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# TIMP-1 and TIMP-2 Levels in Vitreous and Subretinal Fluid

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**Abstract:** To understand the role of tissue inhibitors of metalloproteinase-1 and -2 (TIMP-1 and TIMP-2) in intraocular diseases, levels of TIMP-1 and TIMP-2 were measured by enzyme immunoassay in 47 patients with various ocular diseases: in subretinal fluid of 7 patients with rhegmatogenous retinal detachment and in vitreous of 12 patients with proliferative diabetic retinopathy, 4 with proliferative vitreoretinopathy, 2 with vitreous hemorrhage due to branch retinal vein occlusion, 12 with idiopathic macular hole, 3 with retinal detachment due to high-myopic macular hole, 4 with macular epiretinal membrane, and 3 with choroidal neovascular membrane due to age-related macular degeneration. TIMP-1 levels were significantly higher in subretinal fluid than in vitreous fluid with any diseases ($P < 0.0001$, Mann-Whitney $U$ test). TIMP-1 levels in vitreous fluid of the eyes with proliferative diabetic retinopathy and proliferative vitreoretinopathy were higher than those in vitreous with other diseases ($P < 0.0001$). In contrast, TIMP-2 levels were not elevated in the subretinal fluid and vitreous. TIMP-1, but not TIMP-2, was secreted into the subretinal space in rhegmatogenous retinal detachment, and also into the vitreous in eyes with proliferative diseases, suggesting that TIMP-1 would play a specific role in the process of these diseases. **Jpn J Ophthalmol 1998; 42:377–380** 

**Key Words:** Subretinal fluid, TIMP-1, TIMP-2, vitreous.

## Introduction

Extracellular matrix is a crucial constituent in the eye; for example, forming not only basement membranes but also vitreous gel and interphotoreceptor matrix. The matrix is maintained by a balance between its synthesis and degradation. Matrix metalloproteinases (MMPs) are a group of enzymes involved in the degradation of collagens and proteoglycans.[1] Tissue inhibitors of metalloproteinases (TIMP) inhibit the activity of MMPs by binding to them.[1] These two kinds of proteins play a cooperative role in modulating the degradation of extracellular matrix. Matrix metalloproteinases and TIMPs have been shown to be present in aqueous humor [2,3] and vitreous[4] of the human eye.

Liquefaction of vitreous gel induced by MMPs has been shown to play an important role in the development of proliferative vitreoretinopathy.[5,6] We measured levels of TIMP-1 and TIMP-2 in the vitreous of patients with various diseases and also in the subretinal fluid of patients with rhegmatogenous retinal detachment to understand their roles in normal and abnormal conditions.

## Materials and Methods

Subretinal fluid was collected with an 18-gauge blunt-tipped needle connected to a 1-mL disposable syringe that was placed outside the sclera during drainage from a sclerotomy site in the usual scleral buckling procedure. Nondiluted vitreous fluid was obtained in vitrectomy with a device (Figure 1) that we developed independently of that reported.[7] An infusion cannula was placed first, and a light guide and a vitreous cutter were inserted in the usual three-port system. Before the beginning of irriga-

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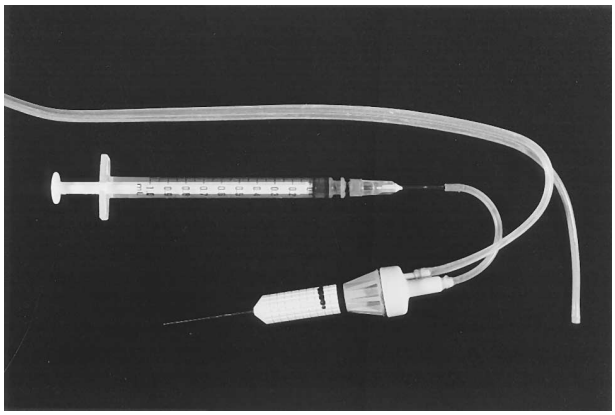

**Figure 1.** Device for collecting undiluted vitreous fluid. Suction tube is connected to 1-mL disposable syringe and fluid is aspirated manually while vitreous gel is being cut without irrigation.

tion, vitreous gel was cut and aspirated manually to a 1-mL disposable syringe connected to the suction tube of a vitreous cutter (Figure 1). Vitreous fluid (0.2 mL) was collected, and then usual vitrectomy was started with irrigation. The samples were frozen at −35°C until use. Informed consent was obtained from each patient, and all the procedures were in accordance with the Declaration of Helsinki.

