Correlation between Interleukin-6 and Thrombin–Antithrombin III Complex Levels in Retinal Diseases

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\textbf{ABSTRACT}

\textbf{Purpose}: This study aims to evaluate and correlate the levels of interleukin-6 (IL-6) and thrombin–antithrombin III complex (TAT) in the vitreous of patients with different vitreoretinal pathologies.

\textbf{Methods}: Vitreous samples were collected from 78 patients scheduled for pars plana vitrectomy at a tertiary medical center. Patients were divided by the underlying vitreoretinal pathophysiology, as follows: macular hole (MH)/epiretinal membrane (ERM) (\(n = 26\)); rhegmatogenous retinal detachment (RRD) (\(n = 32\)); and proliferative diabetic retinopathy (PDR) (\(n = 20\)). Levels of IL-6 and TAT were measured by enzyme-linked immunosorbent assay and compared among the groups.

\textbf{Results}: A significant difference was found in the vitreal IL-6 and TAT levels between the MH/ERM group and both the PDR and RRD groups (\(P < 0.001\) for all). Diabetes was associated with higher IL-6 levels in the RRD group. Different relationships between the IL-6 and TAT levels were revealed in patients with different ocular pathologies.

\textbf{Conclusion}: Our results imply that variations in vitreal TAT level may be attributable not only to an inflammatory reaction or blood–retinal barrier breakdown, but also to intraocular tissue-dependent regulation of thrombin.

\textbf{Introduction}

Inflammatory processes have been implicated in the pathogenesis of numerous retinal diseases.\textsuperscript{1,2} High levels of cytokines in the vitreous humor may be attributable to the local presence of macrophages, fibroblasts, and glial cells\textsuperscript{3} that may enter the vitreous substance from the blood due to breakdown of the blood–retinal barrier (BRB).

Interleukin-6 (IL-6) is a vital part of the inflammatory cascade in humans.\textsuperscript{4,5} Elevated levels of IL-6 have been reported in central and branch retinal vein occlusion, diabetic macular edema, proliferative diabetic retinopathy (PDR), and retinal detachment.\textsuperscript{6}

In recent years, it has become apparent that tight and reciprocal interactions exist between coagulation and inflammation.\textsuperscript{7–16} It is currently unknown if the increase in thrombin formation is due to inflammatory stimulation or if it constitutes an independent response to retinal disease. The present study analyzed the relationship between thrombin–antithrombin III complex (TAT) and IL-6 levels in patients with various retinal diseases.

\textbf{Methods}

The study included 78 patients who underwent pars plana vitrectomy (PPV) between 2013 and 2014. Exclusion criteria were age < 18 years and previous PPV. The study was approved by the institutional review board, and all patients gave written informed consent for participation. Vitreous samples were obtained via standard PPV with the infusion set to air to prevent dilution. Samples were aliquoted and immediately frozen at \(-80^\circ\text{C}\) pending laboratory analysis. IL-6 levels were measured with the Human IL-6 Quantikine\textsuperscript{\textregistered} HS ELISA kit (R&D Systems, Inc., Minneapolis, MN, USA) and TAT levels with the Enzygnost\textsuperscript{\textregistered} TAT micro (Siemens Healthcare Diagnostics, Inc., Malvern, PA, USA). Statistical analysis was performed using Statistica 10.0 (Statsoft, Tulsa, OK, USA).

Further data on the statistical analysis can be found in the supplementary material.

\textbf{Results}

\textbf{Study population}

Seventy-eight patients (Table 1) were divided into three groups per pathologies: group 1: epiretinal membranes (ERMs, \(n = 19\)) and macular holes (MH, \(n = 7\)); group 2: rhegmatogenous retinal detachments (RRDs, \(n = 32\)); and group 3: PDR (\(n = 20\)). Of the 78 samples, 77 were...
analyzed for TAT levels, 63 samples for IL-6, and 62 samples for both.

**TAT and IL-6 levels in ocular pathologies**

TAT level in the ERM/MH group [7.5 µg/L (3.1–29.8 µg/L)] was significantly lower than that in the RRD and PDR groups [136.1 µg/L (49.5–318.2 µg/L) and 67.7 µg/L (20.6–308.8 µg/L), respectively; \( P < 0.001 \) for both] (Figure 1A). Similarly, IL-6 level in the ERM/MH group [6.3 ng/L (4.5–15.6 ng/L)] was significantly lower compared to the RRD and PDR groups [98.3 ng/L (27.9–158.8 ng/L) and 37.5 ng/L (24.1–60.0 ng/L), respectively; \( P < 0.001 \) for both] (Figure 1B). No statistically significant difference in either IL-6 or TAT levels was observed between the RRD and the PDR groups.

**Clinical characteristics and the TAT or IL-6 levels**

To identify patient clinical characteristics significantly affecting the levels of TAT and IL-6, we performed a stepwise multiple linear regression (Supplementary Table 1). In the analysis, RRD and PDR groups were associated with increased TAT levels (compared to the ERM/MH group), which is in line with the results presented in Figure 1A. Additionally, the regression analysis revealed that the RRD group and presence of diabetes were associated with increased IL-6 levels.

Since we found that systemic diabetes affected IL-6 but not TAT, we further investigated the association of patient group and systemic diabetes with the IL-6 levels using a two-way ANOVA. The analysis demonstrated an interaction effect for patient group and diabetes \( (P = 0.047) \), indicating that the effect of diabetes on the IL-6 levels was different in different patient groups. Further post hoc testing revealed that RRD patients with diabetes had significantly higher IL-6 levels than those without diabetes \( (P = 0.042) \), while the ERM/MH patients had similar vitreal IL-6 levels irrespective of whether diabetes was present (Figure 2).

