

Research Article

Analysis of Hypoxia-Inducible Factor-1a and Intercellular Adhesive Molecule-1 on Clinical Findings in Rhegmatogenous Retinal Detachment Patients

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Abstract

The aim of the study is to examine the role of hypoxia-inducible factor-1a (HIF-1a) and intercellular adhesive molecule (ICAM-1) in clinical findings found in patients with rhegmatogenous retinal detachment (RRD). The clinical findings are; the duration of retinal detachment, retinal quadrants involvement, and the severity of proliferative vitreoretinopathy (PVR). A cross sectional study was conducted, with 34 consecutive vitreous sample were obtained from dr. Cipto Mangunkusumo National General Hospital (RSCM), from October 2015 to October 2016. The samples were taken from RRD patient who received vitrectomy in the hospital. There were no significant difference between the ICAM-1 and HIF-1a levels to the clinical findings that is measured; duration of retinal detachment ($p>0.05$), extent of retinal detachment area ($p>0.05$) and severity of PVR ($p>0.05$). However, there is a significant mean difference of ICAM-1 level on the duration of retinal detachment, where < 1 week group has higher mean 49.71, (SD 49.54) than the other group, as well as in the retinal quadrants groups (2nd quadrant 19.42 (SD 2.49) and also in severity of PVR groups (PVR B 35.42, SD 30.13). The results from HIF-1a level on each sub group were insignificant. The level of ICAM-1 and HIF-1a cannot be used as predictor of the clinical findings found in the patients. ICAM-1 levels tend to be high on patients whose diagnosed with retinal detachment early, with smaller area of detachment (2nd quadrant) and lower PVR levels (PVR B). The level of HIF-1a shows a consistent pattern with minimal difference in between the sub groups.

Keywords: rhegmatogenous retinal detachment, hypoxia-inducible factor-1a, intercellular adhesion molecule-1

Analisis Hypoxia-Inducible Factor-1a dan Intercellular Adhesive Molekul-1 pada Temuan Klinis Pasien Ablasi Retina Rhegmatogenous

Abstract

Tujuan penelitian ini adalah mengetahui peran hypoxia-inducible factor-1a (HIF-1a) dan intercellular adhesive molekul (ICAM-1) pada temuan klinis pasien rhegmatogenous retinal detachment (RRD). Temuan klinis tersebut adalah durasi ablasi retina, keterlibatan kuadran retina, dan tingkat keparahan vitreoretinopati proliferasi (PVR). Studi cross sectional ini mengikutsertakan 34 sampel vitreous dari Rumah Sakit Umum Nasional (RSCM) dr. Cipto Mangunkusumo dari Oktober 2015 hingga Oktober 2016. Sampel diambil dari pasien RRD yang menjalani vitrektomi. Tidak terdapat perbedaan bermakna antara kadar ICAM-1 dan HIF-1a terhadap temuan klinis; durasi ablasi retina ($p>0,05$), luas area ablasi retina ($p>0,05$) dan tingkat keparahan PVR ($p>0,05$). Namun, ada perbedaan bermakna rerata tingkat ICAM-1 pada durasi ablasi retina, yaitu kelompok < 1 minggu memiliki rerata lebih tinggi (49,71, SD 49,54) dibandingkan kelompok lain, dan di kelompok kuadran 2 retina (19,42, SD 2,49) dan pada tingkat keparahan kelompok PVR (PVR B 35,42; SD 30,13). Kadar HIF-1a pada masing-masing subkelompok tidak berbeda bermakna. Kadar ICAM-1 dan HIF-1a tidak dapat digunakan sebagai prediktor temuan klinis. Tingkat ICAM-1 yang tinggi pada pasien yang didiagnosis ablasi retina dini, dengan area ablasi lebih kecil (kuadran 2) dan tingkat PVR yang lebih rendah (PVR B). Tingkat HIF-1a menunjukkan pola konsisten dengan perbedaan minimal antara subkelompok.

