·Basic Research·

Relationship between raised intraocular pressure and ischemia-modified albumin in serum and humor aqueous: a pilot study in rabbits

Arzu Taskiran Comez [1], Dilek Ulker Cakir [2], Funda Kirtay Tutunculer [2], Baran Gencer [1], Hasan Ali Tufan [1]

[1]Department of Ophthalmology, School of Medicine, Canakkale Onsekiz Mart University, Canakkale 17020,Turkey
[2]Department of Clinical Biochemistry, School of Medicine, Canakkale Onsekiz Mart University, Canakkale 17020, Turkey

Correspondence to: Arzu Taskiran Comez. Barbaros mah. Plaj sok. Hamidiye sitesi C/9 Merkez-Canakkale, 17020, Turkey. arzucomez@yahoo.com
Received: 2013-04-29 Accepted: 2013-12-31

Abstract

• AIM: To evaluate the relationship between increased intraocular pressure (IOP), ischemia-modified albumin levels in serum (IMA-s) and in humor aqueous ($IMA-_{HA}$) in rabbits.

• METHODS: Twenty-five albino New Zealand rabbits weighing between 2.0 and 2.8 kg were used in this pilot study. With permission from Canakkale Onsekiz Mart University Animal Ethics Committee, the IOP of both eyes of each rabbit were recorded with a Tonopen (Tono-Pen XL, Reichart Inc., Depew, NY, USA) after the application of topical proparacaine 0.5% HCl anesthesia. Blood (4 mL) was collected from the marginal ear vein and an intracameral injection of 2.3 mg/mL sodium hyaluronate and subconjunctival dexamethasone was given in the right eye. Anterior chamber aqueous fluid was obtained using a limbal approach with a 27 gauge needle from both eyes. The left eyes were used as controls. IOP was measured on the 1st, 3rd and 10th day after the initial injection, with Tonopen, IMA-s levels and $IMA-_{HA}$ examined simultaneously.

• RESULTS: Before the injections, IOP was 11.4±3.0 mm Hg in the right eye and 11.3±3.1 mm Hg in the left eye ($P>0.05$). There was a statistically significant difference between IMA-s levels before the IOP increase ($IMA-s_0$) and IMA-s levels on the 1st and 3rd days after the increase in IOP ($P=0.012$ and $P=0.01$, respectively). No difference was observed between $IMA-s_0$ and serum IMA levels on the 10th day ($IMA-s_{10}$) after IOP increase ($P=0.989$). $IMA-_{HA}$ in the right eye in the first day after the injection was positively correlated with IOP($r=0.748$; $P=0.02$).No other correlation is found between any other parameter with $IMA-_{HA}$ levels at any test time. A statistically significant positive correlation was observed between IMA-s values and IOP on the 1st and 3rd days($r=0.398$, $P=0.04$ and $r=0.382$, $P=0.04$, respectively). There was no correlation between IMA-s levels and increased IOP on the 10th day after IOP increase ($r=0.026$, $P=0.902$).

• CONCLUSION: IMA may be an important indicator of acute damage caused by diseases involving ischemic damage to the eye, especially in case of increased intraocular pressure.

• KEYWORDS: ischemia-modified albumin; intraocular pressure; serum; humor aqueous; rabbit; eye
DOI:10.3980/j.issn.2222-3959.2014.03.06

Taskiran Comez A, Cakir DU, Tutunculer FK, Gencer B, Tufan HA. Relationship between raised intraocular pressure and ischemia-modified albumin in serum and humor aqueous: a pilot study in rabbits. *Int J Ophthalmol* 2014;7(3):421-425

INTRODUCTION

Ischemia-modified albumin (IMA) is accepted as an early and sensitive biomarker for identifying ischemic damage before the development of myocardial necrosis [1-3]. Previous studies have demonstrated that IMA is a useful marker for the diagnosis of ischemic events [3-8]. While previous studies on this topic have focused on myocardial ischemia, it has also been reported that IMA levels increase during the acute phase of cerebrovascular diseases, mesenterical and pulmonary ischemia, and in ischemia of the musculoskeletal system [3-8]. IMA is measured using an albumin cobalt-binding (ACB) capacity test. Albumin has a binding capacity for many molecules, including toxic cobalt, copper, and nickel. As a result, albumin acts as a buffer for homeostasis [6]. IMA, a metabolic variant of albumin, emerges in ischemic states [9]. Under pathologic conditions such as ischemia, the metal-binding capacity at the N-terminal of albumin is reduced due to structural changes in the protein. This albumin isoform is known as ischemia-modified albumin. The emergence of this albumin isoform without cobalt-binding capacity is the earliest indicator of ischemia[6].

