The Effect of Age on 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: Overall, 176 eyes of 176 treatment-naive patients with macular edema secondary to diabetes mellitus were enrolled in this study. In the study, the patients were classified into following 4 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 monthly injections were given to all diabetic patients. The efficacy of the ranibizumab treatment on macular edema according to age groups was assessed using optical coherence tomography (OCT) by comparing the central foveal thickness (CFT) and mean visual acuity (VA) changes at baseline and following 3 loading doses.

Results: After three consecutive ranibizumab injection, the mean reduction in CFT was -255.1±123.4, -205.8±99.8, -194.6±119.1 and -191.8±105.7µm, whereas mean improvement in VA were 6.1±0.9, 4.9±0.7, 4.2±0.2, 3.8±0.3 letters in groups 1, 2, 3, and 4, respectively. The VA gain and CFT reduction showed significant intra-group difference in each group ($p<0.001$ in all groups, Paired-samples t-test). In addition, CFT and VA changes were significantly different between age groups (p:0.025 and p:0.009 respectively, Analysis of Covariance, ANCOVA). Moreover, the mean age was correlated with the mean CFT reduction and mean VA gain in the 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 more effective in younger, 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 its global prevalence was estimated as 366 million in 2011 which is expected to reach about approximately 552 million by 2030 [1,2]. Unfortunately, diabetic macular edema (DME) is one of the leading causes of visual impairment, and approximately 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. Until now, macular focal laser photocoagulation and pars plana vitrectomy were available treatment options for DME. Macular laser therapy provided the VA stability and reduced the risk for moderate loss of vision by up to 50%, but could achieve significant visual improvement in only less than 30% of the patients.[5] Hence, there was an unmet need to improve the vision in DM patients. Over time, laser treatment has been surpassed by intravitreal pharmacotherapy. Currently, vascular endothelial growth factor antagonists (anti-VEGF agents including ranibizumab, aflibercept or bevacizumab) are most common agents used in pharmacological therapy. In several multicenter randomized controlled clinical trials (RCTs) on anti-VGEF agents 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] the VA gain was shown in DME patients. The RCTs showed that 3-lines of gain in best-corrected visual acuity (ETDRS) could be possible in 30% of patients treated with these agents. For this reason, expectations of both clinicians and patients regarding visual outcomes have been increased in DME. In the RCTs, patient characteristics such as age are generally similar among study groups.

Based on our understanding, several 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 by advancing age.[16] Therefore, the pharmacodynamics and pharmacokinetic features 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 the changes in anatomical structures that may result from surgical and/or medical treatments during the follow-up period in both objective and subjective manner.[17]

In this study, the primary objective was to investigate the effect of age on the response in DME after 3 monthly ranibizumab injections. In addition, baseline characteristic that may affect the outcome were also assessed in order to ensure more reliable analysis.

MATERIALS AND METHODS

Study Design

The study included 176 of 176 patients who were diagnosed as DME in Kayseri City Hospital from February 2017 to October 2018. In all patients, he right eye 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. The study was approved by Ethics Committee of Kayseri City Hospital. All patients and participants received both oral and written information. The patients included were classified into the 4 groups according to age: group 1 (40-50 years), group 2 (51-60 years), group 3 (61-70 years), and group 4 (>70 years). It was observed that only 3 eye of the 3 patients aged>80 years were included to the study, so we showed no intention to create a separate age group for these patients. All 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, 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 underwent a detailed ophthalmologic examination 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 treatment-naive diabetic macular edema and visual acuity between 35-70 ETDRS letters (approximate Snellen equivalent 20/200 to 20/40, 1.0 to 0.30 logMAR). Intravitreal treatment was planned as three monthly injections with Ranibizumab 0.5 mg/0.05 ml (Lucentis, Novartis pharmaceuticals) with final control including measurement of VA changes (as ETDRS letters) 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 patients during and after the ranibizumab treatment. All injections were performed in operating room under sterile conditions by topical anesthesia using 0.5% proparacaine hydrochloride ophthalmic solution (Alcaine®). In all patients, dropped topical % 0.5 moxifloxacin (Vigamox®; 5 times daily) was prescribed over 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 after three monthly injections via 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). For all variables, normal distribution was determined with Kolmogorov-Smirnov test and homogeneity of variances with one-way ANOVA test. The change in parameters before and after the injection in the groups was analyzed using Paired- samples t-test. Differences in measured parameters between all groups were analyzed using Analysis of Covariance (ANCOVA) test. The age group comparisons 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 considered as statistically significant.

