The Effect of Age on The Ranibizumab Response in Diabetic Macular Edema

Diyabetik Maküler Ödemde Yaşın Ranibizumab Yanıtı Üzerine Etkisi

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ABSTRACT

Purpose: To investigate the effect of age on Ranibizumab (Lucentis®) response in treatment-naive diabetic macular edema.

Materials and methods: One hundred seventy six eyes of 176 treatment-naive patients with macular edema secondary to Diabetes Mellitus were enrolled in this study. The patients in the study were divided into the following four groups according to their ages: group 1 (40-50 years), group 2 (51-60 years), group 3 (61-70 years), and group 4 (>70 years). Three consecutive injections at intervals of 1 month were applied to all diabetic patients. The efficacy of the ranibizumab treatment on macular edema according to age groups was assessed by optical coherence tomography (OCT) by comparing the central foveal thickness (CFT) and mean visual acuity (VA) changes after initial and three loading dose injections.

Results: After three consecutive ranibizumab injection, the mean reduction of CFT in groups 1, 2, 3, and 4 were -255.1±123.4, -205.8±99.8, -194.6±119.1, and -191.8±105.7µm, and mean improvement of VA in groups 1, 2, 3, and 4 were 6.1±0.9, 4.9±0.7, 4.2±0.2, 3.8±0.3 letters respectively. The improvement of VA and reduction of CFT was significantly different in each group ($p<0.001$ in all groups, Paired-samples t-test). The changes of CFT and VA was significantly different between age groups (p:0.025 and p:0.009 respectively, Analysis of Covariance, ANCOVA). Additionally, ages of patients were correlated with the mean reduction of CFT and mean improvement of VA for the whole study group (r:-0.150, p:0.047 for CFT, r:-0.756, $p<0.001$ for VA, Pearson correlation).

Conclusion: In this study, it was observed that the efficacy of ranibizumab treatment was found to be more effective in younger patients in treatment-naive diabetic macular edema patients.

Key words: Age, Central Foveal Thickness, Diabetic Macular Edema, Lucentis, Ranibizumab.

ÖZ

Amaç: Daha önce tedavi almamış diyabetik makula ödeminde yaşın Ranibizumab (Lucentis®) cevabı üzerine etkisini araştırmak.

Gereç ve yöntem: Çalışmaya Diabetes Mellitus'a sekonder maküla ödemi olan ve daha önce tedavi olmamış 176 hastanın 176 gözü dahil edildi. Çalışmadaki hastalar yaşlarına göre aşağıdaki dört gruba ayrıldı: grup 1 (40-50 yaş), grup 2 (51-60 yaş), grup 3 (61-70 yaş) ve grup 4 (> 70 yaş). Tüm diyabetik hastalara 1 aylık aralıklarla üç ardışık enjeksiyon uygulandı. Ranibizumab tedavisinin yaş gruplarına göre maküler ödem üzerindeki etkinliği, optik koherens tomografi (OKT) yardımıyla tedavi öncesi ve üç yükleme dozu enjeksiyonundan sonraki merkezi foveal kalınlık (MFK) değerlerindeki ve görme keskinliğindeki (GK) ortalama değişiklikler karşılaştırılarak incelendi.

Bulgular: Ardışık üç ranibizumab enjeksiyonundan sonra, grup 1, 2, 3 ve 4'teki ortalama MFK azalması -255.1 ± 123.4, -205.8 ± 99.8, -194.6 ± 119.1 ve -191.8 ± 105.7um idi ve grup 1, 2, 3 ve 4'teki ortalama GK iyileşmesi sırasıyla 6.1 ± 0.9, 4.9 ± 0.7, 4.2 ± 0.2, 3.8 ± 0.3 harf idi. GK'nin artışı ve MFK'nın azalması her grup içerisinde anlamlı olarak farklı saptandı (tüm gruplarda $p <0.001$, Paired-samples t-testi). MFK ve GK değişiklikleri yaş grupları arasında anlamlı olarak farklıydı (sırasıyla p: 0.025 ve p: 0.009, Kovaryans Analizi, ANCOVA). Ek olarak, hastaların yaşları MFK'nın ortalama azalması ve tüm çalışma grubu için ortalama GK gelişimi ile korele idi (r: -0.150, p: MFK için 0.047, r: -0.756, GK için $p <0.001$, Pearson korelasyonu).