**TAT and IL-6 in specific pathologies**

To investigate the relationship between TAT and IL-6 in the patient groups, we performed a multiple linear regression (Supplementary Table 2). In this model, the ln(IL-6) × RRD interaction was statistically significant \( (P = 0.040) \), indicating different relationship between the biomarkers in the RRD group compared to the ERM/MH group (Figure 3). Specifically, the results suggest that a one-unit increase in ln(IL-6) is associated with a lesser increase in ln(TAT) in the RRD group than in the ERM/MH group. The ln(IL-6) × PDR interaction was nonsignificant, indicating that the relationship between ln(TAT) and ln(IL-6) in the PDR group did not significantly differ from that in the ERM/MH group. We obtained similar results while controlling for diabetes, anticoagulant treatment, age, and sex (not shown). None of these control variables had a significant influence on the relationship between the TAT and the IL-6 levels.

**Discussion**

BRB inhibits the entry of almost all plasma proteins from the bloodstream into the retinal tissue and vitreous cavity.\(^{17}\) However, during an inflammatory or proliferative insult, blood- or retinal-borne cells enter the vitreous cavity and secrete mediators, such as cytokines.\(^{18}\) Accordingly,
inflammation is elevated in many retinal diseases.\textsuperscript{1,6,19} Levels of IL-6, an inflammatory marker, were found to be increased in patients with RRD, alone or complicated by Proliferative Vitreoretinopathy (PVR) and correlated with the extent of RRD, especially in the subretinal fluid.\textsuperscript{20}

In many systemic diseases, increased IL-6 levels are accompanied by or correlated with increased thrombin levels.\textsuperscript{21} Whether this is true for ocular diseases is still unclear. Thrombin has been found to stimulate human retinal pigment epithelial (RPE) cells to produce IL-6 in addition to a wide variety of other cytokines, chemokines, and growth factors. Subretinal fluid from patients with retinal detachment was found to have a high capacity to generate thrombin activity,\textsuperscript{22} and vitreous fluid from patients with PVR showed increased thrombin activity with increased activation of proinflammatory and profibrotic pathways in RPE cells.\textsuperscript{19} In samples taken from eyes with branch and central retinal vein occlusion, levels of intravitreal thrombin activity were significantly higher than that in healthy controls and apparently adhered to the extent of retinal tissue involved.\textsuperscript{23}

The TAT complex results when thrombin cleaves a scissile bond near the C-terminus of antithrombin III. TAT complex may serve as an acceptable method to investigate the activation of the coagulation system.\textsuperscript{24} Because the TAT complex is kinetically stable, we opted to measure the levels of TAT in the vitreous as an indicator of coagulation cascade activity. In this study, we found TAT and IL-6 levels significantly higher in patients with RRD and PDR than in patients with ERM/MH. The high thrombin levels in RRD and PDR may be explained by either a BRB breakdown and leakage of serum components or de novo secretion from stimulated RPE cells. Thrombin induces the formation of intercellular gaps in RPE monolayers, alters the distribution of cytoskeleton proteins, and promotes the expression of vascular endothelial growth factor (VEGF) in RPE cells.\textsuperscript{25,26} Furthermore, RPE cells provide a procoagulant surface and express mRNA for various factors that are involved in the generation of thrombin from prothrombin, as well as for thrombin receptors.\textsuperscript{27} If, in our study, plasma was the only source of vitreal thrombin, we would expect to find higher levels of TAT in patients with PDR than in patients with RRD. We also did not find systemic treatment with anticoagulation to affect their level.

Despite the difference in the intensity of inflammation and coagulation between the ERM/MH and the PDR groups, our results indicate that induction of inflammation was markedly associated with activation of the coagulation cascade in either group, suggesting close interrelation between the two processes. In contrast, despite a similar intensity of inflammation and coagulation in the PDR and RRD groups, induction of inflammation was less associated with activation of coagulation, suggesting greater independence between the two processes in the RRD group. We also demonstrated that diabetes, which has been linked to increased inflammation,\textsuperscript{28,29} induced elevation in IL-6 levels in patients with RRD but did not affect TAT levels. However, our results indicate that the difference in the relationship between these biomarkers across the patient groups could not be attributed to the presence of diabetes as well as to variation in patients’ age or sex.

These findings suggest that IL-6 and TAT may act differently in retinal diseases and not necessarily in tandem. In cases of RRD and PDR, there is a disrupted BRB unlike in patients with MH and ERM; therefore, we expect higher levels of both TAT and IL-6 in the former conditions compared to the latter, as was found in our study. Yet, if that would have been the only cause for the elevated levels, we would have expected a similar relationship between these biomarkers which was not found in our patients. We theorize that local factors might also be at play in these conditions.

![Figure 2](image2.png)

**Figure 2.** Different effect of diabetes on IL-6 in specific ocular pathologies. IL-6 levels were measured in the vitreous samples from the ERM/MH, RRD, and PDR patients and presented as mean ± SD. The values were normalized using a natural logarithmic transformation and analyzed using a two-way ANOVA followed by Tukey honest significant difference (HSD) post hoc test for unequal sample size.

![Figure 3](image3.png)

**Figure 3.** Association between TAT and IL-6 in specific pathologies. IL-6 and TAT levels were measured in the vitreous samples from the ERM/MH (A), RRD (B), and PDR (C) patients. The values were normalized using a natural logarithmic transformation and presented as scatter charts. Each dot corresponds to a single measurement. The line represents the simple linear regression line.
It remains unclear in which way these two factors are related to each other, whether IL-6 precedes TAT elevation or if another branch of signaling pathway in the inflammatory cascade correlates better with TAT. Further studies are needed to confirm and analyze the role of the coagulation system in the pathophysiology of different retinal diseases.

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Declaration of Interests
The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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