Ischemia-induced injury plays an important role in eye diseases such as glaucoma, including open angle glaucoma, closed-angle glaucoma and normal-tension glaucoma, and central retinal vessel occlusions [9]. However, the data regarding IMA levels in various types of ischemia-associated diseases of the eye, such as glaucoma or retinal vascular diseases are insufficient. In rats, ischemia linked to elevated intraocular pressure (IOP) has been shown to have pathologic properties similar to open-angle glaucoma and central retinal artery occlusion in humans [10,11]. In this study, we evaluated the relationship between raised IOP, serum and humor aqueous IMA in rabbits.

MATERIALS AND METHODS

Materials The current pilot study was conducted between June 2012 and August 2012 in Canakkale Onsekiz Mart University Ophthalmology Department, with contributions from the Medical Biochemistry Department. All procedures were performed in accordance with the ARVO statement for the Use of Animals in Ophthalmic and Vision Research. Twenty-five albino New Zealand rabbits weighing between 2.0 and 2.8 kg were included in the study. The rabbits were housed in a standard animal box kept at a constant temperature of 18-25℃ and with a 12-h light/dark cycle with standard food and water provided. With permission from Canakkale Onsekiz Mart University Animal Ethics Committee, the IOP of both eyes was recorded with a Tonopen (Tono-Pen XL, Reichart Inc., Depew, NY, USA) after topical proparacaine 0.5% HCl anesthesia. Blood(4 mL) was obtained *via* the marginal ear vein of the rabbits. Later, the rabbits were anesthetized with an intramuscular injection of ketamine (25 mg/kg) and xylazine (2 mg/kg), and topical xylocaine before the procedure. A wire lid speculum was used to separate the eyelids. In addition to IOP measurements on 0d prior to the procedure, IOP of both eyes was also measured on 0d (1h after injection), 1, 3, and 10d at the same time of day (between 11:00 and 12:00) to minimize the diurnal variations in IOP. Simultaneously with IOP measurements, 4 mL blood was obtained from the rabbit's marginal ear vein. Blood samples were collected in tubes in the absence of anticoagulants. Serum was separated by centrifugation. Serum samples were frozen at -20℃. Frozen samples were gently vortexed after thawing. IMA absorbance was spectrophotometrically analyzed according to the method described by Bar-Or *et al* [6] and was reported as absorbance units (ABSU). After the IOP measurement, the aqueous humor was collected by a 27-G needle attached to a tuberculin syringe. The needle was introduced into anterior chamber and aqueous humor was withdrawn. The humor aqueous samples were stored at -80℃ until analysis. Intracameral injection of 2.3 mg/mL sodium hyaluronate with subconjunctival dexamethasone injection was administered. The left eyes, which were untreated, were used as controls. The intracameral injection was repeated on 7d.

Methods

Serum ischemia–modified albumin analysis Preparations for the Co (II) albumin-binding protocol involved the addition of 200 μL of animal serum to 50 μL of a 1 gm/L cobalt chloride solution, followed by vigorous mixing and 10-min incubation. Dithiothreitol (DTT) (50 μL of a 1.5 g/L solution) was then added and mixed. After 2min incubation, 1.0 mL of a 9.0 g/L solution of NaCl was added. The absorbance of the assay mixture was read at 470 nm using a spectrophotometer. The blank was prepared similarly with the exclusion of DTT.

Humor aqueous ischemia–modified albumin analysis Humour aqueous IMA were measured using the commercial enzyme-linked immunosorbent assay (ELISA) kit (MyBiosource) with detectable IMA (μ/mL). Unopened test kit was stored at 2-8℃. Samples were stored -80℃. ELISA IMA Detection waveland was 450 nm. The results are reported as Units/milliliter (U/mL).