Sonuç: Bu çalışmada, ranibizumab tedavisinin etkinliğinin, daha önce tedavi almamış diyabetik maküler ödem hastalarında daha genç hastalarda daha etkili olduğu görülmüştür.

Anahtar kelimeler: Diyabetik Makula Ödemi, Lucentis, Ranibizumab, Santral Foveal Kalınlık, Yaş.

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INTRODUCTION

Diabetes mellitus (DM) is a major and chronic healthcare problem in developed and developing countries, and the global prevalence of DM is estimated at 366 million in 2011 which will reach about approximately 552 million by 2030 [1,2] . Unfortunately, diabetic macular edema (DME) is one of the main cause of visual impairment and about 7% of all diabetic patients suffer from this type of visual impairment.[3,4] In some cases, control of hyperglycemia, hypertension and hypercholesterolemia may resolve DME, but often inadequate in most patients. Before now, macular focal laser photocoagulation and pars plana vitrectomy were the treatment options for DME. Macular laser therapy provided the stability of VA and reduced the risk of loss of moderate vision up to 50%, but could achieve significant visual improvement at less than 30% of the patients.[5] Hence, there was an unmet need to improve the vision of the DM patients. In course of time, laser treatment being surpassed by intravitreal pharmacotherapy. Currently, the antagonist of vascular endothelial growth factor (anti-VEGF) (ranibizumab, aflibercept or bevacizumab) are most commonly using as pharmacological treatment agents. Several multicenter randomized controlled clinical trials (RCTs) of vascular endothelial growth factor (VEGF) inhibitors, including the DRCR.net Protocol I,[6] RESTORE,[7] RELIGHT,[8] READ-2,[9] RESOLVE,[10] RISE & RIDE,[11] RESTORE extension,[12] VIVID and VISTA,[13] and DRCR.net Protocol T,[14] exhibited the VA gain in DME patients. These RCTs showed that in 30% of patients treated with these agents, 3-line gain at best-corrected ETDRS VA could be possible. For this reason, both clinician and patient expectations of visual outcome in DME have increased. In these RCTs, patient characteristics such as age are generally similar when the study groups are compared.

As far as we know, various structural and functional changes occur in the vitreous, photoreceptor cells and retinal pigment epithelium (RPE) along with aging. RPE shows a decrease in cell number and a reduction in their functioning, the vitreous undergoes irreversible processes of aging characterized by condensation in the collagen fibrils and changes in the hyaluronic acid components.[15] The internal limiting membrane (ILM) also thickens while aging.[16] Therefore, the pharmacodynamic and pharmacokinetic properties of a drug, such as ranibizumab may change in the vitreous with aging.

Optical coherence tomography (OCT) is used for the detection of retinal diseases and allows us to compare subjectively and objectively the changes in anatomical structures that may result from surgical and/or medical treatments during the follow-up period.[17]

The main study objectives were to investigate the effect of age on the response in DME, after 3 monthly ranibizumab injection. Baseline factors that could affect the outcome were also assessed for more reliable analysis.

MATERIALS AND METHODS

Study Design

One hundred seventy-six eyes of 176 patients who were diagnosed with DME in Kayseri City Hospital from February 2017 to October 2018 were included in the present study. The right eye of each patient was evaluated for the study protocol. Informed consent was obtained as required by bioethical legislation in line with the Declaration of Helsinki for research involving human subjects. Ethics committee approval was obtained from Kayseri City Hospital. All patients and participants received both oral and written information. The patients enrolled in this study were divided into the following four groups according to their ages: group 1 (40-50 years), group 2 (51-60 years), group 3 (61-70 years), and group 4 (>70 years). We observed that only 3 eye of the 3 patients over the age of 80 were included in the study, so we did not need to create a separate age group for these patients. All of the patients were treatment naive and DME was diagnosed by fundoscopic examination, SD-OCT and fundus fluorescein angiography (FFA). SD-OCT was performed by Heidelberg spectralis (Heidelberg Engineering, Franklin, USA).

