Hyperreflective foci as biomarkers for inflammation in diabetic macular edema: Retrospective analysis of treatment naïve eyes from south India

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Purpose: The aim of this study was to analyze the factors associated with hyperreflective foci (HRF) in diabetic macular edema (DME) in treatment naïve eyes. **Methods:** This retrospective observational study included 131 eyes of 91 treatment naïve patients with DME. Details of ophthalmological examination with duration of vision loss and systemic parameters were noted. The spectral-domain optical coherence tomography (SD-OCT) images were analyzed for number and location of HRF and the associated imaging biomarkers. **Results:** Inner retinal (IR) HRF were seen in 88 eyes (67%), outer retinal (OR) in 28 (21%), and subretinal (SR) in 12 (9%). The IR had (7.1 ± 7) HRF, the OR (6.5 ± 4.8), and SR (3.9 ± 2.9). A greater proportion of eyes with HRF also had subretinal fluid (SRF), significantly higher blood pressure and lower serum triglycerides. Univariate linear regression analysis showed women (3 HRF greater vs. men, $P = 0.04$), eyes with cystoid spaces (2.95 more HRF vs. no cystoid spaces, $P = 0.02$), and SRF (2.96 more HRF vs. no SRF, $P = 0.007$) had more HRF, whereas higher triglycerides (1 HRF lesser per 50 mg lower TGL, $P = 0.03$) had lesser. **Conclusion:** Our study highlights the importance of HRF as an imaging biomarker in DME suggesting an inflammatory origin. Long-term observations of large cohorts with automated analysis can give more insights.

Key words: Cystoid macular edema, diabetic macular edema, external limiting membrane, hyperreflective foci, serous retinal detachment

Hyperreflective foci (HRF) were first described in spectral-domain optical coherence tomography (SD-OCT) by Coscas *et al*. in age-related macular degeneration (ARMD).[1] HRF are small punctiform lesions, with reflectivity equal to that or higher than that of the retinal pigment epithelium (RPE), around 20–40 μm in size and found to be distributed throughout the retinal layers in SD-OCT. They cannot be visualized clinically or in fundus photography, autofluorescence, and fundus fluorescein angiography.[2] They have been described in wet ARMD,[1] diabetic macular edema (DME),[2] retinal vein occlusions,[3] central serous retinopathy,[4] MacTel type 2,[5] retinitis pigmentosa,[6] Stargardts disease,[7] Best disease,[8] and choroidal neovascularization due to other etiologies.[9]

DME is the leading cause of vision loss in patients with type 2 diabetes mellitus.[10] Apart from the systemic status of a patient, various SD-OCT biomarkers are known to influence the response to treatment and visual outcome including the presence of cystoid spaces, subretinal fluid (SRF), external limiting membrane (ELM), and ellipsoid zone (EZ) integrity at baseline.[11] Recently, HRF have gained attention as a potential biomarker in the treatment of DME.[4,12] In this study, we evaluate the factors associated with presence of HRF in treatment naïve eyes with DME.

Methods

This retrospective observational study included treatment naïve patients with DME attending the vitreo retinal services

of a tertiary eye care center in Southern India, between January 2018 and April 2019. The study adhered to the tenets of the declaration of Helsinki and institutional ethics committee approval was obtained. Patients with a previous history of treatment for their DME (intravitreal injections or macular laser) were excluded. Patient with a history of cataract surgery within 6 months and other ocular comorbidities such as glaucoma, ARMD, and vein occlusion were also excluded.

Basic demographic details were recorded along with duration of vision loss. All patients underwent detailed ophthalmological examination. The background diabetic retinopathy was graded according to the Modified Airlie House ETDRS classification. Details of systemic illness including diabetes mellitus, hypertension, dyslipidemia, cardiac or renal dysfunctions were documented based on history as provided by patient. Systemic investigations at the baseline included fasting and post-prandial blood sugar levels, glycated hemoglobin (HbA1C), hemoglobin, lipid profile, renal function test, and urine microalbumin levels.