Statistical Analysis IBM SPSS Statistics 19.0 packet program was used for statistical analysis of the collected data. Continuous data were reported as mean±standard deviation, median, interquartile range (IQR) and dispersion interval (minimum-maximum). IOP and IMA initial values were compared with values at other times using the Friedman test and the Wilcoxon Signed Rank test with Bonferroni correction. Spearman correlation analysis was applied to examine variance. Difference tests were performed at 95% confidence level. Statistically significant P-values for Bonferroni-corrected Wilcoxon Signed Rank tests were <0.0125. For other analyses, P<0.05 was accepted as the threshold of statistical significance.

RESULTS

Before injection, mean right eye IOP was 11.4±3.0 mm Hg and mean left eye IOP was 11.3±3.1 mm Hg (P>0.05). Within 1h after 2.3% sodium hyaluronate was injected to the anterior chamber, a significant increase in right eye IOP was observed compared to basal values (11.4±3.0 mm Hg *vs* 38.9±5.1 mm Hg; P<0.0001). On the 1st day after the injection, mean IOP values were 36.9±4.7 mm Hg and 10.4±2.2 mm Hg for right and left eyes, respectively. On the third day, the IOP in the right eye was 34.8±3.2 mm Hg and was 10.3±2.3 mm Hg in the left eye. Ten days after the treatment, right eye IOP was 30.3±2.9 and left eye IOP was 10.5±1.9 mm Hg. Right eye IOP was observed to be significantly higher than basal values at all time intervals (Table 1). There was no difference between basal IOP and IOP measured at any time point in the left eye (Table 2). There was a statistically significant difference between serum IMA (IMA-s_0) values before the IOP increase and IMA values on the 1st and 3rd days after IOP increase (P=0.012 and P=0.01, respectively). There was no statistically significant difference

Table 1 IOP measurements before (0d) and 1, 3 and 10d after right eye intracameral 2.3% sodium hyaluronate injections

Prameters	Mean	SD	Min	Max	Percentiles			*P*
					25	Median	75	
$IOP_{right\ 0}$	11.4	3.0	7.0	18.0	9.0	11.0	13.9	[1]<0.001
$IOP_{right\ 1}$	36.9	4.7	30.9	49.8	33.9	36.8	39.4	[2]<0.001
$IOP_{right\ 3}$	34.8	3.2	30.9	42.7	32.4	34.0	36.9	[3]<0.001
$IOP_{right\ 10}$	30.3	2.9	24.9	35.9	27.4	29.9	31.4	[4]<0.001

[1]*P*-Friedman test; [2]*P*=0-1st day Bonferroni-corrected Wilcoxon Signed Ranks test; [3]*P*=0-3rd day Bonferroni-corrected Wilcoxon Signed Ranks test; [4]*P*=0-10th day Bonferroni-corrected Wilcoxon Signed Ranks test; SD: Standard deviation.

Table 2 IOP measurements before (0d) and 1, 3 and 10d after left eye intracameral 2.3% sodium hyaluronate injections

Parameters	Mean	SD	Min	Max	Percentiles			*P*
					25	Median	75	
$IOP_{left\ 0}$	11.3	3.1	6.0	16.0	8.4	10.9	13.4	[1]0.362
$IOP_{left\ 1}$	10.4	2.2	6.8	15.0	8.3	10.8	11.9	
$IOP_{left\ 3}$	10.3	2.3	6.0	15.8	8.8	9.9	11.5	
$IOP_{left\ 10}$	10.5	1.9	7.0	13.9	8.9	10.0	11.8	

[1]*P*-Friedman test; SD: Standard deviation.

Table 3 Serum ischemia-modified albumin (IMA-s) levels before (0d) and 1, 3 and 10d after intracameral 2.3% sodium hyaluronate injections

Parameters	Mean	SD	Min	Max	Percentiles			*P*
					25	Median	75	
IMA-s_0	0.675	0.113	0.456	0.822	0.593	0.704	0.770	[1]<0.001
IMA-s_1	0.731	0.078	0.598	0.907	0.690	0.734	0.769	[2]0.012
IMA-s_3	0.750	0.068	0.604	0.907	0.722	0.746	0.782	[3]0.01
IMA-s_{10}	0.696	0.063	0.548	0.809	0.679	0.702	0.733	[4]0.989

[1]*P*-Friedman test; [2]*P*=0-1st day Bonferroni-corrected Wilcoxon Signed Ranks test; [3]*P*=0-3rd day Bonferroni-corrected Wilcoxon Signed Ranks test; [4]*P*=0-10th day Bonferroni-corrected Wilcoxon Signed Ranks test.

in IMA values before IOP increase (IMA-s_0) and IMA values on the 10th day (IMA-s_{10}) (P=0.989) (Table 3).

