 and 1.3420 respectively). Moreover, the data indicated that, the increase in the refractive indices in the treated groups without receiving vitamin C is higher than the groups supplemented with vitamin C.

SDS- polyacrylamide gel electrophoresis

The SDS poly acrylamide gel electrophoresis profile (Figure 3) of the control rabbits' lens protein was characterized by the presence of 9 bands, which varies in their molecular weight and intensities. After treatment of vitreous humor with Nd-YAG laser, the mobility and the intensity of the bands were significantly changed. Moreover, the patterns showed pronounced change in the high and low molecular weight regions.

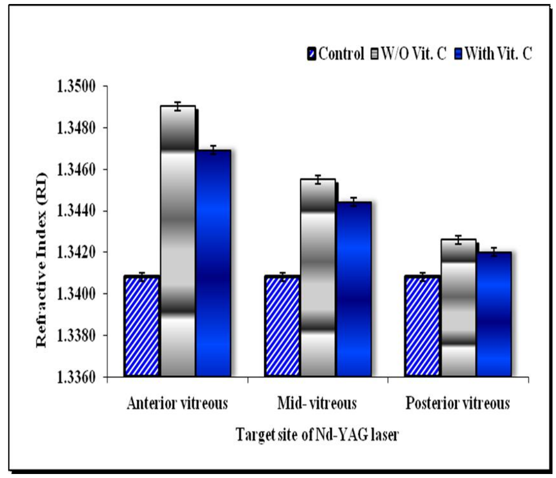

Figure2. Refractive index for lens protein of treated rabbits' vitreous humor with 500 mJ delivered from Q- switched Nd- YAG laser W/O and with vitamin C supplementation.

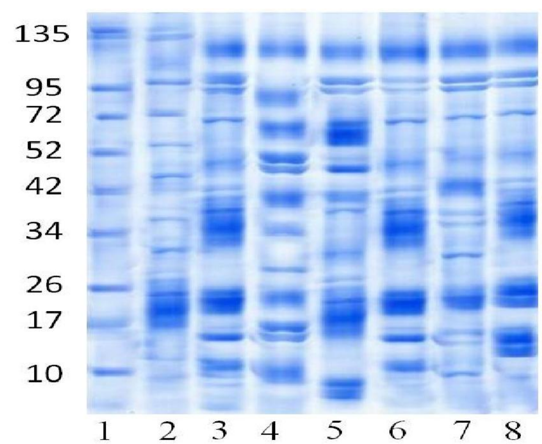

Figure 3. SDS polyacrylamide gel electrophoresis separation; lane (1) represents standard protein, lane (2) represents control group, lanes (3,4&5) represent groups exposed to anterior, middle and posterior vitreous liquefaction by Q- switched Nd-YAG W/O Vitamin C supplementation, lanes (6,7 &8) represent groups exposed to anterior, middle and posterior vitreous liquefaction by Q-switched Nd-YAG with Vitamin C supplementation.

For anterior vitreous treated groups without and with supplementation of vitamin C, the molecular weight of lens protein decreased from 141.8 KD for the control group to 122.4 KD and 129.1 KD respectively (Figure 4). In addition, at low molecular weight region, the molecular weights were shifted towards high molecular weight with the appearance of new fractions between 38.57 and 14.48 KD.

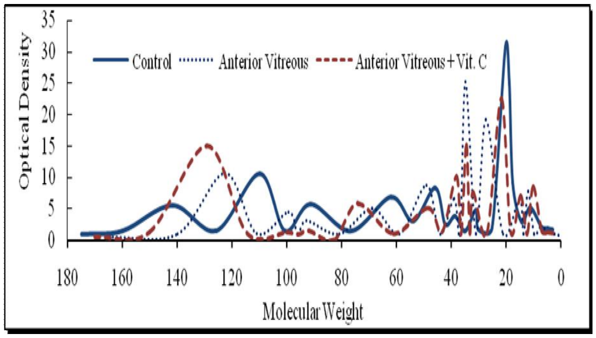

Figure 4. SDS- electrophoresis for the anterior vitreous humor treated with 500 mJ delivered from Q- switched Nd- YAG laser with and W/O Vitamin C supplementation.