Exclusion Criteria

Patients with previous history of vitreoretinal surgery, macular edema secondary to retinal vascular occlusion, wet-type macular degeneration, use of prostaglandin analogues, previous laser photocoagulation and steroid/anti- VEGF treatment, corneal disease, cataract or posterior capsule opacification affecting visual acuity and causing media opacity decreasing OCT image quality were excluded from the study.

Treatment protocol and measurements

All the patients were examined in detail including, best-corrected visual acuity (BCVA) measured by using the ETDRS charts at a distance of 4 m, biomicroscopic examination, stereoscopic fundus examination, FFA if necessary, and SD-OCT before treatment. We included patients with visual acuity between 35-70 ETDRS letters (approximate Snellen equivalent 20/200 to 20/40, 1.0 to 0.30 logMAR,) with the presence of treatment-naive diabetic macular edema. Intravitreal treatment was planned as three monthly injections with Ranibizumab 0.5 mg/0.05 ml (Lucentis, Novartis pharmaceuticals) and last control with measurement of VA as ETDRS letters changes and OCT

scanning. The responses at the end of 3 injections were compared, as it is reported that early results at week 12 were associated with long-term anatomic and visual recovery results.[18] Despite the short follow-up period, no serious ocular and systemic adverse events occurred in any of the patients during and after the ranibizumab treatment. All injections were performed in operating room under sterile conditions by using topical anesthesia, 0.5% proparacaine hydrochloride ophthalmic solution (Alcaine®). All patients dropped topical % 0.5 moxifloxacin (Vigamox®) five times a day for five days after the injection.

In the present study, vertical cross-sectional SD-OCT scans of 5 mm length aligned to the fovea center were used. Central foveal thicknesses were measured from retinal thickness map before and three monthly injections after the treatment with high-resolution SD-OCT.

STATISTICAL ANALYSIS

All analyses were performed using the SPSS for Windows V.22.0 software package (SPSS Inc., Chicago, IL). The variables were presented as mean±standard deviation (SD). The normal distribution of all variables was determined with Kolmogorov-Smirnov test and homogenity of variances with one-way ANOVA test. The change in parameters before and after the injection in the groups was analyzed with paired-samples t-test. Differences in measured parameters between all groups were analyzed with a Analysis of Covariance (ANCOVA) test. The comparison of the age groups were adjusted for baseline VA and CFT values for more reliable analysis. Vision analyses were adjusted for baseline visual acuity and anatomic analyses were adjusted for baseline central foveal thickness. The continuous correlation of age with CFT and VA gain was assessed with Pearsons's correlation analysis. A p-value of less than 0.05 was accepted as significant.

RESULTS

In the present study, 176 treatment-naive DME patients were enrolled and all of the patients had type 2 DM. The sex distributions, baseline CFT and VA as letters of ETDRS of the patients are presented in table 1. Gender distribution, CFT and VA among groups were not significantly different between the groups. As presented in the table 2, after 3 doses of ranibizumab injection, a statistically significant decrease in CFT and a statistically significant increase in VA were detected. The mean changes of the CFT and VA between the pre-treatment phase and after three monthly ranibizumab injection are compared in Table 2. After three monthly ranibizumab injection, the mean reduction of CFT in groups 1, 2, 3, and 4 was -255.1±123.4, -205.8±99.8, -194.6±119.1, and -191.8±105.7 µm, respectively. This reduction of CFT decreased with aging and most prominent in the youngest group (p: 0.025), but the other groups were not different from each other. Also, after treatment, the average increase in visual acuity in groups 1, 2, 3, and 4 was 6.1±0.9, 4.9±0.7, 4.2±0.2, and 3.8±0.3 ETDRS letters, respectively. After adjusting for baseline visual acuity, We found that the increment of VA decreased with aging and the lowest visual increase was observed in the oldest group (p: 0.009). Correlation analyses for all patients of age and the mean changes of CFT and VA between the pre-treatment phase and after three monthly ranibizumab injection are shown in table 3. In all ages, there was a significantly negative correlation between age and the mean reduction of CFT and improvement of VA (for CFT r: -0.150, p:0.047 and for VA r: -0.756, p<0.001). We have not experienced any ocular and/or systemic serious adverse event during the follow-up period.