Image analysis

All patients underwent SD-OCT imaging on Spectralis (Heidelberg Engineering, Heidelberg, Germany). The images were read by a single fellowship trained observer, masked to details of clinical findings and systemic parameters. Scans of

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patients with signal strength less than 20 db were excluded. All the details of DME including presence of vitreoretinal interface changes, cystoid spaces, SRF, integrity of ELM, EZ, and RPE were documented. A distance of 1500 microns from center of fovea was manually marked by calipers nasal and temporal to the foveal center in a single 180° 6-mm horizontal raster line scan centered at the fovea and the number of HRF were counted manually. The location of HRF was also classified and documented as—inner retinal (IR) from the inner limiting membrane to outer nuclear layer, outer retinal (OR) from the ELM to the RPE, and subretinal (SR) if they were present in the space of the neurosensory detachment [Fig. 1]. The dots with the absence of back shadowing and reflectivity equal to RPE were considered as HRF. The larger aggregated foci were correlated with the corresponding infrared image and were considered to be hard exudates, and smaller ones were disregarded as noise.

Statistical analysis

Continuous variables were presented as mean with standard deviation or median with interquartile range (IQR) and categorical variables were presented as proportions (n, %). Group differences between continuous variables were analyzed using the Student's t test or the Wilcoxon rank-sum test when comparing across two groups and using the analysis of variance or the Kruskall–Wallis test when comparing across three groups. Group differences between categorical variables were analyzed using the Chi-square or the Fischer's exact test.
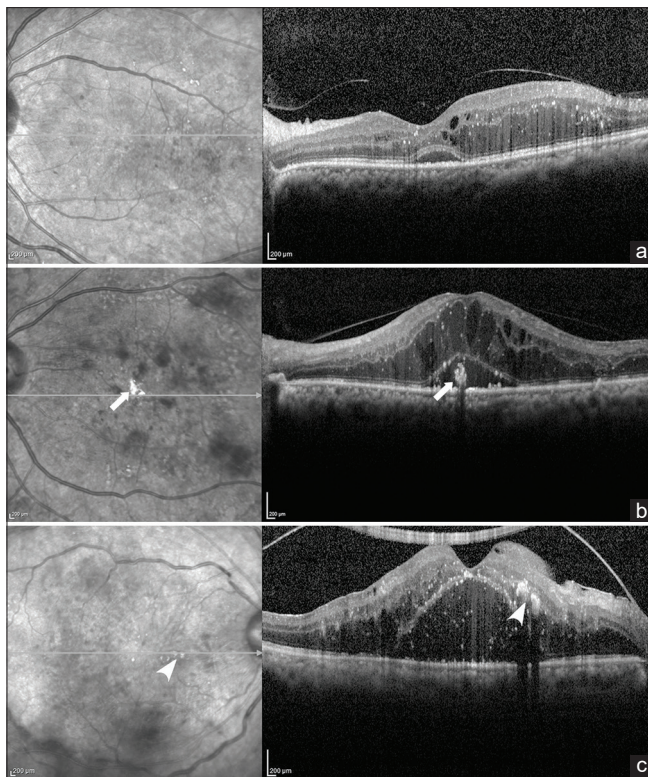

Figure 1: Representative images of SD-OCT scans showing hyperreflective foci (HRF) at various locations: (a) multiple inner retinal HRF, (b) HRF lining cystoid cavities, (c) HRF in inner retina, outer retina and in the subretinal fluid. The aggregated hard exudates in subretinal fluid space (b) (white arrow) and outer retina (c) (white arrow head) show back shadowing and are also visible in the infrared image

IR-HRF were seen more frequently, hence eyes were divided into three groups based on presence or absence of IR-HRF with one group having no HRF. The two other groups with HRF (low HRF and high HRF) were divided based on the median number of HRF. Patient-wise and eye-wise comparisons across these groups were done and presented separately. In view of bilateral disease in many patients, repeated measures linear regression using generalized estimating equation (GEE) method was used to predict factors associated with IR-HRF. Variance inflation was checked and stepwise forward and backward regression was used to identify the multivariable model containing the largest number of statistically significant independent predictors when compared to closely competing models.