Measurements of TIMP-1 and TIMP-2 were done by enzyme immunoassay using a human TIMP-1 kit (Dai-ichi Kagaku Yakuhin, Tokyo) and Biotrak TIMP-2, human ELISA system (Amersham Japan, Tokyo). For TIMP-1, 10 μL of 41-fold-diluted samples or standards, mixed initially with 150 μL of horseradish peroxidase-conjugated antihuman-TIMP-1 monoclonal antibody, were transferred to microplate wells precoated with another antihuman-TIMP-1 monoclonal antibody, and incubated for 30 minutes at room temperature. After three washings with 10 mmol/L phosphate buffer (pH 7.5) containing 50 mmol/L sodium chloride and 0.05% Tween-20, each well was incubated with 0.5% o-phenylenediamine and 0.02% hydrogen peroxide for 15 minutes. Color development was stopped by the addition of 2 mol/L sulfuric acid, and optical density was read at 492 nm with a microplate reader (Bio-Rad Laboratories Japan, Tokyo).

For TIMP-2, 50 μL of 5-fold-diluted samples or standards, premixed with 50 μL of peroxidase-labeled Fab antibody to TIMP-2, were transferred to wells of microtiter plates precoated with anti-TIMP-2 antibody, and incubated for 2 hours. After 4 washings with 10 mmol/L phosphate buffer (pH 7.5) containing 50 mmol/L sodium chloride and 0.05% Tween-20, wells were incubated with 3,3′,5,5′-tetramethylbenzidine/hydrogen peroxide in 20% dimethylformamide for 30 minutes. The reaction was stopped by the addition of 100 μL 1 mol/L sulfuric acid, and optical density was read at 450 nm.

Duplicate measurements were done for each sample, and results were expressed as a mean. The concentrations of TIMP-1 and TIMP-2, used for standard curves, ranged from 51–2000 ng/mL and 8–128 ng/mL, respectively. Serum levels of TIMP-1 and TIMP-2 were measured in 10 normal volunteers with the same kits and expressed as a mean and standard deviation.

## Results

TIMP-1 levels were significantly higher in subretinal fluid of the 7 eyes with rhegmatogenous retinal detachment than in the vitreous of the 40 eyes with various intraocular diseases ($P < 0.0001$, Mann-Whitney $U$ test, Figure 2). TIMP-1 levels in the vitreous of the 12 eyes with proliferative diabetic retinopathy and 4 eyes with proliferative vitreoretinopathy were significantly higher than those in the vitreous of the 24 eyes with other diseases ($P < 0.0001$, $U$ test, Figure 2). There was no statistically significant dif-

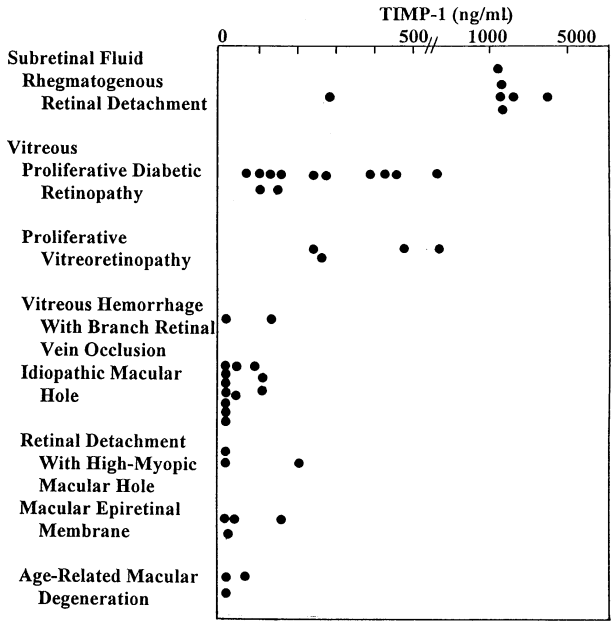


**Figure 2.** TIMP-1 levels in subretinal fluid and vitreous. Subretinal levels are significantly higher than vitreous levels ($P < 0.0001$, Mann-Whitney $U$ test), and vitreous levels in proliferative diabetic retinopathy and proliferative vitreoretinopathy patients are significantly higher than in patients with other diseases ($P < 0.0001$).