DISCUSSION

The pathophysiological mechanism of cellular damage and retinal vascular leakage in DM continues to be understood. Several factors, such as inflammatory cytokines, growth factors, angiogenic agents (VEGF, placental growth factor (PIGF)), intercellular adhesion molecules (ICAMs) are related to vascular permeability increase as a result of the breakdown of the blood-retinal barrier, and dysfunction of the neuroretinal unit. Ranibizumab is a recombinant VEGF antibody and one of the treatment agents used in DME.

Up to the present, the efficacy of ranibizumab treatment for

Table 1. *Gender distributions and baseline central retinal thickness and visual acuity of patients.*

	Group 1 (40-50 years)	Group 2 (51-60 years)	Group 3 (61-70 years)	Group 4 (>70 years)	p
Gender (female/male)	12/11	30/23	30/29	22/19	0.387*
Baseline CRT (µm)	523.7	511.6	518.7	535.9	0.832**
Baseline VA (ETDRS letters)	48.2	49.2	50.5	50.8	0.077**

Abbreviations; **CRT**: central retinal thickness; **VA**: visual acuity; **ETDRS**: Early Treatment Diabetic Retinopathy Study; *Chi –Square test; **one-way ANOVA

Table 2. *Comparison of the central retinal thickness and the mean visual acuity changes before treatment and after 3 loading ranibizumab injections in each and all groups.*

			CFT (µm)	Mean CFT changes (before and three loading dose after treatment)	Mean VA changes (ETDRS letters)	Mean VA changes (before and three loading dose after treatment)
Group 1	(40-50 years)	Baseline	523.7±115.7		48.2±2.9	
		3 loading injections after	268.7±54.7	-255.1±123.4[a]	54.2±2.1	6.1±0.9[a]
		p*	<0.001		<0.001	
Group 2	(51-60 years)	Baseline	511.6±113.4		49.2±3.6	
		3 loading injections after	305.8±65.5	-205.8±99.8[b]	53.7±3.3	4.9±0.7[b]
		p*	<0.001		<0.001	
Group 3	(61-70 years)	Baseline	518.7±137.7		50.5±3.5	
		3 loading injections after	324.1±66.3	-194.6±119.1[c]	54.9±2.9	4.2±0.2[c]
		p*	<0.001		<0.001	
Group 4	(>70 years)	Baseline	535.9±136.1		50.8±3.3	
		3 loading injections after	344.1±89.4	-191.8±105.7[d]	54.6±2.4	3.8±0.3[d]
		p	<0.001*	0.025**	<0.001*	0.009**

Abbreviations; **CRT**: Central retinal thickness, **VA**: Visual acuity, **ETDRS:** Early Treatment Diabetic Retinopathy Study,
* Paired*–samples *t* test, ** Analysis of Covariance (ANCOVA, adjusted p value by baseline VA and CFT)
CRT p $^{a-d}$:0.005, p $^{a-c}$:0.005, p $^{a-b}$:0.018, p $^{b-d}$:0.930, p $^{b-c}$:0.966, p $^{c-d}$:0.998
Mean VA changes p $^{a-d}$<0.001 , p $^{a-c}$ <0.001, p $^{a-b}$<0.001, p $^{b-d}$ <0.001, p $^{b-c}$:<0.001, p $^{c-d}$:0.002