For mid- vitreous treated groups (without and with supplementation of vitamin C), the molecular weight of lens protein decreased to 124.1KD and 123.8 KD respectively (Figure 5). Moreover, there were pronounced changes in the low molecular weight region with the appearance of new protein fractions between 20.8 KD and 7.3 KD for the group treated with vitamin C.

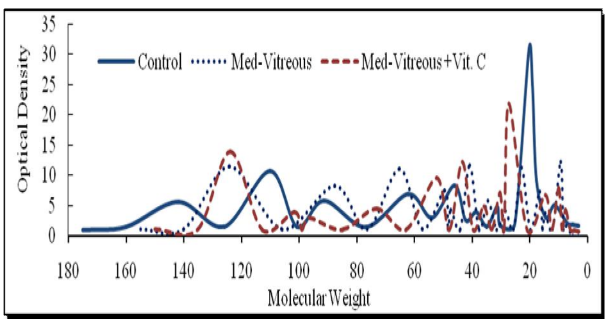

Figure 5. SDS- electrophoresis for the Med-vitreous humor treated with 500 mJ delivered from Q- switched Nd- YAG laser with and W/O Vitamin C supplementation.

For posterior vitreous treated groups (without and with supplementation of vitamin C); the molecular weight of lens protein decreased to 121.5 KD and 129.1 KD respectively (Figure 6). The group supplemented with vitamin C, showed pronounced change in the low molecular weight region with the appearance of new protein fractions between 22 and 42 KD accompanied by increasing in their intensities.

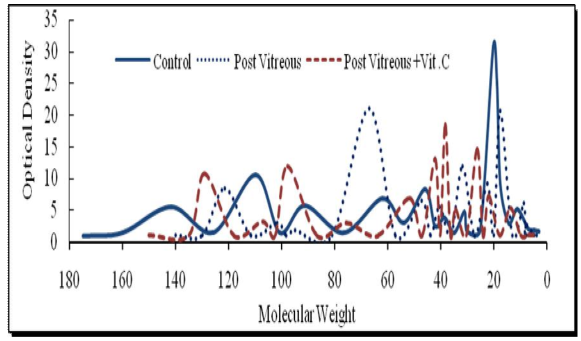

Figure 6. SDS- electrophoresis for the posterior vitreous humor treated with 500 mJ delivered from Q- switched Nd- YAG laser with and W/O Vitamin C supplementation.

Electroretinogram (ERG) measurements

An electroretinogram of a control rabbits is shown in figure 7, showing normal peaks for both the a-and b- waves. The peak amplitude and latency for both waves appear in the normal range according to the reviewed literature. Amplitudes were measured from baseline to the lowest point of the negative peak for the a-wave and from the latter (or baseline, if absent) to the positive peak for the b-wave.

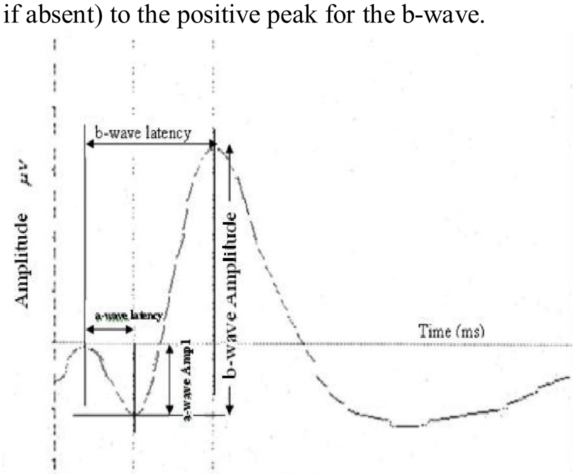

Figure7. Electroretinogram of a control rabbits showing the normal peak amplitude and latency for both a- and b- waves.

All the ERG recordings prove an effectiveness of Nd-YAG laser vitreous liquefaction on the amplitude and latency of both a- and b- waves. After treatment of anterior vitreous with Nd-YAG laser, there were a significant reduction in the a- and b- waves amplitude (Figures 8 and 9) ($P < 0.05$) and a significant delay in the a-wave and b- wave peak latency ($P < 0.05$) when compared to the control group (Figures 10 and 11).

