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MMP-2 and TIMP-2 levels in human serum: relationship with axial length

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高度近视患者血清中 MMP-2 和 TIMP-2 的含量与眼轴长度的相关性

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摘要

目的:检测高度近视患者血清中 MMP-2 和 TIMP-2 的含量并探究它们与眼轴长度之间的相关性。

方法:选取 2015-05/2015-12 在武警总医院就诊的 13 例高度近视患者为观察组,并以同期的 13 例性别、年龄相匹配的正常眼轴患者为对照组。用 A 超测量眼轴长度,ELISA 检测两组患者血清中 MMP-2 及 TIMP-2 含量,并比较两检测指标与眼轴长度值之间的相关关系。

结果:高度近视组血清中 MMP-2 和 TIMP-2 的含量均低于对照组(分别为 $P_1=0.027$ 和 $P_2=0.048$)。MMP-2 含量在高度近视组为 10.21(9.26~11.49)pg/mL,而在对照组为 11.56(11.03~15.14)pg/mL;TIMP-2 含量在高度近视组为 79.69(72.86~93.89)pg/mL,而在对照组中为 93.16(87.54~100.97)pg/mL。TIMP2/MMP2 两组患者之间差异无统计学意义($P=0.216$)。血清中 MMP-2 及 TIMP-2 含量均与眼轴长度呈负相关($r_1=-0.512$, $P_1=0.007$; $r_2=-0.604$, $P_2=0.001$);TIMP2/MMP2 与眼轴长度之间的相关性无统计学意义($r=0.385$, $P=0.052$)。

结论:血清中 MMP-2 及 TIMP-2 的含量随眼轴的增加而降低,且均与眼轴长度呈负相关。

关键词:血清;眼轴长度;MMP-2;TIMP-2

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Abstract

• AIM: To assess the serum levels of matrix metalloproteinase 2 (MMP-2) and tissue inhibitor of matrix metalloproteinases 2 (TIMP-2) in patients with high myopia (HM), and to investigate the relationship between their levels and axial length (AL).

• METHODS: HM patients (13 eyes) (group A) and age-matched healthy volunteers with normal axial length eyes (13 eyes) (group B) who acted as controls were recruited. Blood samples were collected from all subjects in the early morning hours after an overnight fast. MMP-2 and TIMP-2 levels were measured by specific immunoassays (enzyme-linked immunosorbent assay).

RESULTS: The serum MMP-2 and TIMP-2 levels were decreased in the eyes of patients with HM compared with those of controls ($P=0.027$ and $P=0.048$, respectively). The median MMP-2 level was 10.21 pg/ml (range, 9.26-11.49 pg/ml) in the group A and 11.56 pg/ml (range, 11.03-15.14 pg/ml) in the control group. The median TIMP-2 level was 79.69 pg/ml (range, 72.86-93.89 pg/ml) in the group A and 93.16 pg/ml (range, 87.54-100.97 pg/ml) in the control group.

There was no significant difference between the two groups in TIMP2/MMP2 ($P=0.216$) ratio. MMP-2 and TIMP-2 levels were negatively correlated with AL ($r_1=-0.512$, $P_1=0.007$; $r_2=-0.604$, $P_2=0.001$). No correlation was observed between the ratio TIMP2/MMP2 and AL ($r=0.385$, $P=0.052$).

• CONCLUSION: This pilot study reveals that the levels of MMP-2 and TIMP-2 could be detected in the serum. The levels of serum MMP-2 and TIMP-2 were decreased in the eyes with elongated axis, and their levels were nevatively correlated with AL.

• KEYWORDS: human serum; axial length; matrix metalloproteinase 2; tissue inhibitor of matrix metalloproteinases 2

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INTRODUCTION

High myopia (HM), also known as pathological, degenerative, or malignant myopia, is characterized by eyeball excessive elongation and progressive myopia, which predisposes to many pathologic changes involving the sclera, choroid, and retina[1]. Those changes can cause irreversible visual disturbances. Myopia is the commonest ocular abnormalities in the world and imposes a huge pressure on the public health services system and the economy development[2]. A study of 4409 Chinese people under the age of 40 showed that high myopia was the second most common cause of low vision and blindness[3]. To myopia ,the most important ocular parameters is the excessive elongation of the axial length (AL)[4].