All data were recorded using Microsoft Excel and analyzed using STATA 12.1 (I/c, StataCorp, Fort Worth, Texas). All values of $P < 0.05$ were considered statistically significant.

Results

We included 131 eyes of 91 patients in this study. The demographics and baseline systemic parameters of the study cohort are shown in Table 1. The mean age of patients was 55.7 ± 7.8. The patients had diabetes for a mean duration of 11 ± 6.6 years, with mean HbA1C 7.7 ± 1.4. Twenty-three patients (25%) had coexistent hypertension for a mean of 7 ± 6 years, 5 (5%) patients had cardiac illness, 1 had nephropathy, and 15 (16%) had dyslipidemia for a mean of 1.9 ± 1.5 years.

On analyzing eye-wise distributions, IR-HRF was seen in 88 eyes (67%), OR-HRF was seen in 28 eyes (21%), and SR-HRF was seen in 12 eyes (9%). The median number of HRF was 6 (IQR 1–12). Comparison of ocular factors and OCT-based biomarkers across three groups of eyes with IR-HRF is shown in Table 2. A greater proportion of eyes with HRF also had SRF. In eyes with OR-HRF, seven eyes (25%) had absent or discontinuous ELM and seven (25%) had absent or discontinuous EZ. Among eyes with OR-HRF, there were significantly more number of eyes with SRF (24/28, 86%) as compared to 41/103 (40%) eyes that did not have OR-HRF ($P < 0.001$). Only two eyes had HRF exclusively in outer retina, with one case having discontinuous EZ and the other discontinuous ELM. There were no differences in central retinal thickness, BCVA, ELM, and EZ continuity between those with and without IR-HRF or OR-HRF.

On analyzing patient-wise distributions, 68 (75%) patients had IR-HRF in at least one eye and 20 patients (44%) had bilateral IR-HRF. Twenty-six (28%) patients had OR-HRF in at least one eye and only two patients had bilateral OR-HRF. None of the patients had bilateral serous detachment, whereas 12 (13%) had it in at least 1 eye. There was a poor correlation between number of HRF in inner and outer retina ($r = 0.16$) and inner retina and SR space ($r = 0.16$). The inner retina showed the maximum number of HRF (7.1+7), whereas the outer retina (6.5 ± 4.8) and SR space (3.9 ± 2.9) showed slightly lower numbers of HRF. Comparison of systemic factors across three groups of patients with IR-HRF is shown in Table 3. Patients with HRF had significantly higher proportion of cases with coexistent hypertension compared to those who did not have HRF. Additionally, those with higher number of HRF had significantly lower levels of serum triglycerides. No other differences were observed in systemic parameters across groups.

Table 1: Baseline demographics and systemic characteristics of the study cohort

Variable	Value
Age (years)	55.7±7.8
Gender (men)	63 (69%)
Smokers (*n*, %)	12 (13%)
BMI (kg/m²)	28.8±2.8
Mean arterial pressure (mm Hg)	99.1±10.9
On insulin (*n*, %)	10 (11%)
Glycemic control	
Mean fasting blood sugar (mg%)	143.5±65.7
Mean postprandial sugar (mg%)	200.7±81.8
Mean HbA1C (%)	7.7±1.4
Renal parameters	
Urine micro albumin (mg%)	73.1±47.8
Blood urea (mg%)	30.4±9.6
Serum creatinine (mg%)	0.9±0.2
Serum Lipid Profile	
Serum cholesterol (mg%)	187±52
Serum triglycerides (mg%)	147±74
Serum HDL (mg%)	37±8
Serum LDL (mg%)	113±38
Serum VLDL (mg%)	32±23
Cho HDL ratio	5±1.3
LDL HDL ratio	2.8±1.2

BMI=Body mass index

Univariate linear regression analysis using GEE showed that women had more IR-HRF than men (3 HRF greater vs. men, $P = 0.04$), and eyes with cystoid spaces (2.95 more HRF vs. no cystoid spaces, $P = 0.02$) and SRF (2.96 more HRF vs. no SRF, $P = 0.007$) also had more number of HRF [Table 4], whereas patients with higher triglycerides (1 HRF lesser per 50 mg lower TGL, $P = 0.03$) had lesser HRF. On multivariable linear regression with best-fit modeling, these associations persisted with presence of SRF having the highest (3.6 HRF more) and strongest ($P = 0.003$) association with number of IR-HRF. There was no association of HRF with BCVA, glycemic control, and central retinal thickness.

