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ORIGINAL ARTICLE

Evaluation of Outer Retinal Layers in Diabetic Macula Edema Treated with Intravitreal Ranibizumab

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ABSTRAK

Anti-vaskular faktor pertumbuhan endothelial (VEGF) mengurangkan ketebalan choroidal oleh hipoperfusi choroidal di kalangan pesakit diabetes macula edema (DME). Kesan tidak langsung anti-VEGF terhadap lapisan retina luar (ORL) yang dibekalkan oleh sirkulasi choroidal belum dikaji. Kajian ini melihat ketebalan ORL di antara epitelium retina pigmen (RPE) dengan inner-segmen-outer-segmen fotoreseptor (IS/OS) dan RPE dengan external limiting membrane (ELM) sebelum dan selepas suntikan intravitreal ranibizumab (IVR) kepada pesakit diabetes macula edema melibatkan pertengahan fovea. 60 mata (40 pesakit) telah dianalisis. Ketebalan ORL diukur dengan optikal koherens tomografi pada sebelum dan selepas suntikan satu hari, minggu ke-empat dan minggu ke-enam. Min ketebalan RPE-IS /OS menunjukkan keputusan yang signifikan dari masa ke masa (p=0.023) tetapi tidak untuk RPE-ELM (p=0.216). Nisbah ketebalan antara RPE-IS/OS dan RPE-ELM dan ketebalan subfoveal pusat (CST) kedua-duanya menunjukkan keputusan statistik yang signifikan dari masa ke masa dengan p=0.038 dan p=0.000 masing-masing. Kajian mendapati pengurangan awal ketebalan ORL pada hari 1 diikuti oleh peningkatan dalam ketebalan pada minggu 4 dengan pengurangan berikutnya pada minggu 6 diperhatikan. ORL adalah satu aspek yang boleh diterokai dan diberikan penekanan pada pesakit di mana pemberian suntikan IVR harus dipertimbangkan. Kesan jangka panjang IVR kepada ORL bagaimanapun tidak dapat dirumuskan kerana susulan tempoh kajian yang singkat.

Kata kunci: *edema makular, ranibizumab, retina pigmen epithelium, segmen dalam fotoreseptor retina, segmen luar fotoreseptor retina*

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ABSTRACT

Anti-vascular endothelial growth factor (VEGF) reduces choroidal thickness by choroidal hypoperfusion in diabetic macula oedema (DME) patients. Indirect effect of anti-VEGF towards outer retinal layers (ORL) which supplied by choroidal circulation has not been well described. We evaluate the ORL thickness between retinal pigment epithelium (RPE) with inner-segment-outer-segment photoreceptor junction (IS/OS) and RPE with external limiting membrane (ELM) in pre- and post-intravitreal Ranibizumab (IVR) treated eyes with central foveal diabetic macula edema. A total of 60 eyes (40 patients) were analysed. ORL thickness measured with optical coherence tomography at pre- and post-injection day 1, week 4 and week 6. Mean thickness of RPE-IS/OS was statistically significant over time (p=0.023) but not for RPE-ELM (p=0.216). Thickness ratio between RPE-IS/OS and RPE-ELM and central subfoveal thickness (CST) both showed statistically significant result over time with p=0.038 and p=0.000, respectively. We observed an initial reduction of ORL thickness at day 1 followed by increased in thickness at week 4 with subsequent reduction at week 6 was observed. ORL is an aspect that can be explore and emphasized further in patients considered for IVR injections. The long-term effects of IVR to the ORL however could not be concluded due to short follow up period.

Keywords: diabetic macula oedema, ranibizumab, retinal photoreceptor cell inner segment, retinal photoreceptor cell outer segment, retinal pigment epithelium

INTRODUCTION

Diabetic macular oedema (DME) represents leaking and accumulation of plasma protein, lipids and extracellular fluid in the macula due to breakdown of blood retinal barrier (BRB). It deteriorates the neuroglial function in the macula and subsequently compromised the vision (Patz et al. 1973; Klein et al. 1995). DME occurs at any stage of diabetic retinopathy. Diabetic retinopathy occurs due to underlying ischemic retina. Restoration of oxygen supply to the ischemic retina is carried out by vasculogenesis and angiogenesis that is driven by a signal protein, the vascular endothelial growth factor (VEGF) (Rodriguez et al. 2013).