Myopia, especially the pathological type is a disorder that primarily targets collagens within the sclera[5]. One of the characteristics of myopia is abnormal expansion and thinning of the sclera . Today people generally believed that myopia is caused by the sclera extracellular matrix remodeling . The process of sclera remodeling focus on the collagen metabolism and is considered to be the result of excessive degradation of sclera extracellular matrix. Researches on animal models of myopia suggested that proteinases were involved in the scleral remodeling process. Matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs), enzymes which involved in the degradation of extracellular matrix play a role in the pathogenesis of high myopia. In an animal experiment MMP-2 level was higher in the sclera of the myopic eye than the level of the control eye[6]. The domestic and foreign researches have shown that some cytokines such as MMP-2 and TIMP-2 in aqueous humor correlated with ocular axial length. According to a study that MMP-2 and TIMP-2 levels in the aqueous humor were positively correlated with AL[7]. Wang *et al*[8] reported that in the defocused eyes the expression of TIMP-2 was significantly lower and the expression of MMP-2 was significantly higher in the sclera than in the contralateral eyes. Zhuang *et al*[9] recently reported that the vitreous levels of MMP-2 and the ratio MMP-2/TIMP-2 in HM were significantly higher than in the control group. In recent years, numerous attempts were done to investigate the relationship between the various cytokines and ocular axial length.

However, the previous researches were mainly based on the cytokines and proteinases in aqueous humor and vitreous body cavity. To date, there is limited data on levels of proteinases in serum of subjects with high myopia. The purpose of our study was to investigate the changes of MMP-2 and TIMP-2 levels in the serum of patients with high myopia.

SUBJECTS AND METHODS

Subjects The present study was performed in accordance with the Declaration of Helsinki. All subjects gave informed consent for participation in the study and the Institutional Review Board at the Beijing Armed Police General Hospital approved the research. This prospective case-control study consisted of thirteen patients with high myopia patients and thirteen age-matched healthy volunteers with normal axial length eyes as controls during the period from May 2015 to Nov. 2015. Patients with AL ≥ 26 mm and spherical equivalent of ≥-6.0 D in both eyes were included as high myopia group (group A)[10]. Volunteers with 22 mm<AL<26 mm and -0.5 D≤ spherical equivalent ≤+0.5 D in both eyes were defined as controls (group B). The AL was measured with a ophthalmology ultrasonic diagnosis apparatus (SW-2100, suoer A/Bscan, China). All participants were healthy subjects with no other ophthalmic diseases (for example, glaucoma, uveitis, progressive retinal disease), systemic diseases, such as kidney diseases, liver disease, hematologic diseases, diabetes mellitus, immune diseases, or history of drug use and previous laser or intraocular surgery. Venous blood samples from all subjects were drawn in the early morning after overnight fasting at Beijing Armed Police General Hospital, Beijing. All blood samples were allowed to clot before centrifugation. The blood was obtained in the standard blood tests, and was centrifuged for 15min at 2000 rpm. Serum were removed and stored at -80°C until assayed.

Methods This study adopted the method of prospective case-control study. We chose the patients with high myopia without other eye diseases as the study group; for comparison, we chose non-myopic volunteers with similar systemic condition as the control group. Serum concentrations of MMP-2 and TIMP-2 were measured by two experienced technicians in department of clinical laboratory using enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Abingdon, England) accordingly to the manufacturer recommendations. The serum samples were diluted 5-fold for the measurement of MMP-2 and 50-fold for the determination of TIMP-2. The intra-assay coefficient of variation (CV%) of MMP-2 is reported by the manufacturer to be 3.8% at a mean concentration of 11.2 ng/ml, SD=0.420, and that of TIMP-2 is reported to be 4.4% at a mean concentration of 1.23 ng/ml, SD=0.054. The detection limits were 33 pg/ml for MMP-2 and 11 pg/ml for TIMP-2 respectively.

