Relationship of MMP-2 and TGF-β2 levels in human vitreous with axial length

Ting-Ting Zhao, Meng-Zhu Wu, Jing Jin, Shu-Nong Xie, Ying Fan

Department of Ophthalmology, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200080, China

Correspondence to: Ying Fan. Department of Ophthalmology, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200080, China. mdfanying@sjtu.edu.cn

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Abstract

- **AIM:** To investigate the relationship of matrix metalloproteinase-2 (MMP-2) and transforming growth factor-beta 2 (TGF-β2) levels in human vitreous with axial length (AL) of patients with high myopia.
- **METHODS:** The concentrations of MMP-2 and TGF-β2 levels were tested by enzyme-linked immunosorbent assay (ELISA). Fifty-five human vitreous samples of 55 patients were collected during vitrectomy surgery, and were divided into two groups according to their spherical equivalent (SE) and AL. High myopia group (25 cases): AL≥26.00 mm, and control group or non-high myopia group (30 cases): AL<26.00 mm.
- **RESULTS:** The MMP-2 levels in vitreous of high myopia group (96.87±55.95 ng/mL) was significantly higher than that of control group (77.24±41.81 ng/mL, \(P<0.05\)), but not correlated with AL (\(r=0.088, P=0.544\)). While the vitreous TGF-β2 concentration was negatively correlated with AL (\(r=-0.344, P=0.014\)), and there was significant difference of TGF-β2 vitreous levels between high myopia group (3729.08±1890.88 pg/mL) and control group (3926.00±1333.88 pg/mL, \(P<0.05\)).
- **CONCLUSION:** MMP-2 and TGF-β2 in human vitreous may play a critical role in human high myopia development, and the TGF-β2 appears to be associated with AL.

**KEYWORDS:** matrix metalloproteinase-2; transforming growth factor-beta 2; vitreous body; high myopia; axial length

**INTRODUCTION**

Myopia is the most common visual disorder in the worldwide, especially in the Asia countries\(^{1-3}\). High myopia is often defined as refractive error<-6.00 D or axial length (AL) ≥26.00 mm, with complications including posterior staphyloma (PS)\(^{1-4}\), choroidal neovascularization (CNV)\(^{5,13}\), macular hole (MH)\(^{6,14-16}\), retinal detachment (RD)\(^{17-18}\), macular hole retinal detachment (MHRD) or macular retinoschisis\(^{10-21}\) and so on. These may result from its pathology characteristics: sclera thinning, remodeling and axial elongation\(^{22-24}\). In myopic eyes, sclera remodeling and axial elongation are associated with changes of its extracellular matrix (ECM)\(^{25}\), and controlled by a number of little chemical molecules, in which, the major two are matrix metalloproteinase-2 (MMP-2) and transforming growth factor-beta 2 (TGF-β2).

MMPs are a group of zinc-dependent proteinases that can degrade collagens, gelatins, ECM molecules, which composed by collagenases, stromelysins, gelatinses and membrane type-MMPs\(^{26-37}\). Among above, gelatinases, including MMP-2, also called gelatinase A, can be found in various ocular tissues of human and animals\(^{29}\). Although numbers of human and animal experiments have suggested that the expression of MMP-2 (mRNA or protein) increases during myopia developing and decreases when recovery\(^{25,27,38-39}\). However, there are also studies suggested no association between myopia and MMP-2\(^{28,33,40}\), this comes out controversy. TGF-β plays a critically important role in regulating ECM turnover\(^{41-45}\). There are five isoforms, that are, TGF-β1, TGF-β2, TGF-β3, TGF-β4 and TGF-β5 in total, among them, the first three have been demonstrated expression in few tissues in mammal animals, include sclera, retina and choroid, in addition, TGF-β2 is the main subtype and a normal composition in human vitreous\(^{46-49}\). Nevertheless, the previous researches primarily instructed the two molecules: MMP-2 and TGF-β2 (mRNA or protein) in aqueous humor, sclera, retina, choroid etc. of human or animals, maybe, just investigating the changes of MMP-2 and TGF-β2 levels in patients with high myopia. The purpose of our study was to explore the relationship between MMP-2 and TGF-β2 levels in human vitreous and AL of high myopic patients, furthermore, to explicit the action of MMP-2 on myopia.
SUBJECTS AND METHODS

Ethical Approval  Informed consent was obtained from all individual participants included in the study, the study followed the principle of the Declaration of Helsinki (2008) and was approved by the Ethics Committee of Shanghai First People’s Hospital of Shanghai Jiao Tong University.