As compared to laser treatment, inhibition of VEGF has clinically improved visual acuity and avoid visual loss (Clinical Practice Guidelines 2015). READ-2 and RESTORE study both concluded intravitreal injection with Ranibizumab gives superior benefit over standard laser therapy in DME patients, rapidly improved and sustained best corrected visual acuity (BCVA) over 6 and 12 months' period respectively (Nguyen et al. 2009; Mitchell et al. 2011). Numerous clinical data favor the usage of anti-VEGF for

DME. However, there were cases describing improvement of macular oedema without visual recovery. Disruption of inner segment-outer segment (IS/OS) junction serves as alternative pathogenesis than macular thickening for visual improvement with 49% to 75% incidence rate (Otani et al. 2010; Shin et al. 2012). In these cases, the visual loss maybe due to photoreceptor damage evidenced by disruption of IS/OS junction from optical coherence tomography (OCT).

VEGF is the main regulator of angiogenesis and has a protective role against apoptotic neuroretinal cell death in ischaemic retina (Nishijima et al. 2007). VEGF enhance neuroprotection by increasing blood flow to neuronal tissue (Martínez-Vila et al. 2001). The probability exists that VEGF inhibition in short term can help improve vision by reducing macular oedema but at the expense of increased neuroretinal apoptosis with or without capillary dropout at macula (Manousaridis et al. 2012). Therefore, although VEGF inhibition has been proven to improved macula oedema, neutralization of VEGF was found to exert detrimental effect on the choriocapillaries (Kniggendorf et al. 2016).

Anti-VEGF reduced choroidal thickness in DME patients with pre-existing thinner choroid. Alteration of choroidal blood flow and hemodynamics leads to vessels constriction and photoreceptor death (Kniggendorf et al. 2016). Kurihara et al. (2012) demonstrated vision loss in adult mice by targeted deletion of VEGF-A. Complete ablation of choriocapillaries was observed at day 3 post inactivation of VEGF. As the outer retinal layer (ORL) is almost entirely supplied by the choroidal circulation, progressive dysfunction of the retina especially cone photoreceptors was observed.

Currently, there is no study which observed the effect of anti-VEGF directly on the choroidal and outer retina layers in the human eyes. Studies were mainly on animal models which may not reflect truly the actual reaction in the human eyes. In this study, we observe the effect of VEGF inhibition on ORL using OCT scanning with the enhanced depth imaging (EDI) setting.

MATERIALS AND METHODS

This was a prospective interventional study conducted in Ophthalmology Department, Universiti Kebangsaan Malaysia Medical Centre (UKMMC) from December 2014 to August 2015. Research approval was obtained from Universiti Kebangsaan Malaysia Research Ethics Committee (FF-2015-179). Informed consent was obtained from research subjects.

Inclusion criteria were centrally involved DME for more than 3 months (evidenced by OCT scans with good signal strength). Following were the exclusion criteria i.e. poor media with signal strength less than 25, macular oedema due to other causes, concurrent vitreoretinal interface diseases such as vitreomacular traction and epiretinal membrane, recent intraocular surgery or laser therapy less than 3 months and refractive error more

than -6 diopters. BCVA were measured using the standard Snellen chart and subsequently converted to logarithm of minimal angle of resolution (logMAR) score. Funduscopic examination was performed and OCT measurement (EDI setting) of macular thickness was done.

Scanning was done with Spectralis (Heidelberg Engineering, Heidelberg, Germany) SD-OCT machine with EDI setting using high resolution of 6 radial line cross-sectional B-scans. Imaging was obtained with good pupillary dilatation (6mm or more). Measurement was taken at each 6-radial line and mean thickness at each line was taken. Three retinal layers which were the retinal pigment epithelium (RPE), IS/OS junction and external limiting membrane (ELM) were delineated using automatic segmentation. These three layers represent the ORL. Thickness was measured from RPE-IS/OS junction and RPE-ELM. RPE-IS/OS junction represents the photoreceptor cells while RPE-ELM layer represents both the photoreceptor and Muller cells. These areas were measured manually using a caliper provided by the software. Central subfoveal thickness (CST) was also documented.

