Evaluation of bioclinical markers to predict short term response to intravitreal anti-VEGF in treatment-naive diabetic macular edema and the “Fried Egg” sign : a novel OCT feature

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Article

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Abstract

Objective: To determine the incidence of suspended scattering particles in motion (SSPiM) and to assess baseline bioclinical markers in treatment-naive diabetic macular edema (DME) which can predict the short term response to intravitreal anti-VEGF.

Design: Prospective observational study.

Participants: Treatment-naive DME patients from January 2022 to June 2022 were included in the study.

Methods: Treatment-naive DME cases were included and followed up for three months. Best corrected visual acuity (BCVA), SSPiM, HbA1c levels, hyperreflective dots (HRD), sub retinal fluid (SRF), central subfoveal thickness (CSFT), optical density ratio (ODR) of the largest cyst near the fovea, type of cyst (uniformly hyperreflective or fragmented intracystic hyperreflectivity called "fried egg" sign) at baseline were studied to predict response to treatment after anti-VEGF.

Results: 123 eyes of 88 patients were included in the study. Incidence of SSPiM was 64.5%. The mean incidence of poor responders was 35%. BCVA, ODR, hyperreflective cysts and presence of SSPiM were not associated with response to anti-VEGF. Higher CSFT, SRF, higher number of HRD, presence of SSPiM in the outer nuclear layer (ONL) and outer plexiform layer (OPL) were associated with short term good response to anti-VEGF. High HbA1c and presence of "fried egg" sign in the hyperreflective cysts were associated with poor response.

Conclusion: Higher CSFT, SSPiM in the ONL and OPL, and higher HRD were associated with good response to anti-VEGF. High HbA1c and "fried egg" sign was associated with poor response to anti-VEGF. BCVA, presence of SSPiM and ODR were not associated with anti-VEGF response.

Introduction

Diabetic macular edema (DME) is the most common cause of visual morbidity in diabetes mellitus with a prevalence of 5.5%. (1) Anti-VEGF agents revolutionized the treatment of DME with 30–70% of patients achieving ≥10-letter improvement and 10–40% of patients achieving ≥15-letter improvement in best-corrected visual acuity (BCVA) after one year of treatment. (2–4) Up to 50% cases do not respond well and are labeled as anti-VEGF poor responders/ persistent DME. (5),(6) No consensus is available at present and poor responders are defined as less than 10–15% decrease from baseline central subfoveal thickness (CSFT) after a minimum of three to six monthly intravitreal anti-VEGF injections.(7) Early control of DME is a predictive factor for vision at the end of two years and hence identifying this subset of poor responders earlier can salvage vision. (8)

Several clinical and optical coherence tomography (OCT) markers have been studied to predict poor responders, namely hyperreflective dots (HRD), suspended scattering particles in motion (SSPiM), size and type of cysts, disorganization of inner retinal layers (DRIL), foveal vascular density and area of foveal...
avascular zone. (9–12) Suspended scattering particles in motion (SSPiM) is a newly described OCT marker which is characterized by the presence of flow signals corresponding to non-vascular components on en face optical coherence tomography angiography (OCTA). The incidence and its response to treatment is not well understood. (13) This study aims at determining the incidence of SSPiM and its response to treatment as well as to correlate baseline best corrected visual acuity (BCVA), HbA1C, presence of SSPiM, location of SSPiM, central subfoveal thickness (CSFT), number of HRD, optical density ratio (ODR) and type of intraretinal cyst with response to anti-VEGF treatment.

**Materials And Methods**

All consecutive patients with treatment-naive DME from January 2022 to June 2022 were included. All patients received complete ophthalmic examinations at their visit, including BCVA measurement (expressed as logMAR units), slit-lamp examination, dilated fundus examination, OCT, OCTA imaging and HbA1C at every visit. Repeat injections were advised based on standard protocols for retreatment. Patients were advised to come for monthly follow up.

The study was approved by Aravind Eye Care system research ethics committee board and adhered to the tenets of the Declaration of Helsinki (Project code: RES2021002CLI). Informed consent was taken from all patients and the images were anonymized. OCT and OCTA images were taken using a Spectralis OCT imaging device (Heidelberg Engineering, Heidelberg, Germany). OCTA examinations were done using a 6-mm volume scan pattern centered on the fovea. Enface OCTA images were exported from the Spectralis OCT after projection artifact removal. Exclusion criteria included patients with renal disease, cardiac failure, presence of other ocular inflammatory and vascular diseases, hard exudate plaque at the fovea, schitic macular edema, chronic DME (duration of more than 6 months), and vitreoretinal interface abnormalities like vitreomacular traction and epiretinal membrane.