Table 3. *Correlation analyses for age and the mean changes in central foveal thickness and VA between the before treatment and after 3 loading ranibizumab injections for all patients.*

	Age (years) n:176	
	r	p*
CFT (µm)	-0.150	0.047
Mean VA changes (letters)	-0.756	<0.001

Abbreviations; **CRT**: Central retinal thickness, * Pearson correlation

DME has been documented in various RCTs [7, 11, 19-21]. However, the effects of different treatment regimens on matched groups were assessed in these studies and demographic characteristics such as age were similar between groups, so the effect of age on the treatment response was not evaluated. Altunel et all. investigated the effect of age on dexamethasone implant response in branch retinal ven occlusion and found that the effectiveness of dexamethasone implant treatment decreased with aging. [22] This study presents new evidence on the effect of age on ranibizumab response in treatment-naive DME patients. When evaluating the effectiveness of the ranibizumab treatment on DME between the age groups, we found that the reduction in CFT thickness was the most prominent in the youngest (40-50 years) group, but we could not detect significant difference in the anatomical improvement among the group 2 and 3, group 2 and 4, and group 3 and 4. The efficacy of treatment may be higher in young patients and similar after a certain age. Additionally, the present study also showed that the least visual improvement (3.8 letters) following treatment with ranibizumab was in the oldest group patients. Visual recovery may be limited as the age progresses in treatment naive DME. There was a stronger correlation between mean VA change and age than CFT change-age relationship (r:-

0150 vs r:-0.756). The background for this finding might be attributable to decreased retinal cell functioning, age-related RPE changes, the thickening the ILM and vitreous degeneration and imply that information about the age of the patients needs to be differentiated between age groups about the expected treatment response.

As we know, a lower baseline VA and a higher CFT create a potential for greater CFT reduction and greater VA increase; however, baseline CFT and VA values were similar between age groups in this study and additionally, baseline CFT and VA values were used as covariants to increase the reliability of the analysis. To the best of our knowledge, there is no literature on the relation of the age group with ranibizumab treatment response in naive DME patients. In fact all of the anti-VEGF's may act similarly, ranibizumab may be more effective in younger patients even may be better in older patients than aflibercept or bevacizumab, but there is no study comparing the efficacy of anti-VEGF's with respect to age groups in the literature. To make a real conclusion about anti-VEGF molecules, a head-to-head comparison is needed and features will be identified that were associated with a relatively better outcome could help patients and physicians in their choice of treatment and their expectations.

Firstly, we predict that the ILM will thicken with aging therefore an intravitreal molecule, such as anti-VEGF, may not effectively diffuse into the sensorial retina and have a decrease in activity on the retina. It is reported that vitreoretinal interface abnormalities such as epiretinal membran (ERM) were associated with a worsened visual and anatomic response in DME.[21] Therefore, even if ERM is not present, the thick ILM may be a barrier for the drug molecule in the vitreous. Secondly, the health of the photoreceptor layer is related to the RPE integrity and with aging RPE shows some changes, including increased pleomorphism and lipofuscin content, decreased melanin content and the number of cells. These findings might be attributable to limited VA improvement in the elderly group in this study. Thirdly, an important structural change with aging is seen in the vitreous compartment. With aging, vitreous builds up irreversible changes in collagen fibrils and hyaluronic acid, and gel formations are reduced.[15] It is reported that vitreous degeneration was present in 80% of the population older than 60 years old.[23] With aging, cross-linking of the collagen fibers increase and that causes decreasing solubility, increasing stiffening, and resistance to enzymatic degradation, as a result, the collagen concentration in the gel vitreous increases.[24] It is believed that liquefaction of the vitreous and a clustering of collagen causes irregularities of the hyaluronic acid and collagen molecules. Lacunar spaces form in the vitreous as a result of these changes. It is reported that the elimination of VEGF-A molecule and anti-VEGF agents from the vitreous is not due to degradation but results from being eliminated into the anterior chamber at a constant but individual rate and they are cleared by draining into the peripheral circulation.[25] In addition, the ocular half-life of a large molecule such as anti-VEGF's will be about 4 times the calculated value of its vitreous diffusion time. The radius of the vitreous chamber and diffusion coefficient of the molecule affects the diffusion time. In vitrectomized eyes, the viscosity within the vitreous chamber should be reduced; the diffusion rates of anti-VEGF's and VEGF-A should be increased and consequently elimination rates of these entities will increase also. [26] For this reason, we believe that the solubility and release of anti-VEGF molecule in the vitreous might change with aging and it would be better to study about the solubility of the ranibizumab in elderly patients in-vivo is needed.