Statistical Analysis The serum levels of MMP-2 and TIMP-2 did not follow a normal distribution based on Kolmogorov-Smirnov and Shapiro-Wilk tests. Therefore, nonparametric statistical analyses were used. Data were expressed as median

Table 1 Clinical characteristics of the study subjects

Variant	Total	Group A	Group B	*P*
Number	26	13	13	–
Age (a)	50.27±23.50	46.92±17.52	53.62±28.62	0.359[a]
Gender (M/F)	16/10	7/6	9/4	0.500[a]
AL (mm)	26.02±2.26	27.38±2.39	24.66±0.95	0.006[b]

Group A: high myopia group, AL ≥26 mm; Group B: controls group, 22 mm <AL<26 mm; [a]$P>0.05$: there was no statistically significant difference; [b]$P<0.05$: there was statistically significant difference; AL: Axial length.

Table 2 The levels of serum MMP-2 and TIMP-2 in two groups

Variant	Group A	Group B	*Z*	*P*
TIMP-2(pg/ml)	79.69 (72.86, 93.89)	93.16 (87.54, 100.97)	-1.992	0.048[a]
MMP-2(pg/ml)	10.21 (9.26, 11.49)	11.56 (11.03, 15.14)	-2.201	0.027[a]
TIMP-2/MMP-2	7.93 (5.56, 10.50)	7.68 (6.66, 8.74)	-1.293	0.216

MMP-2: Matrix metalloproteinases 2; TIMP-2: Tissue inhibitor of matrix metalloproteinases 2; [a]$P<0.05$: there was statistically significant difference.

and range (25th and 75th percentiles) in continuous variables that are not normally distributed, such as the levels of MMP-2 and TIMP-2, or as mean ± SD for normal distributed continuous variables, such as age and AL. The Mann-Whitney U test was employed to compare two analyzed groups. The relationship between AL and MMP-2, TIMP-2 and TIMP-2/MMP-2 were determined by Spearman's rank correlation test. Comparison of sex and ratio TIMP-2/MMP-2 distribution in two groups was analyzed by the Kruskal-Wallis test. Comparison of age and AL distribution in two groups was analyzed by the paired t-test. Statistical analyses were carried out using the SPSS 19.0 software for windows (SPSS Inc., Chicago, IL, USA). The differences were considered statically significant when a 2-tailed $P<0.05$ for this analysis.

RESULTS

The mean patients age was 50.27±23.50y (mean ± SD). No statistically significant difference was found in gender ($Z=1.220$, $P=0.500$) and age ($t=-0.953$, $P=0.359$) distributions between the two groups. The difference of axial length between the two groups was extremely significant ($t=3.315$, $P=0.006$). The demographic and clinical features are summarized in the Table 1. Serum MMP-2 and TIMP-2 Levels MMP-2 levels in the serum of the patients with HM (median, 10.21 pg/ml; range, 9.26-11.49 pg/ml) were significantly lower than those of the controls (median, 11.56 pg/ml; range, 11.03-15.14 pg/ml; $P=0.027$) (Table 2). Moreover, the patients with HM had significantly lower serum.

TIMP-2 levels than the controls [(median, 79.69 pg/ml; range, 72.86-93.89 pg/ml) vs (median, 93.16 pg/ml; range, 87.54-100.97 pg/ml); $P=0.048$] (Table 2). However, There was no significant difference between the two groups of serum ratio TIMP-2/ MMP-2 ($P=0.216$) (Table 2).

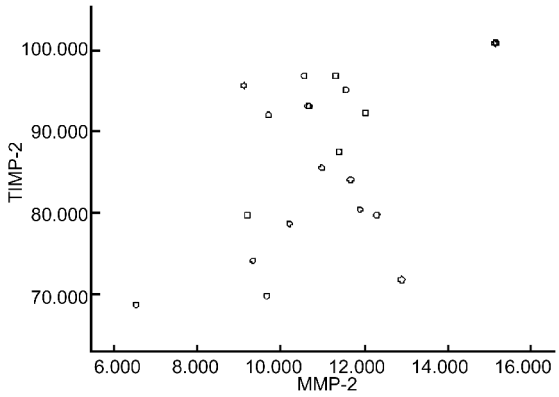

Figure 1 Correlation between TIMP-2 and serum MMP-2.

Relationship Among MMP-2, TIMP-2 and AL The serum TIMP-2 concentration was significantly positively correlated with the MMP-2 (correlation coefficient $r=0.441$, $P=0.024$; equation of the regression line: TIMP-2 concentration (pg/ml) = 2.812×MMP-2 concentration (pg/ml) + 55.973) in all subjects. (Figure 1).