We evaluated the thickness ratio by dividing the thickness between RPE-IS/OS junction with RPE-ELM after first intravitreal Ranibizumab injection in DME patients. Measurements for all values were taken from vertical line, horizontal line and 2 lines on each 4 quadrants; superior nasal, inferior nasal, superior temporal and inferior temporal quadrants and the mean were calculated from each location. At every quadrant, 3 points of measurements were taken; one point on the subfovea and two points 500 mm on either side of the fovea (Figure 1).

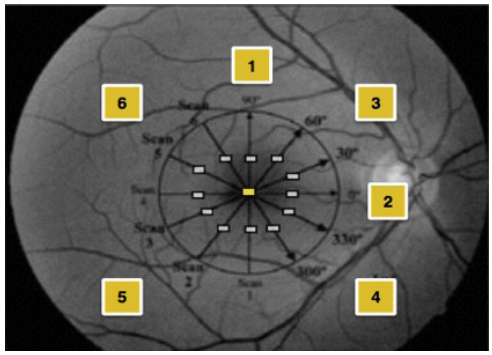

Figure 1: Division of macular region into 6 quadrants with 3 points of measurements'; one point on the subfovea and two points 500 µm on either side of the fovea, as shown above 1, vertical line; 2, horizontal line, 3, superior nasal quadrant; 4, inferior nasal quadrant; 5, inferior temporal; 6, superior temporal 3-6 (2 lines at each quadrants)

STATISTICAL ANALYSIS

Data was analyzed with SPSS version 21.0. General linear model (repeated measure ANOVA) was used for repeated measurements of RPE-IS/OS, RPE-ELM thickness, CST and at pre-injection, post-injection day 1, week 4 and week 6.

RESULTS

A total of 60 eyes from 40 patients (26 males, 14 females) were included in this study. Mean age was 62.95 ± 7.4 years with 24 Malays (60%), 7 Chinese (17.5%) and 9 Indians (22.5%). Baseline measurement of ORL thickness (RPE-IS/OS junction and RPE-ELM), ORL thickness ratio were summarized in

Table 1: Mean ORL thickness ± SD values and p value at pre-injection, post-injection day 1, week 4 and week 6

Variables	Mean thickness and volume ±SD (mm/mm^3)				p value
	Day 0	Day 1	Week 4	Week 6	
RPE-IS/OS	36.87 ± 3.14	36.12 ± 3.19	37.28 ± 3.50	35.60 ± 4.82	**0.023**
RPE-ELM	60.50 ± 3.71	60.27 ± 3.36	61.13 ± 3.40	59.87 ± 4.90	0.216
RPE-IS/OS: RPE-ELM	0.609 ± 0.35	0.599 ± 0.30	0.609 ± 0.32	0.593 ± 0.51	**0.038**
CST	413.17 ± 108.0	402.80 ± 125.8	367.33 ± 119.3	338.47 ± 80.76	**0.000**

RPE: retinal pigment epithelium; IS/O: inner segment/outer segment of photoreceptors; ELM: external limiting membrane; CST: central subfoveal thickness; A: RPE-IS/OS junction; B: RPE-ELM

Table 1.

For the RPE-IS/OS junction, there was reduction of thickness at day 1 post injection compared to before treatment. Subsequently, the thickness increased at week 4 followed by reduction of thickness at week 6. The result was statistically significant over time (p=0.023). Similar trend was observed for the RPE-ELM thickness (Figure 2). There was 0.23 mm reduction of thickness at day 1 post-injection for the RPE-ELM. The reduction was less compared to the changes at the RPE-IS/OS junction which was 0.75 mm. Repeated measures showed no significant changes over time for RPE-ELM (p=0.216).

Overall, ratio of RPE/IS-OS thickness to RPE-ELM reduced over time significantly (p = 0.038). On the other hand, CST showed progressive reduction up to week 6 post-injection. Mean CST decreased from 413.17 ± 108.0 mm at pre-injection to 338.47 ± 80.76 mm at 6 weeks' post injection (p <0.05) (Figure 3). Maximum change was observed between day 1 to week 4 with 35.47 mm difference in thickness.