Discussion

HRF are an imaging biomarker in many retinal pathologies, but, there is still no clear consensus as to their exact nature.[2,11,12] In diabetics, they have been described in OCT of patients with and without DR.[13] Very few HRF were also seen in normal controls.

In our study, maximum HRF were seen in the inner retina. This is in concurrence with previous studies.[14] Previous *in vivo* studies have shown activation of retinal microglial cells which lie in inner retina, in diabetic retinopathy.[15] Lee *et al*.[16] showed an increase in CD14 levels in aqueous humor of patients with DME and in diffuse macular edema. They noted an increase in number of HRF in the inner retina supporting the possibility that HRF could be derived from activated microglial cells. Vujosevic *et al*.[13] showed the presence of HRF in diabetics with early DR but no DME and also in diabetics without DR, located mainly in inner retina. They postulate that these HRF indicate

an inflammatory response to early microglia activation; and are not formed due to lipid extravasation.

We saw OR-HRF in 28 eyes. HRF was shown to be associated with all patterns of DME and final visual outcomes were dependent on OR-HRF.[14] Uji *et al*.[17] showed that OR-HRF were associated with ELM and EZ disruption, postulating a disrupted ELM allowing migration of HRF from inner to outer retina. In our study, we found more HRF in the inner retina, with disrupted or discontinuous ELM as well as EZ. A discontinuous ELM predicted more HRF in univariate analysis. It is possible that a degenerative process in diabetic retina with an inflammatory component causes microglial activation, ELM disruption, and photoreceptor degradation. As our cohort is of treatment naïve DME, we would possibly see migration of HRF to outer retina from inner retina in these cases posttreatment.

The presence of SRF strongly correlated with HRF in our cohort, as in previous studies.[14] Various inflammatory biomarkers present in the vitreous fluid and are associated with increased vascular permeability and severity of DME.[18] The levels of ICAM 1 are associated with the height of SRF, indicating that increased vascular permeability by inflammatory mediators results in SRF.[19] In eyes with DME treated with ranibizumab and dexamethasone, a greater reduction in number of HRF as well as SRF was seen in the dexamethasone group.[11] An inflammatory pathology in occurrence of HRF is plausible considering these studies and our findings.

Presence of cystoid spaces was strongly predictive of more HRF in univariate & multivariate analysis. Ischemia and inflammation leads to the activation of intercellular adhesion molecule ICAM 1, which facilitates tethering, slow rolling and transepithelial migration of leucocytes which eventually plug the deep capillary plexus leading to the fluid imbalance.[20] It is possible that inflammation contributes to the formation of cystoid spaces as well as HRF.

In our cohort, the duration of vision loss and baseline BCVA was comparable in all the three groups of IR-HRF, thereby refuting their occurrence as a sign of disease chronicity. Previous studies have shown that increased HRF at baseline indicates lesser tissue integrity with poor visual acuity at presentation and poor treatment outcomes.[21]

Prior studies have shown poor glycometabolic control to be associated with more HRF, suggesting HRF may be a marker of disease severity.[21,22] In our study the presence of HRF at baseline did not correlate with glycometabolic control. Since we included cases with DME, there may not be significant differences in the glycemic status. We observed a higher prevalence of hypertension in the group with more HRF. Hypertension can influence the blood–retinal barrier and increase fluid leakage and impair fluid reabsorption.[22]

HRF have been described as precursors of hard exudates of protein or lipid origin, distributed throughout all layers of retina, becoming confluent at the border of the outer nuclear and the outer plexiform layers when they become detectable in infrared and fundus imaging.[2] This was concurred by Ota *et al*.[23] who showed the correlation between HRF and hard exudates in 50% of patients and also showed their aggregation to form subfoveal hard exudates with rapid regression