Patients and Samples  This study was an observational and case-control study. Fifty-five samples of 55 individuals received vitrectomy for retinal detachment or MH, which were all collected from Mar. 2015 to Nov. 2015. There were 24 males and 31 females in total with ages ranging from 18 to 80y. Their average age is 58.53y. Comprehensive ocular examinations were received, which including detailed ophthalmoscopic examinations, B-scan ultrasonography and optical coherence tomography (OCT). AL was measured with an IOL-Master (Optical Biometry, Master 500, Carl Zeiss Meditec AG, Germany). According to AL, the subjects were divided into two groups (high myopia group, 25 cases and control group or non-high myopia group, 30 cases). The exclusion criteria were ocular traumas, CNV, proliferative vitreoretinopathy, endophthalmitis and any other history of ocular surgery. The samples were collected into marked sterile tubes during vitrectomy prior to intraocular infusion and immediately stored at -80℃ until analysis.

Measurement of MMP-2 and TGF-β2  All the vitreous samples were assayed with enzyme-linked immunosorbent assay kit (ELISA kits; R&D System, USA). According to the manufacturer’s instructions, briefly, diluted human vitreous or rebuilt standard samples incubated for 2h at room temperature, repeated aspirating and washing with wash buffer for a total of three times, conjugate added and washed also, stopped with stop solution and measured by a microplate reader at a wavelength of 450 nm.

Statistical Analysis  The results from two groups were analyzed with SPSS 17.0 statistical software. The original data of MMP-2/TGF-β2 levels and ages of two groups were normally distributed by the Kolmogorov-Smirnov test. The relationship between AL and MMP-2/TGF-β2 or ages and MMP-2/TGF-β2 was used Spearman correlation analysis. The MMP-2/TGF-β2 levels and ages were expressed as mean±standard deviation (SD) and analyzed with t-test. The χ² test was used to analyzed the comparison of sex distribution. A two-tailed P value of less than 0.05 was considered statistically significant.

RESULTS

The Characteristics of Sex, Ages and Axial Length  There was no significant difference of age (t=0.006, P=0.94) or gender (χ²=1.528, P=0.216) between high myopia group and non-high myopia group, furthermore, no statistically significant correlation between the age and AL was found (r=-0.249, P=0.081; Table 1).

Levels of MMP-2 and Axial Length  The MMP-2 could be detected in all vitreous samples and MMP-2 concentration of high myopia group was significantly higher than control group (P=0.037), nevertheless, there was no significant correlation between AL concentration and MMP-2 (r=0.088, P=0.544) in all subjects (Table 1, Figure 1).

Levels of TGF-β2 and Axial Length  The TGF-β2 could be detected in all vitreous samples, there was significant difference of TGF-β2 between the two groups (P=0.030), in addition, there was significant correlation between AL concentration and TGF-β2 (r=-0.344, P=0.014; Table 1, Figure 2).

Relationship between Concentrations of MMP-2/TGF-β2 and Age/Gender  No significant correlation was found between MMP-2 (r=-0.106, P=0.451) or TGF-β2 (r=0.014, P=0.923) and age, either, there was no significant difference of MMP-2 (F=0.057, P=0.812) or TGF-β2 (F=2.007, P=0.163) between males and females.