Kata kunci: rhegmatogenous retinal detachment, hypoxia-inducible factor-1a, intercellular adhesion molekul-1

Introduction

Retinal detachment is the separation of the neurosensory retina from the underlying retinal pigment epithelium (RPE).¹⁻³ It is further classified into rhegmatogenous, tractional, and exudative form. Rhegmatogenous retinal detachment (RRD) affects the population as much as 1 in 10.000 individuals each year. Most cases reported that retinal detachment only occurs unilaterally, while bilateral cases can be found approximately 10% of total cases. RRD usually occurs in patients within age group of 45-65 years old.² RRD characterized by retinal break and accumulation of liquefied vitreous under the neurosensory retina due to vitreoretinal traction. RRD cases occurs in the presence of both; partially liquefied vitreous and presence of vitreoretinal traction.² Proliferative vitreoretinopathy (PVR) is one of the most common RRD complications, with formation of fibrocellular membrane and intraretinal fibrosis.^{1,4} The fibrotic membrane disturbs the wound healing process with surrounding cell inflammation, migration, proliferation, and immune cell invasion.⁷

In the case of retinal detachment, the photoreceptors as well as the RPE will receive the most damage. Retinal detachment also increases the risk of hypoxia at third quarter of retina's outer part, includes outer plexiform layer, outer nuclear layer, photoreceptor, and RPE, as the choroid capillary supply these structures.⁵ Photoreceptors, which is a visual sensing element of the retina, receive oxygen and nutritional support from the underlying retinal pigment epithelium (RPE).⁶ Retinal detachment will result in photoreceptor hypoxia and their time-dependent death. The major regulators of the cellular response to the hypoxic conditions are the hypoxia inducible factors (HIF).⁷ Hughes et al.⁷ explains that in normal human retina, there is active constitutive signalling of HIF-1 α which indicates a physiological role of HIF-1 α in the retina. Under normal oxygen condition, the natural course of HIF-1a is marked for degradation with Prolyl Hydroxylase Domain (PHD) and Factor Inhibiting HIF protein (FIH proteins).^{8,9} However, a drop in the oxygen tension will cause PHD and FIH to be inactivated thus allowing the HIF-1a to stay in the cytoplasm. The increase in HIF-1a will result in activation of stress response and genes that is needed for the cells to survive.^{9,10} Rhegmatogenous retinal detachment (RRD) is the separation process of the photoreceptors from retinal pigment epithelium (RPE) which causes an inflammatory reaction in the retina.^{6,11} Inflammatory

reactions that take place in the retina can cause the formation of oxidative stress as well as the state of hypoxia on the retina. The inflammatory state of the retina causes an increase in ICAM-1 and HIF-1 α which will play a role in extravasation of inflammatory cells to the retina.¹¹

Intercellular adhesion molecule-1 (ICAM-1) that is mentioned above, is one of the adhesion molecules that regulates leucocyte recruitment to inflammation area.¹² Webster et al. showed that level of ICAM-1 was four times higher in retinal ablatio than normal condition. Limb et al.¹³ In other study, also mentioned that increment of ICAM-1 in vitreous was up to 97,7 ng/mL in patients with PVR cases after surgery. These studies shows, despite of the absence of actual inflammation as the disease, such as uveitis, any disruption to the normal physiology will results in inflammatory cascade. Further understanding on the biochemical analysis of ICAM-1 and HIF-1a levels on RRD patient is needed. This report is expected to give information for further strategy to manage and treat RRD. Therefore, we aimed to investigate the concentration level of HIF-1a and ICAM-1 related to clinical status of RRD patients. Clinical status in this study include duration of retinal detachment, PVR severity, retinal quadrants involvement.

Metode

Study Design

A cross-sectional study was conducted from October 2015 to October 2016. This study was held in Vitreoretinal Division, Department of Ophthalmology, Dr. Cipto Mangunkusumo National General Hospital (RSCM), Jakarta. ICAM-1 was examined at Pathology Clinic Laboratory and HIF-1a was examined at Biochemistry Laboratory, Faculty of Medicine, Universitas Indonesia, RSCM. This study has received ethical clearance from Ethic Research Committee (594/UN2.F1/ETIK/2016). All patients have given written informed consent prior enrollment to this study. Consecutive vitreous sampling was performed and 34 samples were obtained during pre-operation. Data regarding patient's demography and duration of retinal detachment were obtained through history taking.