**Outcome measures**

The primary outcome measures include to determine the incidence of SSPiM and to assess the response of SSPiM to anti-VEGF as well as to correlate baseline BCVA, HbA1c, CSFT, presence of SSPiM, location of SSPiM, number of HRD, ODR and type of intraretinal cyst with response to anti-VEGF. Response to treatment was defined as greater than 10% decrease in the CSFT four weeks after anti-VEGF injection. For patients who received 2 and 3 injections response was defined as greater than 10% decrease in CSFT at 8 and 12 weeks respectively.

**Image analysis**

The Heidelberg image viewer software with automated segmentation was used to assess the location of the SSPiM. For counting of HRD, single raster line scans of 6mm passing over the fovea were exported to Image J. Gamma function was used to reduce noise in the region of interest which is the outer nuclear layer. A plugin was created to count HRD size ranging from 0-40 microns by Otsu thresholding (Supplement image1). (14) Optical density ratio (ODR) was calculated by dividing the mean pixel value of
the largest cyst closest to the fovea with the mean pixel value of the vitreous cavity by using the technique described by Baek et al. The largest cyst near the fovea was classified as being uniformly hyperreflective, or hyperreflective with a fragmented intracystic cavity which we describe as a "fried egg" sign. The observer was blinded for anti-VEGF response. Interobserver variability was done for a sample of 25 cases.

**Statistical analysis**

Statistical analysis was done with SPSS software version 23.1. Quantitative variables are described as means (standard deviations [SD]), and categorical variables are described as numbers and percentages. For the association of continuous variables with response after injections, independent t test was done and for that of categorical variable, Chi square test and Fisher's exact test was used. To find the possible cutoff of test values for continuous variables, Receiver operating characteristics curve (ROC) with area under the curve (AUC) to depict degree of separability was used. Logistic regression model was done for the baseline predictive factors. P value of < 0.05 was taken as significant.

**Result**

A total of 88 treatment-naive DME patients presented during the study period. Baseline characteristics are represented in supplement table 1. Out of 123 eyes taken into the study 119 (96.7%) eyes received at least one intravitreal injection, 48 (39%) eyes received two monthly injections and 21(17%) eyes received three monthly injections. Incidence of SSPiM was 64.5%. The total number of poor responders in those who received one, two and three injections were 38 (31%), 17 (35%) and 6 (40%) respectively. There was no significant difference in baseline characteristics data between the two groups except for cardiac disease which was more commonly absent in good responders. (Table 1) BCVA, ODR, hyperreflective cysts and presence of SSPiM were not associated with response to anti-VEGF. HbA1c was not associated with response initially but had a significant difference in patients who received three monthly injections (Responders 7.38% ±1.68%, Nonresponders 10.8% ± 2.5%, p < 0.006). Responders had a higher CSFT at baseline (545.2µm ± 139.6) compared to poor responders (461µm ± 114, p value < 0.001). HRD were significantly higher in responders (26 ± 15) when compared to poor responders 19 ± 13 (p < 0.012) after the first injection and lost their significance after two and three injections. (Table 2) Presence of SSPiM in the outer nuclear layer (ONL) and outer plexiform layer (OPL) was consistently associated with initial responders (p < 0.012) whereas presence of SSPiM in the inner nuclear layer was not significantly different between the two groups. (Table 3) This significance was not noted in patients with more than one injection. 23 (28%) of cases with SSPiM (52% responders and 48% were poor responders) persisted post injection with 12(15%) of cases disappearing, 16 (20%) reducing and 4(5%) turning into hard exudate plaques. (Fig. 1 and supplement Fig. 2) The interobserver variability for HRD and SSPiM Chronbach Alpha was 0.8 and 0.7 respectively. Presence of "fried egg" sign in the hyperreflective cysts was significantly associated with poor response both at one month(37% versus 63%, p <0.001) and at 2 months(35% versus 64%, p <0.009), but lost its significance at 3 months follow up. (Fig. 2)(4) ROC curve
with AUC did not yield cut off values of statistical significance. Logistic regression analysis was done which showed no additional significance.