Table 2: Comparison of ocular and OCT parameters with varying amounts of inner retinal HRF

Variable	No HRF (*n*=43)	Low HRF (*n*=24)	High HRF (*n*=64)	P
HRF number	0	4.3±1.3	12.8±5.4	NA
Diabetic retinopathy stage				
Mild-moderate	14 (33%)	10 (42%)	24 (38%)	0.87
Severe NPDR	7 (16%)	4 (17%)	10 (16%)	
Very severe NPDR	2 (5%)	1 (4%)	3 (5%)	
PDR	19 (44%)	8 (33%)	26 (41%)	
Lasered PDR	1 (2%)	1 (4%)	0	
Mean BCVA (logMAR)	0.32±0.26	0.36±0.28	0.32±0.22	0.95
Duration of symptoms (months)	2.1±1.8	2.0±2.3	2.3±2.3	0.74
OCT features				
Mean CRT	451±164	431±146	463±144	0.48
Mean SRF height (μm)	147±85	127±66	171±104	0.65
Mean HRF in Outer retina	7.5±6.3	6.1±4.8	6.6±4.9	0.94
Presence of SRF	13 (30%)	13 (54%)	39 (61%)	0.007
Mean HRF in SRF	0	3.3±1.5	4.1±3.4	0.99
VR interface anomaly	7 (16%)	3 (12%)	4 (6%)	0.24
Diffuse macular edema present	15 (35%)	12 (50%)	35 (55%)	0.13
Cystoid spaces present	31 (72%)	17 (71%)	53 (83%)	0.31
ELM discontinuity	9 (21%)	4 (17%)	13 (20%)	0.82
ELM absent	5 (12%)	1 (4%)	7 (11%)	
EZ discontinuity	8 (19%)	5 (21%)	18 (28%)	0.34
EZ Absent	5 (12%)	0	30 (50%)	
Subfoveal hard exudates	4 (9%)	1 (4%)	4 (6%)	0.71
DRIL present	2 (5%)	4 (17%)	7 (11%)	0.22

HRF=Hyperreflective foci, CRT=Central retinal thickness, SRF=Subretinal fluid, ELM=External limiting membrane, EZ=Ellipsoid zone, DRIL=Disorganization of inner retinal layers

Table 3: Comparison of systemic parameters with varying amounts of inner retinal HRF

Variable	No HRF (*n*=23)	Low HRF (*n*=27)	High HRF (*n*=41)	P
Number of HRF*	0	5.8±1.9	15.2±5.2	NA
Age	54.5±8.1	55.8±8.1	56.3±7.6	0.67
Gender (% men)	19 (83%)	18 (67%)	26 (63%)	0.28
BMI	29.1±2.5	28.4±3.1	28.9±2.9	0.66
MAP (mm Hg)	100.7±8.4	97.9±10.4	99.1±12.5	0.57
Coexistent hypertension	1 (4%)	9 (33%)	13 (31%)	0.02
FBS (mg%)	149±55	137±45	144±82	0.32
PPBS (mg%)	203±72	213±64	190±96	0.52
HbA1c (%)	8.0±1.5	7.7±1.3	7.5±1.5	0.18
% on Insulin	2 (9%)	4 (15%)	4 (10%)	0.76
Urinary microalbumin (mg%)	78±53	70±50	72±43	0.84
Blood urea (mg%)	28±10	29±8	32±10	0.21
Creatinine (mg%)	0.93±0.2	1.0±0.3	0.95±0.2	0.89
Serum cholesterol (mg%)	176±40	201±63	183±49	0.28
Serum triglyceride (mg%)	146±45	179±99	125±59	0.03
HDL (mg%)	37±5	36±8	37±8	0.89
LDL (mg%)	105±37	117±32	115±41	0.76
VLDL (mg%)	31±11	34±16	32±32	0.25
% Dyslipidemia	2 (9%)	7 (26%)	6 (15%)	0.28
DM duration (years)	11±8	11±7	11±6	0.74