There was a statistically significant positive correlation between IMA-s and IOP values on the 1st and 3rd days after treatment (r=0.398, P=0.04 and r=0.382, P=0.04, respectively). However, no correlation was found between IMA-s values and IOP on the 10th day after IOP increase (r=0.026, P=0.902).

The mean IMA value in the humor aqueous (IMA-$_{HA0}$) in the right eye before injection was 7.1±1.1 U/mL and in the left eye 7.2±1.0 U/mL; P=0.903). In the 1st day after the injection, 20 samples from the right eye and 22 samples from the left eye could be obtained; with mean IMA-$_{HA1}$ 7.3±1.4 U/mL in the right eye and 7.0±0.8 U/mL in the left eye; P=0.498). On the 3rd day after the injection the mean of the IMA-$_{HA3}$ of the 18 right eye samples obtained was 7.3±1.4 U/mL; and the mean of 21 samples obtained from left eyes was 7.4±0.6 U/mL (P=0.765) On the 10th day, the mean of the 22 samples from the right eyes was 7.5±0.9 U/mL and the mean of the 19 samples from the left eyes was 7.4±1.4 U/mL (P=0.779) A strong positive correlation was found between IMA-$_{HA}$ and the IOP values of the right eye in the 1st day (r=0.748, P=0.02). No correlations was found between IMA-$_{HA}$ and IMA-s at any time interval.

DISCUSSION

Glaucoma is a complex eye disease that may cause ischemia at the head of the optic nerve, characterized by retinal ganglion cell degeneration and vision-field loss. Glaucoma may lead to optic atrophy, resulting in full vision loss if left untreated [12]. Vision loss due to glaucoma is caused by degeneration of retinal ganglion cells and axons. While the degeneration mechanism is not fully understood, high IOP is widely accepted as the most important and the only treatable risk factor. Various studies about the effects of elevated IOP on retinal ganglion cells have used experimental glaucoma models [10-13]. In animal models, IOP increase, episcleral vein cauterization, translimbal photocoagulation, limbal hypertonic "saline" injection, Indian ink in the anterior chamber, sodium hyaluronate, latex microspheres, microspheres and hydroxypropyl methylcellulose (HPMC) mix injection, and subconjunctival dexamethasone injection are methods commonly performed to produce IOP increase [14-18]. Each of these methods has advantages and disadvantages. For this pilot study, we used easily administered anterior chamber sodium hyaluronate injection with subconjunctival dexamethasone injection repeated once every week, resulting in sufficient IOP elevation. The disadvantage of this method is that multiple sodium hyaluronate injections are required to

maintain sufficient and continuous IOP elevation. However, sodium hyaluronate does not result in diurnal IOP changes, does not affect blood flow in the eye, and is easily obtained. Thus we believe the advantages outweigh the disadvantages of this method.

Previous studies have used the Tonopen, the pneumotonometer and cannulation for IOP measurements[14-17]. In this study, Tonopen was successfully used for IOP measurements. The narrow tip of the Tonopen made it easy to apply in rabbits. IOP, in both humans and animals, changes with the circadian rhythm, rising in evening darkness and falling with morning light [19]. In order to standardize the effects of light, a diurnal pattern of lighting was arranged. All rabbits were kept in darkness for 12h and brightness for 12h during each 24-h cycle.

IMA is accepted as an early and sensitive biomarker for ischemic damage before the development of myocardial necrosis [1-3]. Serum IMA increases within minutes after the initiation of ischemia, and remains elevated for 6-12h and returns to a baseline range 12-24h after myocardial ischemia[20].

Shen *et al* [21], in a study of patients applying to emergency services with acute chest pain, demonstrated that IMA sensitivity in the first 3h was 86.1%. The same study on IMA levels in acute coronary syndrome diagnosis reported a peak in IMA levels in the 1st day of hospitalization, and a gradual reduction and a return to baseline values within 14d[21].