**Discussion**

In the Diabetic Retinopathy Clinical Research Network (DRCR.Net) Protocol T, 51–73% of eyes had persistent DME, 12 weeks after initiating anti-VEGF therapy.\(^{(16)}\) Such eyes are defined retrospectively as persistent DME or poor responders. Identification of this subset of patients becomes critical due to long term poor prognosis of patients with chronic DME. Gonzalez et al. in a post hoc analysis of DRCR. Net protocol I showed that eyes with suboptimal early BCVA response had poorer long-term visual outcomes than eyes with pronounced early response.\(^{(8)}\) Our study has tried to assess baseline bioclinical markers to predict short term response to intravitreal anti-VEGF agents.

HbA1c levels were significantly associated with poor responders who received 3 monthly injections. Though the number of eyes which received 3 injections were low (21) the difference was still significant. Multiple retrospective studies have shown that the mean decrease in CSFT and improvement in BCVA was negatively correlated with HbA1c. This was not the case with large prospective studies like RIDE, RISE, VISTA and VIVID.\(^{(17–19)}\) Our mean HbA1c was 7.8 ± 1.9, similar to that of VIVID and VISTA. The reason for the difference is not clear.

Higher CSFT and presence of SRF has been consistently associated with good response to anti-VEGF treatment as shown in DRCR trials.\(^{(6, 8, 11)}\) A recent study by Turski A et al. also showed a short term good response for anti-VEGF with higher CSFT.\(^{(11)}\) We had a similar response to anti-VEGF treatment with higher CSFT and patients with SRF more likely to show good response at their first and second injection.

HRD are hypothesized to be inflammatory in origin, though the exact pathogenesis is not known. HRD are known to occur in other macular diseases like macular telangiectasia type 2 and could be of neurodegenerative origin.\(^{(20)}\) Hwang et al. showed higher HRD were associated with poor response though the effect size was small with poor responders having 16.06 ± 6.6 and responders having a marginally increased count of 11.17 ± 4.83 HRD. Our study shows that higher HRD(26 ± 15) favors a good response to anti-VEGF initially. The loss of significance later could be attributed to low sample size in patients receiving 3 injections. Recent systematic review on HRD was not conclusive of its role in VEGF response though reduction in number was noted following intravitreal anti-VEGF or steroids.\(^{(9)}\) This is the first study to use a semi-automated software to count the HRD. The interobserver variability for this technique was 0.83. Use of a semiautomated software to count HRD adds to the strength of this study. Other studies counted HRD manually and to define HRD between 20–40 µm would be a tedious task.\(^{(9)}\)

SSPiM was present in 65% of cases during the study period and there was no significant difference between responders and poor responders. Kashani H et al. first described this entity in DME, vein occlusion, choroidal neovascular membrane and a case of macroaneurysm as a presence on non vascular flow signal on enface OCTA.\(^{(13)}\) The origin of SSPiM is unclear. The authors hypothesized the
presence of lipoproteinaceous debris within the pockets of intra retinal fluid causes brownian motion resulting in non vascular flow signals in OCTA. Longitudinal follow up was present only in 8 cases, 5 of which developed into a hard exudate plaque after anti-VEGF treatment. Ahn et al. showed poor response to anti-VEGF agents in DME with SSPiM but in their study vitreoretinal interface disorders were not excluded. Poor response can be attributed to the presence of epiretinal membrane or vitreomacular adhesion. (10) In our study there was a high incidence of SSPiM at baseline and was associated with a good response to anti-VEGF agents. The number of eyes having persistent SSPiM in responders and poor responders were almost equal.

ODR was measured to study if cysts with intraretinal hyperreflectivity responded poorly to anti-VEGF. Ahn et al. measured the ODR of the cyst with SSPiM and found a significantly higher ODR when compared to other cysts. (10) We tried to measure ODR using a similar technique in Image J and tried to correlate ODR with treatment response. No such difference was noted between the ODR of responders and poor responders.