*Eye with greater number of inner retinal HRF considered for this analysis. BMI=Body mass index, MAP=Mean arterial pressure, FBS=Fasting blood sugar, PPBS=Post prandial blood sugars

Table 4: Univariate and multivariable linear regression with generalized estimating equations

Variable	Interval	Univariate analysis		Multivariable Analysis	
		β coeff	95% CI	β coeff	95% CI
Age	10 year increment	-0.41	-2.0-1.2	-	-
Gender	Female vs. male	2.91	0.1-5.7**	2.97	0.2-5.7**
Duration of symptoms	1 month increment	0.32	-0.2-0.9	-	-
BMI	1 kg/m^2 increment	-0.03	-0.4-0.4	-	-
MAP	10 mm Hg increment	0.22	-0.9-1.4	-	-
Hypertension	Vs. No Hypertension	2.34	-0.2-4.9*	-	-
FBS	25 mg increment	-0.02	-0.5-0.5	-	-
HbA1c	1 mg% increment	-0.51	-1.3-0.2	-	-
Cholesterol	50 mg increment	-0.15	-1.3-1.0	-	-
Triglycerides	50 mg increment	-0.94	-1.6 to -0.2**	-1.02	-1.7 to -0.4**
Duration of DM	1 year increment	0.003	-0.2-0.2	-	-
CRT	50 μ increment	0.28	-0.2-0.7	-	-
Cystoid spaces	Vs. No cysts	2.95	0.6-5.3**	3.25	1.1-5.5**
ELM discontinuity	Vs. Normal ELM	1.76	-0.4-3.9*	-	-
DRIL	Vs. no DRIL	3.13	-2.3-8.5	-	-
SRF	Vs. No SRF	2.96	0.5-5.5**	3.57	1.3-5.9**
BCVA	0.1 logMAR decrement	0.64	-4.1-5.3	-	-

BMI=Body mass index, MAP=Mean arterial pressure, FBS=Fasting blood sugar, ELM=External limiting membrane, DRIL=Disorganization of inner retinal layers, SRF=Subretinal fluid

of edema on treatment. Patients with hard exudates have more deranged lipid profile and are likely to benefit from lipid-lowering drugs.[24] We found that there was lower level of triglycerides in our cases with more HRF. Our data indicate that HRF are not precursors to hard exudates and are more likely to be sequalae of inflammation and degeneration.

The unique finding in our study was the increased prevalence of HRF among female patients compared to males ($P = 0.02$). There is evidence in the literature that menopause causes a state of low-grade systemic inflammation.[25] Srinivasan *et al*.[26] have shown that postmenopausal women are more likely to have dry eyes which could be considered as a surrogate of ocular inflammation. Though we have not recorded the menstrual history, the mean age of our patients is 55.7 ± 7.8 years, indicating a post-menopausal age group. The higher incidence of HRF in women in our cohort may also indicate the added role of post-menopausal inflammation to the already existing inflammation in DR.

The limitations of our study are its retrospective and cross-sectional nature. A longer follow-up with treatment can provide more insights into how HRF behave over time, and help correlation to pathology and significance in treatment decision making.

HRF are an imaging finding that is gaining more attention with better imaging technology. There is a lack of consensus on its source of origin, with some reports favoring a lipid origin with HRF being precursors of hard exudates, whereas others point towards an inflammatory hypothesis. In our study, we saw more inner HRF, and SRF strongly correlated with the number of HRF. Lower triglycerides were seen in eyes with more HRF. Although glycemic control was not significant, hypertension had positive correlation with increased HRF. We feel an inflammatory origin of HRF in diabetic retinas,

with degenerative process is more likely cause. As HRF are an imaging finding seen at various retinal layers a heterogenous origin is possible. Further studies with molecular analysis of HRF can elucidate their origins and role in treatment response. More long-term observations of large cohorts, with automated analysis of HRF pre and posttreatment can give insights to its pathogenesis and significance in treatment.

Conclusion

Our study highlights the importance of HRF as an imaging biomarker in DME suggesting an inflammatory origin.

Conflicts of interest

There are no conflicts of interest.

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