LogMAR visual acuity was statistically significant over time and between each time points (p < 0.000).

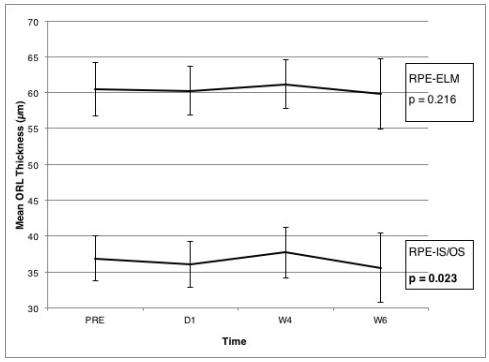

Figure 2: Mean outer retinal layer thickness (µm) at pre-injection, day 1 post-injection, week 4 and week 6. (ORL, outer retinal layer; RPE, retinal pigment epithelium; IS/OS, inner segment/outer segment of photoreceptors; ELM, external limiting membrane)

Paradoxical relationship was observed between RPE-ELM with LogMar visual acuity. However, the correlation was weak and not statistically significant. (r=-0.041, r^2=0.02, p=0.527). No correlation observed between the RPE-IS/OS thickness with LogMAR visual acuity (r=-0.008, p=0.899). Positive correlation was observed between visual acuity and CST (r=0.311, r^2= 0.853, p<0.05).

DISCUSSION

Intravitreal Ranibizumab injection (IVR) has been used widely for the treatment

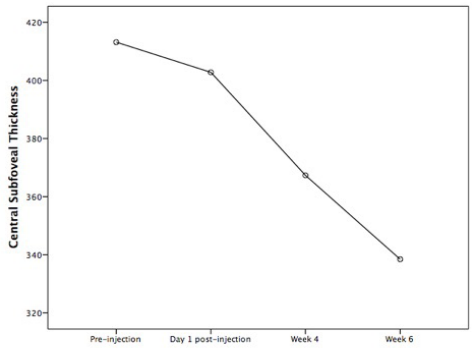

Figure 3: Central subfoveal thickness (μm) at pre-injection, day 1 post-injection, week 4 and week 6

of DME. Previous studies supported the benefit of injection with marked improvement of BCVA and reduced central retinal thickness (Mitchell et al. 2011). OCT imaging with EDI protocol has tremendously improved resolution and image acquisition speed with software advancement resulting in a better scan quality. It increases signal sensitivity by averaging up to 100 B-scans at the same location (Branchini et al. 2013; Ho et al. 2011). Hence, allowing detail interpretation of each individual retinal layers and choroid.

Previous studies also demonstrated the effect of anti-VEGF choroidal thickness. Anti-VEGF injection reduces choroidal thickness by possibly causing choroidal angiopathy or hypoperfusion (Laíns et al. 2014; Rayess et al. 2015; Yiu et al. 2014). Choroidal circulation supplies oxygen and nutrient to the outer retina (Gaudreault et al. 2005). Currently, there is lack of study evaluating the direct effect of choroidal hypoperfusion and anti-VEGF treatment towards the outer retinal layers. We evaluated the effect of anti-VEGF (Ranibizumab) treatment on the ORL thickness.

RPE-IS/OS junction represents the photoreceptor cells whereby RPE-ELM represents both photoreceptor cells and Muller cells. Interestingly, we noted an initial reduction in thickness at day 1 post-IVR injection for both RPE-IS/OS and RPE-ELM. DME itself creates a hypoxic condition. There was an over expression of VEGF factor during the first week of hypoxia (Penn et al. 2008). Possible hypothesis was even on day 1, VEGF inhibition reduced fenestrations of choriocapillaris which directly caused functional and morphological damage to RPE and photoreceptors' (Peters et al. 2007). Hence, possibly reduction in thickness at day 1.