Inclusion and Exclusion Criteria

Patients with RRD who presented to the Kirana-RSCM during October 2015 to October 2016, which indicated for vitrectomy were included in this study. Patients with prior history of laser photocoagulation (LP)/surgery/injection, uncontrolled hypertension

(systolic >180 mmHg or diastolic >110 mmHg), kidney failure (hemodialysis patients), coagulation disorder or consume anticoagulant one week before procedure, history of stroke, and auto immune disorder (rheumatoid arthritis, systemic lupus erythematosus, or systemic sclerosis) were excluded from the study. Patients with severe media opacity were excluded.

Vitrectomy

Vitrectomy was precluded by general anesthesia and performed with standard three-port pars plana technique. Any vitreous hemorrhage, vitreous fibroproliferative tissue and membrane was removed by vitrectomy cutter and intraocular forceps. Endophotocoagulation laser was performed in each eye with power of 250-350 mw, duration of 150-200 ms, interval of 150-200 ms and number of laser shots of 100-700.

Vitreous and Plasma Samples

Undiluted vitreous fluid samples (0.5 – 1.0 ml) were obtained from 34 patients with RRD during pars plana vitrectomy (PPV). Vitreous samples were collected with 1 ml syringe which connected to vitreous cutter, before saline intravitreal infusion was performed. Samples were hand transferred into sterile tubes, put into a container filled with ice, and immediately centrifuged at 13.000 G for 5 minutes at temperature of 4 °C. Supernatants were frozen at -80 °C in micro tubes and rapidly put in the container filled with blue ice/dry ice until assayed.

Laboratory Assay

HIF-1 α level was measured from the vitreous samples by Department of Biochemistry, FKUI. The concentrations of immunoreactive HIF-1 α were measured by using enzyme-linked immunosorbent assay (ELISA). A two-step sandwich-type immunoassay protocol was performed, microwell plates were coated with HIF-1 α antibody and another anti-HIF-1 α antibody detection which labelled with horseradish. The intra-assay CV was

3.3% and the inter-assay coefficient of variation (CV) was 3.5%. The vitreous concentrations of ICAM-1 were measured by ELISA for ICAM-1 and performed by the Department of Clinical Pathology, FKUI-RSCM. The assay procedure was run based on the manufacturer's standards.

Statistical Analysis

Statistical analyses were performed with Statistical Package for Social Sciences Software for Windows version 20.0 (SPSS Inc, Chicago, IL). Analysis of normality of data distribution was performed by Saphiro-wilk with p value of more than 0.05 was considered normally distributed. The value of ICAM-1 and HIF-1 α then recorded in mean or median depended on the normality of data distribution. Statistical analysis is considered significant with p value less than 0.05. Unpaired numerical comparative analysis was used for comparing the value of ICAM-1 and HIF-1 α values based on variables of duration of retinal detachment, PVR grade, and retinal quadrant involvement.

Results

A vitreous sample was collected from 34 eyes that underwent vitrectomy surgery were analysed. The 34 samples were analysed for its ICAM-1 and HIF-1 α levels to see whether both markers are associated with the clinical findings found in the patients. Patients' characteristics are reported in table 1. The samples consist of 11 women (33%) and 23 men (67%). The parameters of clinical findings are duration of RRD, the extent of retinal detachment area, and the PVR grading. Each of the parameters were classified into 2 groups. Duration of RRD to vitrectomy was divided into ≤ 1 week groups and > 1 week group. While the extent of retinal detachment area, grouped into patients with retinal detachment area that covers 2 quadrants and 4 quadrants. For the PVR parameter, the samples were grouped into PVR Grade B group and PVR Grade C group.

Table 1. Characteristics of Subjects

Characteristics	Frequency (n=34)	%
Age group		
<18 years old	2	8.82
18-<40 years old	9	2.94
40-<60 years old	15	20.59
≥60 years old	8	23.53
Gender		
Male	23	67.65
Female	11	32.35
Laterality		
OD	16	47.06
OS	18	52.94
Duration of ablatio		
≤1 week	3	8.9
>1 week	31	91.1
Retinal quadrants involvement		
2 nd quadrants	2	5.9
4 th quadrants	32	94.1
PVR severity		
PVR B	13	38.24
PVR C	21	58.82

An independent samples t-test was conducted to compare the level of ICAM-1 as well as HIF-1a based on the duration of the RRD to the vitrectomy procedure. The duration of RRD further classified

into 2 groups; patients whose underwent vitrectomy less than or equal to 1 week and patients whose underwent vitrectomy more than 1 week after diagnosis. The result can be seen in Table 2.