The present study has some limitations. First, this study was conducted with ranibizumab, but the efficacy of aflibercept and bevacizumab molecules with respect to age groups is unknown and is still open to research. We observed that CFT reduction and visual improvement were more pronounced in the youngest group but it still needs to be investigated whether the result will be similar after a certain age group. Additionally, we have looked at the relationship between short-term treatment response and age, but it is still need to investigate other potential factors that may affect anatomical and functional outcomes in the studies with longer follow-up.

Consequently, treatment with ranibizumab modality provides effective CFT reduction and VA improvements in all age groups with treatnment-naive DME in the early period. Furthermore, we observed that the effectiveness of the ranibizumab treatment was more prominent in the youngest group. This condition might be related to changes with aging such as decreased retinal cell functioning, age-related changes in the RPE, the thickening in the ILM, vitreous degeneration and increase the diffusion coefficient. After future studies with different anti-VEGF agents with respect to age groups and determining the individual intraocular flows, individualized treatment regimes may be possible according to age groups.

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

REFERENCES / KAYNAKLAR

1. Whiting, D., Guariguata, L., Weil, C. and Shaw, J. (2011) IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. *Diabetes Res Clin Pract* 94: 311–21.
2. Guariguata L, Whiting DR, Hambleton I, et al. Global estimates of diabetes prevalence for 2013 and projections for 2035. Diabetes Res Clin Pract 2014;103:137–49.
3. Yau JW, Rogers SL, Kawasaki R, et al; Meta-Analysis for Eye Disease (META-EYE) Study Group. Global prevalence and major risk factors of diabetic retinopathy. Diabetes Care 2012;35:556–64.
4. Kempen JH, O'Colmain BJ, Leske MC, et al. The prevalence of diabetic retinopathy among adults in the United States. Arch Ophthalmol 2004;122:552–63.
5. Early Treatment Diabetic Retinopathy Study Research Group: Photocoagulation for diabetic macular edema: Early Treatment Diabetic Retinopathy Study report number 1. Arch Ophthalmol 1985;103:1796–806.
6. Elman MJ, Aiello LP, Beck RW, et al. Diabetic Retinopathy Clinical Research Network. randomized trial evaluating ranibizumab plus prompt or deferred laser or triamcinolone plus prompt laser for diabetic macular edema. Ophthalmology 2010;117(6):1064–77.
7. Mitchell P, Bandello F, Schmidt-Erfurth U, et al. The RESTORE study: ranibizumab monotherapy or combined with laser versus laser monotherapy for diabetic macular edema. Ophthalmology 2011;118(4):615–25.
8. Pearce I, Banerjee S, Burton BJ, et al. Ranibizumab 0.5 mg for diabetic macular edema with bimonthly monitoring after a phase of initial treatment: 18-month, multicenter, phase IIIB RELIGHT study. Ophthalmology 2015;122(9):1811–9.
9. Nguyen QD, Shah SM, Khwaja AA, Channa R, Hatef E, Do DV, et al. Two-year outcomes of the Ranibizumab for Edema of the macula in Diabetes (READ-2) Study. Ophthal- mology 2010;117(11):2146–51.
10. Massin P, Bandello F, Garweg JG, Hansen LL, Harding SP, Larsen M, et al. Safety and efficacy of ranibizumab in diabetic macular edema (RESOLVE Study): a 12-month, randomized, controlled, double-masked, multicenter phase II study. Diabetes Care 2010;33(11):2399–405.