There was retention of VEGF in choriocapillaris and Bruch's membrane at day 1 post VEGF inhibition evidenced by overlapping of IR antibodies between bevacizumab and VEGF in choroidal vessels wall (Heiduschka et al. 2007). Sufficient VEGF was produced within 24 hours' period (Heiduschka et al. 2007). After 24 hours, anti-VEGF effect sets in and the condition reversed. VEGF neutralization occurred and ischemic reperfusion injury was probably initiated. Ischemic reperfusion injury causes swelling of retinal cells and axonal damage secondary to focal ischemia and accumulation of axoplasmic material due to flow obstruction (Hombrebueno et al. 2015). Reactive gliosis from astrocytes and Muller cells also occurred and reflected by up-regulation of glial fibrillary acidic protein (GFAP) antibody (Hombrebueno et al. 2015).

These postulations could reflect the increased thickness of ORL at week 4 where the concentration of anti-VEGF was the highest (Bakri et al. 2007).

Ranibizumab is a humanized antigen-binding fragment directed against VEGF with a molecule size of 48kDa (Gaudreault et al. 2005). The maximum concentration was achieved at day 3 (2.6 to 4 days) when administered intra-vitreally into monkeys and 2.88 days in rabbit (Bakri et al. 2007). Hence, VEGF inhibitory effect may not be seen at day 1 post-IVR injection. Reduction in thickness of both RPE-IS/OS junction and RPE-ELM from week 4 to week 6 could be due to steady loss of VEGF inhibition and sign of neurorecovery. This occurred after week 4 as the efficacy and duration of action of IVR was up to 28 days (4 weeks) as reported by Shah & Del Priore (2009). After week 6, the ORL thickness had reduced further from the baseline level.

There was significant reduction in thickness ratio of RPE-IS/OS junction compared to RPE-ELM. This signifies RPE-IS/OS thickness was affected more than RPE-ELM layer. RPE-IS/OS represent photoreceptors which received its blood supply from the choroidal circulation. RPE-ELM represent the Muller cells which exhibit dual relationship with large retinal blood vessels and choroidal vasculature (Sorrentino et al. 2016). Hence, RPE-ELM were less affected during choroidal hypoperfusion by anti-VEGF. VEGF also plays an important role for photoreceptor differentiation and survival as suggested by in-vitro experiment of rats by Yourey et al. (2017). Hence, the reduction in thickness was seen more at RPE-IS/OS junction compare to RPE-ELM. For CST, there was significant reduction between the pre- and post-injection values at week four and week six. This finding supported by study conducted by Abou Shousha (2015) showing reduction in CST at 1 week and 1 month post-IVR injection in DME which was significantly reduced compared to the pre-injection CST.

Between RPE-ELM and LogMAR, there was negative correlation. As the ORL thickness reduced, visual acuity (VA) worsened due to apoptosis of photoreceptor cells as suggested by Ebneter et al. (2016). There was a strong correlation between VA with ELM status and IS/OS junction as reported by Ito et al. (2013). We found no correlation between the thickness of RPE-IS/OS junction with LogMAR acuity. Oishi et al. (2010) observed higher correlation for ELM with BCVA compared to IS/OS junction with BCVA. Positive correlation was observed between CST and LogMar. RESTORE study concluded anatomic improvements in central retinal thickness leads to significant BCVA improvements (Mitchell et al. 2011). No correlation was observed between CST and RPE-IS/OS junction or CST and RPE-ELM (Mitchell et al. 2011).

No previous study has reported the effect of single IVR injection over different time points on the outer retinal layers' thickness in DME patients. The strength of this study was based on the specific selection of cases of naïve eyes without prior anti-VEGF treatment. Our study observed

the changes of ORL prospectively as opposed to other studies which were done retrospectively. Limitation of this study is the short period of follow up. Hence, the effect of repeated injection of IVR to the outer retinal layer thickness cannot be concluded.

CONCLUSION

This study concludes significant change of ORL specifically RPE-IS/OS junction following single intravitreal Ranibizumab injection. However, ORL changes did not correlate with CST and BCVA. We postulated an initial insult to the outer retina upon VEGF inhibition which then slowly regained morphological normality signifying neurorecovery. In the future, it may be useful to study the effect of repeated anti-VEGF injections towards the outer retina.

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