Table 2. Comparison of ICAM-1 and HIF-1a to Duration of RRD to Vitrectomy

Biomarker	Duration of RD	n	Mean	Std. Deviation	p-value	95%CI	
						Lower	Upper
ICAM-1	≤ 1 week	3	49.7073	49.54377	0.264	-16.89	59.56
	> 1 week	31	28.3697	29.39653			
HIF-1a	≤ 1 week	3	1.1437	0.55455	0.720	-2.68	1.87

The group statistics table represents a significant difference in the level of ICAM-1 in patients whose diagnosed less than or equal to 1 week (49.71, SD 49.54), in comparison to patients whose diagnosed more than 1 week (28.37, SD 29.39). This represented in the number of mean, where ≤1 week group has almost double the number of >1 week group. However, for the HIF-1a level, the results show no significant difference as the mean between the two groups are similar, as shown in the ≤ 1 week group mean result 1.14 (SD 0.55) and > 1 week group mean result of 1.54 (SD 1.90).

The independent t-test result shown no significant difference in the scores for < 1 week group and >1 week group conditions to the level of ICAM-1 ($t(32)=1.13$, $p\text{-value}=0.264$). Similar to the ICAM-1 level, both group that is tested has no significant difference in the levels of HIF-1a, with $p\text{-value}$ of 0.720 ($t(32)=-0.36$). Another independent samples t-test was conducted in order to compare the level of ICAM-1 and HIF-1a in patients with retinal detachment in 2nd quadrant and 4th quadrant area. There is a significant difference between the 2nd quadrant group (19.42, SD 2.49) and 4th

quadrant group (30.93, SD 32.04) in the ICAM-1 category. Meanwhile, there is only minimal gap in the mean results of 2nd quadrant group and 4th quadrant group; 0.83 SD 0.38 and 1.56 SD 1.87 respectively.

Table 3 represents the result of independent t-test on ICAM-1 and HIF-1a levels based on the area of retinal detachment. The results show insignificant result on both ICAM-1 (t(32)=2.-0.50, p-value=0.620) and HIF-1a (t(32)=-0.54, p-value=0.591).

Table 3. Comparison of ICAM-1 and HIF-1a to Area of RRD

Biomarker	Area of RD	n	Mean	Std. Deviation	p-value	95%CI	
						Lower	Upper
ICAM-1	2 nd quadrant	2	19.4150	2.48902	0.620	-58.33	35.3
	4 th quadrant	32	30.9298	32.04001			
HIF-1a	2 nd quadrant	2	.8255	0.38537	0.591	-3.47	2.01
	4 th quadrant	32	1.5564	1.87679			

Table 4 reveals the independent samples t-test analysis on ICAM-1 and HIF-1a results based on the severity of PVR. The sample group was divided onto 2 groups; patients with Grade B PVR and patients with Grade C PVR. From the group statistics, the ICAM-1 level difference between the PVR Grade B group and PVR Grade C group

was significant. The mean difference on the PVR Group B is 35.42 (SD 30.13) while the PVR Grade C group has 27.05 (SD 32.11). On the other hand, the mean difference between the 2 groups is not significant on the HIF-1a level, with PVR Grade B group has lower mean (1.45 SD1.86) than PVR Grade C group (1.55 SD 1.85).

Table 4. Comparison of ICAM-1 and HIF-1a to PVR Severity

Biomarker	PVR Severity	n	Mean	Std. Deviation	p-value	95%CI	
						Lower	Upper
ICAM-1	PVR B	13	35.4209	30.13545	0.455	-14.19	30.92
	PVR C	21	27.0529	32.10858			
HIF-1a	PVR B	13	1.4475	1.86352	0.872	-1.41	1.22

Based on Table 4, the result from analysis reveals that there is no significant difference on both ICAM-1 and HIF-1a levels based on the degree of PVR. Both ICAM-1 and HIF-1a results in p-value > 0.05; with p-value of 0.455 for ICAM-1 (t(32)=0.76) and p-value of 0.872 for HIF-1a (t(32)=-0.163).