Though ischemic events in the eye are common, there are few papers in the literature regarding IMA and the eye. Turk *et al* [22] determined that IMA may be a useful marker in monitoring diabetic retinopathy. Chang *et al* [9] compared blood IMA levels in primary open angle glaucoma (POAG) patients and healthy controls and reported that IMA levels were significantly elevated in POAG patients. The same study determined that IMA may be a new marker to show oxidative damage in POAG[9].

We observed a statistically significant difference in mean serum IMA values measured before IOP increase and on the 1st and 3rd days after intracameral sodium hyaluronate injection. These results, while supporting Chang *et al*[9], suggest that an increase in IOP may result in acute ischemia in the eye and this ischemic state is associated with increased serum IMA, especially in the acute period.

This study is the first study showing the presence of IMA in the humor aqueous in the rabbit eye although the amount is very low compared to serum. We could not show correlations between serum IMA and humor aqueous IMA as well as between IOP values except with the IOP measurement in the first day. However, we think that the strong correlation we assessed between IMA-$_{HA}$ and IOP at the 1st day needs further reseach with larger study population.

While our study is the first to show a relationship between IMA and acute increase in IOP, it has certain limitations. The first is that multiple injections of sodium hyaluronate were required to maintain sufficient IOP elevation, increasing the risk of damage to the cornea and surrounding tissues. Another limitation may be the lack of a control group monitored under the same conditions with no induced IOP increase. Specifically, it is not known whether intracameral injection alone or sodium hyaluronate injection alone might increase IMA (in the absence of a rise in IOP). Furthermore, variables such as body hydration status, serum albumin levels, arterial pressure, and exercise might have influence the results, and these variables could not be standardized. Also, there is no accepted consensus about the serum IMA cut-off levels for rabbits or humans, even there is no information about the presence of IMA in the humor aqueous in the literature; therefore, we cannot state that the serum IMA levels and humor aqueous IMA levels of the rabbits before intracameral sodium hyaluronate injection were normal, nor can we state that these IMA levels may be translated to humans.

In conclusion, this pilot study is the first study showing the presence of IMA in the humor aqueous in rabbits. We report statistically significant correlation between IOP and humor aqueous IMA levels on the 1st day and statistically significant correlation between IOP and serum IMA levels on the 1st and 3rd days of acute IOP increase in rabbits.

IMA may be an important marker of acute damage in diseases causing ischemic damage to the eye, especially glaucoma. However, its diagnostic value and use as a routine biochemical marker should be assessed in further studies.

ACKNOWLEDGEMENTS

We thank Assist. Prof. Sibel Cevizci, M.D. from ÇOMU Medical Faculty, Department of Public Health, for her valuable contributions to the statistical analysis of study data.

Foundation: Supported by Canakkale Onsekiz Mart University, Scientific Research Project Center (No.2010/206)

Conflicts of Interest: Taskiran Comez A, None; **Cakir DU,** None; **Tutunculer FK,** None; **Gencer B,** None; **Tufan HA,** None.

REFERENCES

1 Cho DK, Choi JO, Kim SH, Choi J, Rhee I, Ki CS, Lee SC, Gwon HC. Ischemia-modified albumin is a highly sensitive serum marker of transient myocardial ischemia induced by coronary vasospasm. *Coron Artery Dis* 2007;18(2):83-87

2 Anwaruddin S, Januzzi JL Jr, Baggish AL, Lewandrowski EL, Lewandrowski KB. Ischemia-modified albumin improves the usefulness of standard cardiac biomarkers for the diagnosis of myocardial ischemia in the emergency department setting. *Am J Clin Pathol* 2005;123(1):140-145

3 Kang SY, Suh JT, Lee W. Clinical usefulness of ischemia modified albumin in acute coronary syndrome. *Korean J Lab Med* 2005;25 (5): 306-311

4 Gunduz A, Turedi S, Mentese A, Karahan SC, Hos G, Tatli O, Turan I,

Ucar U, Russell RM, Topbas M. Ischemia modified albumin in the diagnosis of acute mesenteric ischemia: a preliminary study. *Am J Emerg Med* 2008;26(2):202-205