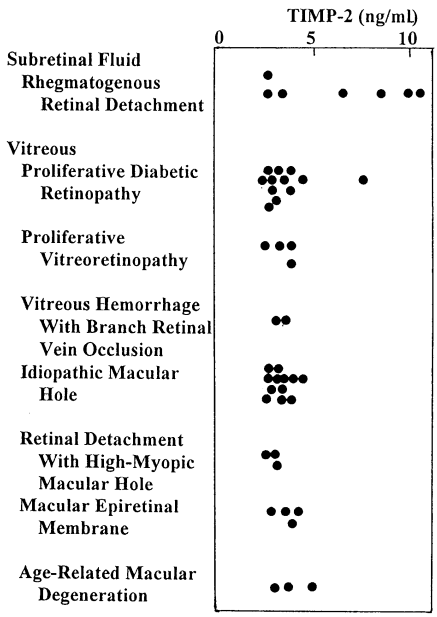


**Figure 3.** TIMP-2 levels in subretinal fluid and vitreous. Subretinal levels are higher, although not significantly, than vitreous levels (Mann-Whitney $U$ test, $P = 0.1299$). There is no elevation of TIMP-2 levels in vitreous.

ference in TIMP-1 levels between vitreous samples of other diseases. The elevated TIMP-1 levels in the subretinal fluid and vitreous were higher than its level in normal human serum (163 ± 25 ng/mL as a mean and standard deviation).

TIMP-2 levels in subretinal fluid were somewhat higher, although not significantly, than those in vitreous ($U$ test, $P = 0.1299$, Figure 3). The vitreous levels of TIMP-2 were not elevated in any diseases (Figure 3). The TIMP-2 levels in both subretinal fluid and vitreous were lower than its level in normal human serum (54.5 ± 13 ng/mL as a mean and standard deviation).

## Discussion

TIMP-1 levels were higher in subretinal fluid and vitreous than in serum, whereas TIMP-2 levels were lower in subretinal fluid and vitreous than in serum. These facts indicate that the elevation of TIMP-1 in subretinal fluid and vitreous could not be attributed to breakdown of the blood-ocular barrier and that TIMP-1, but not TIMP-2, is specifically secreted by intraocular tissues.

Higher levels of TIMP-1 in the subretinal fluid could be attributed to its production by retinal pigment epithelial cells. Retinal pigment epithelial cells in culture have been shown to secrete both MMPs and TIMPs.[8–11] Interphotoreceptor matrix is also known to contain MMPs.[12–14] Higher levels of TIMP-1 in the subretinal fluid in rhegmatogenous retinal detachment would reflect its enhanced production by retinal pigment epithelial cells in order to protect interphotoreceptor matrix from degradation, and thereby to protect photoreceptors. The subretinal fluid in rhegmatogenous retinal detachments, especially in long-standing ones, has a high concentration of proteins due to the absorption of fluid by retinal pigment epithelial cells. The high levels of TIMP-1 in the subretinal fluid may otherwise simply result from this concentration of proteins.

The vitreous of proliferative diabetic retinopathy or proliferative vitreoretinopathy patients contained higher levels of TIMP-1 than was the case with other diseases. The normal human vitreous has been shown to contain MMP-2, whereas the vitreous of patients with diabetes mellitus has the additional activity of MMP-9.[4] Because TIMP-1 is an inhibitor of MMP-9,[1] the elevation of TIMP-1 in diabetic vitreous would have a specific role in counterbalancing the elevation of MMP-9. Otherwise, fibrovascular tissue would secrete a high level of TIMP-1, together with MMP-9.

In conclusion, the present data suggest that specific elevation of TIMP-1 levels in vitreous and subretinal fluid would be an indicator for the remodeling of extracellular matrix and would provide a background for understanding the process of intraocular diseases. The results in this pilot study with a limited number of patients should be confirmed by a further study including a larger number of patients.

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