Discussion

In this study, majority of subjects with RRD is at the age group of 40-60 years old (44%) and males (67%). Similar results shown in previous study by Chen et al.¹¹ in Taiwan, the average age of patients with RRD was 47 years old and highest percentage of age group was 50-69 years old. Another previous study by Antunica¹⁴ in Croatia also showed that prevalence of RRD was higher in older age group. The duration between first onset of RRD and vitrectomy procedure in this study varies, with majority of the sample had their vitrectomy

more than 1 week after the onset. Study by Rose¹⁵ reveals a correlation between the duration of retinal detachment and post-operative retinal sensitivity. The study shows patients that underwent vitrectomy under 28 days have better outcome than patients who underwent the procedure more than 28 days since the onset.

ICAM-1 and HIF-1a to duration of Rhegmatogenous Retinal Detachment

On the result section, we have seen that there is no significant difference in the duration of the retinal detachment to the ICAM-1 levels. However, between the 2 duration groups, ≤1-week group and >1-week group there is a significant difference. The ≤1-week group has a significantly higher mean difference, almost double up the mean difference of the >1 week. This result was in line with previous study by Chen et al¹⁵ that stated despite of the

assumption that the ICAM-1 level will be higher with the increase of the duration, it is proven that there is no statistical correlation was found. The higher mean difference in the ≤ 1 week group represent the early cytokine-induce inflammatory process that occurs in hours to days after the onset of retinal detachment. During the ≤ 1 -week the inflammation might be at the peak, thus the higher ICAM-1 levels, whereas the >1 -week the inflammation process might be slowed down. Following the RRD, the breakdown of blood-retinal barrier will follow. This leakage of blood will promote inflammatory reaction and proliferation of retinal glial and RPE cells. ICAM-1 acts as an inflammatory factor that plays a critical role in signal transduction and adhesion during inflammatory response.^{8,15} However, this process is not only determined by ICAM-1, but along with other inflammatory cytokines, thus further research on the cytokines during the inflammatory process is needed.

There is no significant difference in the HIF-1a levels to the duration of the retinal detachment. The mean difference between patients diagnosed ≤ 1 -week and those in >1 -week group was minimal. The minimal difference between the 2 subgroups represents a constant hypoxic condition due to the detachment. As mentioned before, HIF-1a will increase in the presence of hypoxic condition, which inhibits the PHD and HIF protein excretion.^{8,9} Thus, as long as the detachment was not resolved, the hypoxic condition will persist, which result in a maintained HIF-1a level in the vitreous.¹⁶ A study in the animal, represents a similar result with this study. The study highlight a marked increase in the level of HIF-1a and HIF-2a protein at 1 day post retinal detachment. However, the level decreased by the third day.²²

ICAM-1 and HIF-1a to area of Rhegmatogenous Retinal Detachment

Studies conducted by Chen et al⁶ showed an increase in ICAM-1 expression in RPE cells in RRD. The study also mention that duration and extent of RRD has a direct involvement in the prognosis and outcome of RRD after vitrectomy. This can be caused by the presence of irregular lamellar debris in the sub-retinal area which causes disruption of healing in a longer duration. Hypertrophy of Muller cells in the outer plexiform layer can cause the production of glial lesions which can cause disruption of photoreceptor regeneration.²³⁻²⁵ There are lack of study in terms of HIF-1a relation to the extent of RRD in human, as most study was

conducted in animals. However, the result from the previous study was not found in the current study. In this study, both ICAM-1 and HIF-1a levels was insignificant to the extent of the retinal detachment. The highest values of HIF-1 α and ICAM-1 were found in retinal detachment at 4th quadrants. The mean difference of ICAM-1 level between the 2 subgroups are significant, greater area of damage will trigger greater inflammatory response. However, the HIF-1a levels are relatively similar despite of the extent of damage. Nevertheless, limitations from this study such as the small amount of samples and other confounding factors might contribute to the insignificant result.