RESULTS

The study included, 176 patients with treatment-naive DME. There was type 2 DM in all patients. Table 1 presents sex distributions, baseline CFT and VA as letters of ETDRS of the patients. Gender distribution, CFT and VA showed no significant difference among groups. As presented in the Table 2, a significant decrease was observed in CFT whereas a significant increase was detected in VA after 3 doses of ranibizumab injection. Table 2 presents comparison of mean the CFT and VA changes between the pretreatment phase and after three monthly ranibizumab injection. After three monthly ranibizumab injection, the mean CFT reduction was -255.1±123.4, -205.8±99.8, -194.6±119.1, and -191.8±105.7 µm in groups 1, 2, 3, and 4, respectively. The extent of the CFT reduction was decreased by advancing age. It was most prominent in the youngest group (p: 0.025), but there was no significant difference among remaining groups. Again, after treatment, the mean VA gain was 6.1±0.9, 4.9±0.7, 4.2±0.2, and 3.8±0.3 ETDRS letters in groups 1, 2, 3, and 4, respectively. After adjusting for baseline visual acuity, we found that the extent of VA gain was decreased by advancing age. The smallest visual gain was observed in the oldest group (p: 0.009). Table 3 presents correlation analyses for patients from all age and the mean CFT and VA changesbetween the pretreatment phase and after three monthly ranibizumab injection. In all ages, there was a significant negative correlation between age and the mean CFT reduction and VA gain (CFT r: -0.150, p:0.047; VA r: -0.756, p<0.001). No ocular and/or systemic serious adverse event was observed during the follow-up period.

DISCUSSION

Our understanding about pathophysiological mechanism of cellular damage and retinal vascular leakage in DM has been evolving. 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.

The efficacy of ranibizumab treatment for DME has been

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

documented in various RCTs so far[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 al. 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 evaluated the effectiveness of the ranibizumab treatment on DME between the age groups, we found that the CFT reduction was the most prominent in the youngest group (40-50 years), but we detected no 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 by advancing agee in treatment naive DME. There was a stronger correlation between mean VA change and age than those observed between mean CFT change age (r:- 0150 vs. r:-0.756).

The background for this finding might be attributable to decreased retinal cell functioning, age- related RPE changes, the thickened ILM and vitreous degeneration, implying that information about the age of the patients needs to be differentiated between age groups about the expected treatment response.

As known, 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 study on the relationship of the age group with response to ranibizumab treatment in naive DME patients in the literature. In fact, although all the anti-VEGF agents may act in a similar manner, ranibizumab may be more effective in younger patients and even better in older patients than aflibercept or bevacizumab; however, but there is no available 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 identifying features that were associated with a relatively better outcome could help patients and physicians in treatment choice and their expectations.

Firstly, we predict that the ILM will thicken with aging; therefor,e an intravitreal molecule such as anti-VEGF, may not effectively diffuse into the sensorial retina and have a decreased activity on the retina. It has been reported that vitreoretinal interface abnormalities such as epiretinal membran (ERM) were associated with a worser visual and anatomic response in DME.[21] Therefore, even if ERM is lacking, 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, causing causes decreased solubility, increased stiffening, and resistance to enzymatic degradation; thus, 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 are formed 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 elimination 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 longer than 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 could be reduced; the diffusion rates of anti-VEGFs and VEGF-A could 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 .

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 treatment-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 increased 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.

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