Fragmented intracystic hyperreflectivity which we call “fried egg” sign was noted in 64% of cases among the poor responders. In cases with SRF, there was an initial resolution of the SRF without further improvement in CSFT. Liang et al. first described such cysts in a case series where they showed 4 cases with solid appearing cysts responding poorly to anti-VEGF agents and intravitreal steroids(21). Cysts with SSPiM are hyperreflective too but uniformly so. These cysts are unique with a central hyperreflectivity equal to that of the OPL with a surrounding clear space which gives it an appearance of a “fried egg”. One of the eyes with marked SSPiM lost its flow signal on OCTA and became a hyper reflective cyst with “fried egg” sign at eight weeks of follow up following intravitreal anti-VEGF.(Fig. 2) 13% of eyes with “fried egg” sign showed corresponding signal in the enface OCTA though flow signal in the B scan was minimal. We also noticed the appearance of “fried egg” sign following intravitreal anti-VEGF after which the patient responded poorly. (13) We hypothesize that the intraretinal fluid causing brownian motion of the lipoproteinaceous debris may reduce with time resulting in cysts with the lipoproteinaceous remnants only, without flow signals. This process may not happen with all SSPiM cysts, some tend to disappear or form hard exudate plaques with repeated intravitreal anti-VEGF.

Our study included only treatment-naive DME and excluded patients with chronic DME, renal or cardiac failure and those with vitreo macular interface disorders. Such patients are known to respond poorly to anti-VEGF(22). Hence our cohort can be considered as primary poor responders. The DME in these patients may be due to alternate pathways like the angiopoietin or kallikrein pathway. (23, 24)

To the best of our knowledge this is the first prospective study reporting the incidence of SSPiM in DME. (10, 13, 25) HRD were counted with a semi-automated software with low inter observer variability thus avoiding errors from manual counting. All the other studies used manual counting over a single line scan passing through the fovea. (9) We also report “fried egg” sign, a novel OCT marker which is present more commonly in anti-VEGF poor responders.
Limitations

Patients received different types of anti-VEGF agents and subgroup analysis for each injection was not done due to low sample size in each group. HbA1c data was available only for 56% of cases. 33% of eyes were lost to follow up before 3 months were completed. This study reflects the scenario in a developing country where a single day spent in the hospital translates to loss of wages for that day. Thresholding for HRD calculation was uniform for all the cases but reduction of noise using gamma function was done manually by an experienced retina specialist. Though interobserver variability was low, standardization has not been done for reproducibility.

Conclusion

Higher CSFT, SSPiM in the ONL and OPL, and higher HRD were associated with good initial response to antVEGF. High HbA1c and presence of “fried egg” sign were associated with poor response to anti-VEGF. BCVA, presence of SSPiM and ODR were not associated with anti-VEGF response.

Declarations

Future considerations

With the development of drugs which have a combined action against VEGF-A and angiopoietin like Faricimab(26), future randomized control studies should aim at identifying primary poor responders to anti-VEGF more effectively and earlier, so that they are started on alternate injections.

Acknowledgements

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References


Tables

Tables 1 to 3 are available in the Supplementary Files section.

Figures
**Figure 1**

Enface OCTA and corresponding structural B scan of 3 patients. A, E, I. Pre-injection enface OCTA signal of suspended scattering particles in motions (SSPiM) B, F, J. Corresponding cyst with flow signal. C and D shows the absent SSPiM signal and resolved cyst respectively. G and H show increase in SSPiM signal and increase in size of cyst respectively. K and L show slight decrease in SSPiM signal and reduction in size of cyst respectively.
Figure 2

Enface OCTA and corresponding structural B scan of 2 patients. A and B show the pre-injection enface OCTA signal of scattering particles in motion (SSPiM) and the corresponding cyst with flow signal respectively. C and D shows presence of reduced enface OCT signal due to hyperreflective material inside the cyst with markedly reduced flow signal ("fried egg" sign ) after 1st anti-VEGF injection respectively. E and F shows no change in the size and characteristics of the cyst after 2nd injection .G and H show the pre injection enface OCTA and the corresponding cyst with fragmented hyperreflective material with minimal flow signal ("fried egg" sign ).I and J show minimal response to 2 monthly anti-VEGF agents.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.Differenceinpatientcharacteristicsineyesbetweenrespondersthroughpoorresponders.xlsx
- Table3.Bioclinsmmarkerspredictingshortterm injectionresponseat4weeks8weeksand12weeks.xlsx