11. Nguyen QD, Brown DM, Marcus DM, et al. Ranibizumab for diabetic macular edema: results from 2 phase III randomized trials: RISE and RIDE. Ophthalmology 2012;119(4):789–801.
12. Schmidt-Erfurth U, Lang GE, Holz FG, et al. Three-year outcomes of individualized ranibizumab treatment in patients with diabetic macular edema: the RESTORE extension study. Ophthalmology 2014;121(5):1045–53.
13. Korobelnik JF, Do DV, Schmidt-Erfurth U, et al. Intravitreal aflibercept for diabetic macular edema. Ophthalmology 2014; 121(11):2247–2254.
14. Wells JA, Glassman AR, Ayala AR, et al. Diabetic Retinopathy Clinical Research Network. Aflibercept, bevacizumab or ranibizumab for diabetic macular edema. N Engl J Med 2015; 372(13):1193–203.
15. Salvi SM, Akhtar S, Currie Z. Ageing changes in the eye. Postgrad Med J. 2006;82:581–7.
16. Le Golf MM, Bishop PN. Adult vitreous structure and postnatal changes. Eye 2008;22:1214–22.
17. Health Quality Ontario. Optical coherence tomography for age-related macular degeneration and diabetic macular edema: An evidence-based analysis. Ont Health Technol Assess Ser. 2009;9:1–22.
18. Gonzalez VH, Campbell J, Holekamp NM, Kiss S, Loewenstein A, Augustin AJ, Ma J, Ho AC, Patel V, Whitcup SM, Dugel PU, Early and Long-term Responses to Anti-Vascular Endothelial Growth Factor Therapy in Diabetic Macular Edema: Analysis of Protocol I Data, American Journal of Ophthalmology (2016)
19. Elman MJ, Bressler NM, Qin H, et al. Expanded 2-year follow- up of ranibizumab plus prompt or deferred laser or triamcinolone plus prompt laser for diabetic macular edema. Ophthalmology 2011;118:609–14.
20. Diabetic Retinopathy Clinical Research N, Elman MJ,Qin H, Aiello LP, Beck RW, Bressler NM et al. Intravitreal ranibizumab for diabetic macular edema with prompt versus deferred laser treatment: three-year randomized trial results. Ophthalmology 2012; 119(11): 2312–8.
21. Bressler SB, Qin H, Beck RW, Chalam KV, Kim JE, Melia M et al. Factors associated with changes in visual acuity and central subfield thickness at 1 year after treatment for diabetic macular edema with ranibizumab. Arch Ophthalmol 2012; 130: 1153–61.
22. Orhan Altunel, Altan Göktaş, Necati Duru, Ayşe Özköse, Hasan Basri Arifoğlu & Mustafa Ataş (2016): The Effect of Age on Dexamethasone Intravitreal Implant (Ozurdex®) Response in Macular Edema Secondary to Branch Retinal Vein Occlusion, Seminars in Ophthalmology, 1-6.
23. Okasala A. Ultrasonic findings in the vitreous body at various ages. Av Graef Arch Klin Exp Opth. 1978;207:275–80.
24. Hamlin CR, Kohn RR. Evidence for progressive age-related structural changes in post-mature human collagen. Biochem Biophys Acta. 1971;236:458–67.
25. Saunders DJ, Muether PS, Fauser S. A model of the ocular pharmacokinetics involved in the therapy of neovascular age-related macular degeneration with ranibizumab. Br J Ophthalmol. 2015; 99(11):1554-9
26. Hutton-Smith LA, Gaffney EA, Byrne HM, et all. A Mechanistic Model of the Intravitreal Pharmacokinetics of Large Molecules and the Pharmacodynamic Suppression of Ocular Vascular Endothelial Growth Factor Levels by Ranibizumab in Patients with Neovascular Age-Related Macular Degeneration. Mol Pharm. 2016;13(9):2941-50.