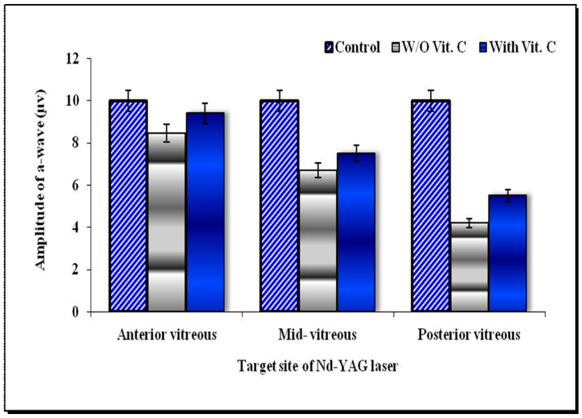

Figure 8. a- wave amplitudes for different groups treated with 500 mJ delivered to vitreous humor of rabbit from Q- switched Nd-YAG laser W/O and with vitamin C supplementation.

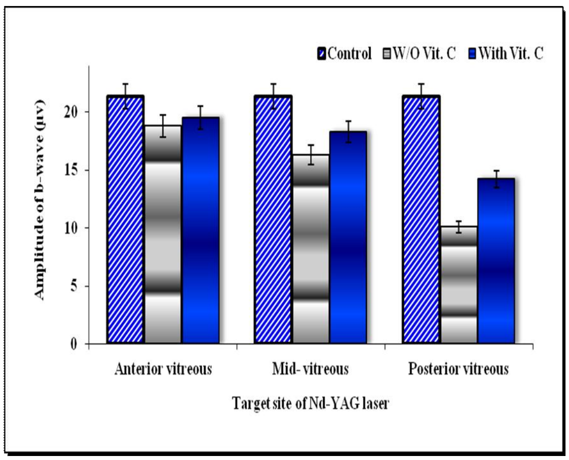

Figure 9. b- wave amplitudes for different groups treated with 500 mJ delivered to vitreous humor of rabbit from Q- switched Nd- YAG laser W/O and with vitamin C supplementation.

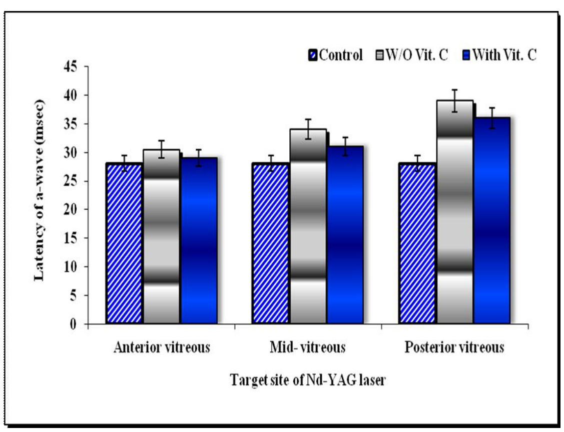

Figure 10. a- wave latencies for different groups treated with 500 mJ delivered to vitreous humor of rabbit from Q- switched Nd- YAG laser W/O and with vitamin C supplementation.

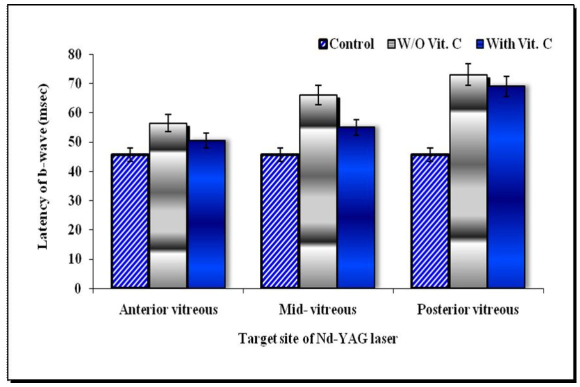

Figure11. b- wave latencies for different groups treated with 500 mJ delivered to vitreous humor of rabbit from Q- switched Nd- YAG laser W/O and with vitamin C supplementation.

It was noticed that these effects increased in case of treatment of the mid- vitreous; the percentage difference was 33% for a-wave and 23.76 % for b-wave amplitude i.e. the a-wave is more affected with ND-YAG laser than b-wave.