ICAM-1 and HIF-1a to PVR Severity

PVR refers to a formation of epiretinal and subretinal membrane formation which causes tangential retinal traction and fixed retinal fold formation. The main characteristic of PVR is the retinal folds and its rigidity, which overall reduces the retinal mobility. Proliferative vitreoretinopathy was stratified into 3 grades; Grade A, Grade B, and Grade C. PVR Grade A (minimal) characterized by a diffuse distribution of vitreous haze and tobacco dust, some clumps might also found in the inferior surface of the retina. While PVR B (moderate) characterized by wrinkling of the inner retinal surface, decrease in the vitreous gel mobility, rolled edges of the retinal breaks, as well as retinal stiffness. And the las grade, PVR Grade C (marked) diagnosed when a rigidity of full-thickness retinal folds was found. Usually it is accompanied with a severe vitreous condensation and strands.^{2,24,26}

A formation of PVR origins from the normal wound-healing response to inflammation, migration, and proliferation of resident ocular cells and invading immune cells. Increased ICAM-1 levels have been shown to play a role in the occurrence of PVR post vitrectomy.²³ Other studies also state ICAM-1 has an involvement in the pathogenesis of PVR particularly in the phase of retinal apoptosis, during the early phase of retinal detachment.²⁴ Based on the immunohistochemical studies that has been done, it is known that the presence of fibroblast growth factor (FGF), insulin growth factor (IGF) as well as vascular endothelial growth factor (VEGF) are common in the PVR membranes. Moreover, levels of Interleukin-1 (IL-1), Interleukin-6 (IL-6), interferon gamma (IFN-gamma) as well as intercellular adhesion molecule (ICAM-1) have been shown to be elevated in the vitreous of PVR patients. These findings

suggest an inflammatory component during PVR formation.²⁴ The ICAM-1 level in the vitreous of PVR patients are found to be upregulated by IL-1, TNF-alpha or IFN-gamma. ICAM-1 plays role in regulating the leukocyte migration into the area of inflammation, thus enhancing the cytokine-mediated inflammatory reactions after the onset of retinal detachment.^{24,26} Previous studies conducted by Roybal et al.²⁷ showed an increase in ICAM-1 level alongside other cytokines, such as IL-6 and VEGF, in accordance to increase in the severity of PVR. This shows the potential use of ICAM-1 in detecting the severity of PVR.

This study found that there is no significant correlation between the PVR and levels of ICAM-1. Current findings on this study was supported by a previous study on animal¹⁰ where it shows an upregulation of proinflammatory genes starting from hours to days after the onset of retinal detachment. The animal study was referred to, as it was far more controlled in comparison to other studies done in humans. On the other parameters, the levels of ICAM-1 in PVR Grade B are significantly higher than the PVR Grade C. These findings contradict the findings from Roybal et al.²⁷ The lack of analysis on the confounding factors as well as the small size of sample may contribute to the result. On the other hand, this study found no significant result between the HIF-1a levels and the degree of PVR. Both PVR grades, PVR grade B and PVR grade C also only has a small difference in terms of the mean difference. This result means that the HIF-1a levels was maintained at a certain level regardless of PVR grades. However, there are no studies that show a correlation between HIF-1 α and the occurrence of PVR, thus further research on this subject to confirm the results is needed.

Conclusion

Despite the known inflammatory process and hypoxic condition that occurs in RRD, this study has found no significant difference between the level of ICAM-1 and HIF-1a to the clinical findings of RRD. However, we can see the trend for ICAM-1 levels, where the level is higher in patients whose duration between onset of RRD to vitrectomy less or equal to 1 week, in patients with extent of retinal detachment area up to 4 quadrants, as well as patients with PVR Grade B. The higher ICAM-1 levels represent the inflammation process that peaked in the early phase (hours to days) after the onset. On the other hand, the HIF-1a results

across the sub groups only have slight differences, because of the constant hypoxic stimulation that is still ongoing. Thus, further comprehensive study in this field is needed.

Ethics approval

This study has received ethical clearance from Faculty of Medicine, Universitas Indonesia Ethic Research Committee (594/UN2.F1/ETIK/2016)

Author contributions

AAV, TDG, RD, and SWAJ conceived and designed the analysis, collected the data, contributed data, performed the analysis, and wrote the paper

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