Table 1 Main characteristics of 55 patients from two groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>High myopia group (n=25)</th>
<th>Control group (n=30)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>57.72±12.26</td>
<td>60.21±14.23</td>
<td>0.940*</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>12/13</td>
<td>12/18</td>
<td>0.216*</td>
</tr>
<tr>
<td>Axial length (mm)</td>
<td>28.69±1.93</td>
<td>23.57±1.08</td>
<td>0.020*</td>
</tr>
<tr>
<td>MMP-2 concentration (ng/mL)</td>
<td>96.87±55.95</td>
<td>77.24±41.81</td>
<td>0.037*</td>
</tr>
<tr>
<td>TGF-β2 concentration (pg/mL)</td>
<td>3729.08±1890.88</td>
<td>3926.00±1333.88</td>
<td>0.030*</td>
</tr>
</tbody>
</table>

MMP-2: Matrix metalloproteinase-2; TGF-β2: Transforming growth factor-beta 2. *Independent t-test; *Pearson χ² test.

Figure 1 Correlation between AL and MMP-2.
between MMP-2 concentration in human aqueous humor and
humor with high myopia, significantly positive correlation lies
MMP-2 expression increased in human vitreous or aqueous
addition, previous studies have provided strong evidence that
deprivation, different time course and recovery
between MMP-2 (mRNA and protein) and myopia scleral
human have constituted evidence of significant association
Researches of animal (tree shrew and chicken) model and
was one bone of contention comparing with previous reports.
between MMP-2 concentration and AL for all subjects, this
myopia group, moreover, there was no significant correlation
high myopia was statistically significant higher than non-high
in the first place, MMP-2 concentration of human vitreous in
and measured these with ELISA and draw several conclusions,
in high myopia was statistically significant higher than non-high
myopia group, moreover, there was no significant correlation
between MMP-2 concentration and AL for all subjects, this
was one bone of contention comparing with previous reports.
Researches of animal (tree shrew and chicken) model and
human have constituted evidence of significant association
between MMP-2 (mRNA and protein) and myopia scleral
remodeling. Animal experiments have illustrated the MMP-2
mRNA level up- and down-regulation changes during form
deprivation, different time course and recovery. In addition,
previous studies have provided strong evidence that
MMP-2 expression increased in human vitreous or aqueous
humor with high myopia, significantly positive correlation lies
between MMP-2 concentration in human aqueous humor and
AL. In our study, results showed elevated MMP-2 level in
human vitreous of high myopia and no further AL correlation.
MMP-2 protein findings were consisted with preceding
researches (mRNA or protein) generally, we speculated that
tissue and race specificities maybe the sources for discrepancy.
Several antecedent studies found that MMP-2 (single-
nucleotide polymorphisms; SNP analysis) did not appear to
be associated with myopia or refractive error with the dye
terminator-based SNaPshot method including Han Chinese
high myopia, Japanese high myopia and Caucasian ethnicity
myopia. Another research discovered MMP-2 gene was
involved in myopia of Amish families but not in Ashkenazi
families using the same genetic analysis means. The results
suggested that tagging different promoter SNPs that carrying
common sequence or not and environmental heterogeneity
probably came out various associations with myopia.
In the next place, TGF-β2 level of high myopia group was
significantly lower than non-high myopia group, furthermore,
there was a significantly negative correlation between TGF-β2
concentration and AL. TGF-β2 is the critical mediator of
scleral remodeling during myopia development, it mainly
led to reducing synthesis and increasing resolution of scleral
collagen. Researches of TGF-β2 concentration in human
myopia subjects are controversial, significantly higher in
human aqueous humor and positively correlated with AL
were found but no significant difference in human vitreous
between high myopia and control group, the difference
may be generated by tissue diversity and measuring method.
Previously, studies in animal models showed that retinal and
choroid TGF-β2 mRNA level was not altered while reduced
abidingly or fluctuant in the scleral at specific myopia scleral
remodeling stages. It was probably that the early
decrease of scleral TGF-β2 was an indication for retinoscleral
signal guiding ocular myopia change. These were not in
conformity with our study, which could be attribute to tissue
and race specificities, detection method may also accounted for
part factors.
In conclusion, this study implied that the protein levels of
MMP-2 and TGF-β2 changed in the human vitreous of patients
with longer AL. The concentration of MMP-2 increased
while TGF-β2 decreased in high myopia subjects, in addition,
it implied that longer AL patients had lower level TGF-β2.
MMP-2 and TGF-β2 played critical roles in high myopia
cleral remodeling. Our study will provide a strong evidence
for the research concerned high myopia clinically.

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