In addition, the effect of posterior vitreous treated with laser appear in a form of significance delay in the latency of both a- and b- waves ($P< 0.01$). Moreover, there was a significance reduction in the amplitude of both a-wave and b-wave. The percentage difference of the two waves is 58 % and 52.76 % respectively. It was found that the amplitude of the waves reduced nearly to half of the control.

As summarized in the figures, it was noticed that there was some sort of improvement in ERG wave after intake of vitamin C. That is clear in treatment of an interior vitreous, the percentage difference of the two parameters of a-wave are 6% and - 3.57 % for its amplitude & latency respectively, and 8.79% &-10.7% for b-wave. It was observed that, there was a significant improvement in anterior vitreous than in the mid- vitreous treated groups. In case of the treatment of posterior vitreous, the least improvement was observed. The percentage difference of the amplitudes of a-wave, and b-wave are 45% and 35.85 % and the latencies were -28.57%, and -51.32 % respectively compared with control.

4. Discussion

This study has been undertaken to consider the possible hazardous side effect of Q- switched Nd-YAG laser photo disruption on the lens and retina after anterior, middle and posterior vitreous liquefaction and to identify the protective role of vitamin C against laser oxidative damage.

In the present work, after treatment of the anterior, middle and posterior vitreous with 500 mJ of Nd-YAG laser, the total soluble lens proteins are markedly decreased than the control by 57.8%, 49.3% and 43.9%. In a previous studies by Lerman *et al*. (1984) and Michael *et al*. (1996) they reported that the Nd-YAG laser whether focused on the posterior lens capsule or mid- vitreous, causes considerable alteration of the molecular structure of the vitreous and lens, associated with retinal hole formation and detachment. Moreover, the decrease of biosynthesis of lens crystallin was followed by their aggregation, which then leads to opacity of the lens and cataract formation. In addition, there are continuing decreases in protein content in the groups supplemented with vitamin C before laser treatment (40.2%, 34.8%, and 30.85 respectively). It is noticed that, the decrease in the latter groups is lesser than the first one due to the ability of vitamin C to scavenge free radicals and the high level of vitamin C in the eyes of diurnal animals suggested that the vitamin might protect against oxidative or photo-oxidative damage (Garland 1991, Rose *et al*.1998).

The transparency of the lens crystallin depends on the regular or ordered spacing of its cells and proteins. Disturbance of this order, such as change in molecular weight, protein aggregation, or mechanical deformability in collagenous-engineered tissues results in local changes of refractive index, that cause light scattering.

The present study shows that, the refractive index is elevated after vitreous treatment with Nd-YAG. The elevation in refractive index is directly proportional to the decrease of soluble lens protein. This increase in refractive index which accompanied by an aggregation of soluble lens protein is in accordance with the previous work of Clark (1994) who reported that, in the lens there is a linear relationship between the refractive index and the concentration of protein.

The structural changes in the lens crystallin due to Nd- YAG laser is also evidenced by SDS polyacrylamide gel electrophoresis. It is clear from the scanning curves for all treated groups (Fig. 4, 5 and 6), different lens crystallin fractions exhibit a different behavior from control. There are changes in the high and low molecular weight regions, electrophoresis mobility and intensity of different peaks representing different crystallin fractions. Such changes may be due to lens protein oxidation. Nd-YAG has been easily oxidize proteins, giving rise to disulphide bond formation, and induce cross-linking, therefore, enhancing protein aggregation and resulting in protein modifications indicative to oxidative damage and protein cross-linking. There is ample clinical evidence that destruction of the vitreous gel contributes to nuclear cataract formation (Brown *et al*. 1992) and the increased risk of nuclear cataract is avoided when laser treatment is performed in a manner that preserves the gel structure of the vitreous body (Sawa *et al*. 2005). It was proposed that, when the vitreous is usually in the gel state, oxygen diffused into the vitreous humor from the vessels in the ciliary epithelium and metabolized by retinal tissue (Stefansson *et al*. 1990, Buerk *et al*.1993, Harocopos *et al*. 2004).