5 Mitsudo S, Brandt LJ. Pathology of intestinal ischemia. *Surg Clin North Am* 1992;72(1):43-63

6 Bar-Or D, Lau E, Winkler JV. A novel assay for cobalt-albumin binding and its potential as a marker for myocardial ischemia-a preliminary report. *J Emerg Med* 2000;19(4):311-315

7 Gunduz A, Turkmen S, Turedi S, Mentese A, Yulug E, Ulusoy H, Karahan SC, Topbas M. Time-dependent variations in ischemia-modified albumin levels in mesenteric ischemia. *Acad Emerg Med* 2009;16 (6): 539-543

8 Gunduz A, Turedi S, Mentese A, Altunayoglu V, Turan I, Karahan SC, Topbas M, Aydin M, Eraydin I, Akcan B. Ischemia-modified albumin levels in cerebrovascular accidents. *Am J Emerg Med* 2008;26 (8): 874-878

9 Chang D, Sha Q, Zhang X, Liu P, Rong S, Han T, Liu P, Pan H. The evaluation of the oxidative stress parameters in patients with primary angle-closure glaucoma. *PLoS One* 2011;6(11):e27218

10 Smith GG, Baird CD. Survival time of retinal cells when deprived of their blood supply by increased intraocular pressure. *Am J Ophthalmol* 1952;35(5:2):133-136

11 Buchi ER, Suivaizdis I, Fu J. Pressure-induced retinal ischemia in rats: an experimental model for quantitative study. *Ophthalmologica* 1991;203 (3):138-147

12 Quigley HA, Nickells RW, Kerrigan LA, Pease ME, Thibault DJ, Zack DJ. Retinal ganglion cell death in experimental glaucoma and after axotomy occurs by apoptosis. *Invest Ophtalmol Vis Sci* 1995;36(5):774-786

13 Frankfort BJ, Khan AK, Tse DY, Chung I, Pang JJ, Yang Z, Gross RL, Wu SM. Elevated intraocular pressure causes inner retinal dysfunction before cell loss in a mouse model of experimental glaucoma. *Invest Ophthalmol Vis Sci* 2013;54(1):762-770

14 Levkovitch-Vebin H, Quigley HA, Martin KR, Valenta D, Baumrind LA, Pease ME. Translimbal Laser photocoagulation to the trabecular meshwork as a model of glaucoma in rat. *Invest Ophthalmol Vis Sci* 2002; 43(2):402-410

15 Urcola JH, Hernandez, M, Vecino E. Three experimental glaucoma models in rats: comparison of the effects of intraocular pressure elevation on retinal ganglion cell size and death. *Exp Eye Res* 2006;83(2):429-437

16 Morrison JC, Moore CG, Deppmeier LM, Gold BG, Meshul CK, Johnson EC. A rat model of chronic pressure induced optic nerve damage. *Exp Eye Res* 1997;64(1):85-96

17 Moreno MC, Marcos HJ, Oscar Croxatto J, Sande PH, Campanelli J, Jaliffa CO, Benozzi J, Rosenstein RE. A new experimental model of glaucoma in rats through intracamaral injection of hyaluronic acid. *Exp Eye Res* 2005;81(1):71-80

18 Moreno MC, Campanelli J, Sande P, Sánez DA, Keller Sarmiento MI, Rosenstein RE. Retinal oxidative stress induced by high intraocular pressure. *Free Radic Biol Med* 2004;37(6):803-812

19 Krishna R, Mermoud A, Baerveldt G, Minckler DS. Circadian rhythm of intraocular pressure: a rat model. *Ophtalmic Res* 1995;27(3):163-167

20 Sinha MK, Gaze DC, Tippins JR, Collinson PO, Kaski JC. Ischemia modified albumin is a sensitive marker of myocardial ischemia after percutaneous coronary intervention. *Circulation* 2003,107(19):2403-2405

21 Shen XL, Xing C, Han LL, Lin L, Lin LF, Deng YL, Pu XD, Hu XZ. Diagnostic value of ischemia-modified albumin in patients with acute coronary syndrome. *Zhonghua Xinxueguan Bing Zazhi* 2007;35 (9): 814-817

22 Turk A, Nuhoglu I, Mentese A, Karahan SC, Erdol H, Erem C. The relationship between diabetic retinopathy and serum levels of ischemia-modified albumin and malondialdehyde. *Retina* 2011;31 (3): 602-608