The present work hypothesized that, when the vitreous liquefies, oxygen accumulate in the vitreous. The more that oxygen is mixed with the vitreous fluid, the more opportunity it will have to react with vitamin C lowering its concentration, slowing the consumption of oxygen, permitting more oxygen to reach the lens, and causing cataract. This is consistent with the results of previous intervention trials, which found that dietary supplementation with vitamin C, did not protect against nuclear cataract (Gritz *et al*.2006).

Because the propagation of the laser damage is posterior, special care must be considered to avoid damaging to an intact crystallin lens. When working near the lens, the laser must be focused anterior to the treatment area, and energy values close to threshold must be used. A safe distance between the target tissue and the neighboring tissues has been estimated to be 2 to 5 mm. Because of the location of vitreous floaters, most often in the anterior vitreous or mid – vitreous, they can be treated with the Nd-YAG laser while keeping a safe distance from the lens and the retina (Benhamou *et al*. 1998).

In addition to that, Nd-YAG vitreolysis caused significant electrophysiological changes. The b-wave, which is mainly related to the bipolar cell currents and less to Muller cells (Frishman 2006), showed a reduction in its amplitude and appeared with more Latency. The a-wave that reflects the photoreceptor activity was significantly reduced indicating that, Nd-YAG laser exerts an effect on the retina that begins particularly in photoreceptors layer and inner retinal layers. It was noticed that, the closer the targeted area is to the retina, the greater the effect. So it was concluded that, in posterior vitreolysis there was an obvious decline in a- and b- waves amplitude and delay in their peak latencies. The latter results clarify the affected inner nuclear layers as well as photoreceptors layers. The ERG results in this study were in agreement with previous studies (Branes 1968, Vogel *et al*. 1994, Hikichi *et al*. 1995) demonstrating vitreolysis affects on the retina; this effect is in the form of retinal detachment. These results correlate with another finding by Kolb *et al*. (2011) which clarified that ERG of retinal detachment reduced and represented the healthy part of the retina. The observed changes in the ERG wave may be attributed to the ability of Nd-YAG laser photo disruption to the formation of shock waves, which causes tissue disruption in the vitreous as discussed before. Since, the vitreous is attached to the retina at the inner limiting membrane (Vogel *et al*. 1994) where the terminations of Müller cells are contributing component (Matsumoto *et al*. 1984, Hikichi *et al*. 1995) and playing a role in ERG generation. Hence, it can be said that, the vitreous has an integral role in a variety of retinal diseases and it may have a direct impact on retinal oxygenation (Snead 2008). Another reason is that Nd-YAG Laser induced production of free radicals in vitreous humor (Krebs 2007). These free radicals play an important role in liquefaction of vitreous. This liquefaction exerts stress on retina especially at photoreceptor layer and that associated with vitreotinal pathology (Ueno 1995, Robredo *et al*. 2005). The obtained results are in agreement with Baudouin *et al*.(1999) who studied an experimental model of vitreoretinopathy obtained by direct production of free radicals in the vitreous body and its role in reduction of both a-and b- waves. Finally, lens opacity or the cataract which induced as a result of laser may be behind the dissatisfactory changes in the ERG, and that is proved by the obtained results.

The present work finds some sort of improvement of ERG waves with supplementation of vitamin C. This improvement is reversely proportional with target site from the retina. It was suggested that, the improvement of ERG may be due to the role of vitamin C in reducing the activity of some of free radicals in vitreous and retina and that may help the vitreous to improve its characteristics (Tam *et al*. 2004). Additionally, the improvement may be due to the newly regenerated photoreceptors, which are continuously replenishing themselves (Kolb *et al*. 2011). The obtained results agree with Baudouin *et al*. (1999) and Robredo *et al*. (2005) which showed that the antioxidant treatment could improve the progression of some retinal disorder.

Conclusion

The conclusion that well supported from this investigation is that Q-switched Nd-YAG could contribute to lens opacity and retina dysfunction. The results suggested a possible role in the development of cataract on the rabbits lenses used. It causes functional changes to the lens crystallin results in the development of protein aggregates that scatter light with resulting formation of opacity and eventually form a cataract. It is also concluded that, the formation of vitreous liquefaction may have a great effect on the retina. Based on interactions between laser and vitreous humor, it should be possible to formulate strategies to delay or prevent cataracts. Further investigations are required to find new techniques for floaters therapy to improve the traditional ones and make a significant step toward preventing this disease.

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