The serum MMP-2 concentration was significantly negatively correlated with AL (correlation coefficient $r=-0.512$, $P=0.007$; equation of the regression line: MMP-2 concentration (pg/ml) = -0.656×AL(mm) + 28.453) in all subjects (Figure 2); The serum humor TIMP-2 concentration was significantly negatively correlated with AL (correlation coefficient $r=-0.604$, $P=0.001$; equation of the regression line: TIMP-2 concentration (pg/ml) = -2.856×AL(mm) + 162.262) in all subjects (Figure 3); However, no correlation was observed between the ratio TIMP-2/ MMP-2 and AL ($r=0.385$, $P=0.052$).

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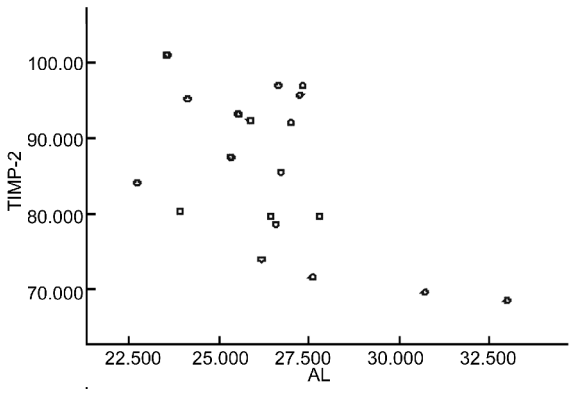

Figure 2 Correlation between axial length and serum MMP-2 level.

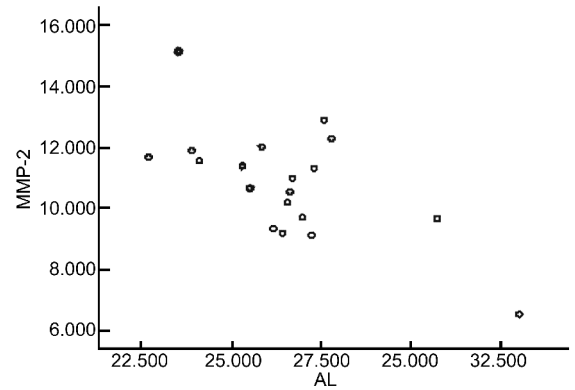

Figure 3 Correlation between axial length and serum TIMP-2 level.

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DISCUSSION

High myopia may cause a number of serious secondary complications, such as retinal hemorrhage, retinal detachment, and retinal hole. These complications may lead to an irreversible damage of vision and negatively impact patient's quality of life. So to study the pathogenesis of high myopia has attracted much attention for ophthalmologists. In recent years experiments on animals have provided the majority of our understanding of the pathogenesis of high myopia. But, there are very few studies on levels of cytokines and proteinases in humans with HM.

To our knowledge, this is the first study to detect the MMP-2 and TIMP-2 levels in human serum with high myopic eyes and show that levels of them were lower than healthy volunteers used ELISA kits.

It has been demonstrated that the pathogenesis of high myopia is associated with active scleral elongation and remodeling caused by reduced scleral collagen accumulation, scleral thinning, and loss of scleral tissue[9,11-12], which involve diminished production of the extracellular matrix[13]. Studies on high myopia in animal models have shown that scleral remodeling was one of the most important causes in the development of myopia[14]. Some enzymes (such as MMPs), and TIMPs[15] take part in the process of sclera extracel-lular matrix (ECM) remodeling. MMPs are a family of zinc-dependent endopeptidases that can break down all the ECM components[16]. Among them, MMP-2 (gelatinase A) is the most important enzyme in this group, which can degrade type IV collagen and its activity is mostly regulated by TIMP-2. MMP-2 have been shown to be expressed in the human sclera and are potential participants in scleral remodeling[17,18]. When MMP-2 activity increase, ECM will be degraded in the sclera, with potential scleral weakness, and eventually lead to the elongation of AL and the development of myopia[8,15]. TIMPs are a group of four multifunctional proteins . Their main role is not only to inhibit the function on MMPs, but also to maintain the balance between ECM protein synthesis and degradation. An imbalance between the activities of MMPs and TIMPs may take part in the pathogenesis of HM[9], which is the result of scleral ECM remodeling.

In the current study, levels of MMP-2 and TIMP-2 in the serum of the patients with HM were significantly lower compative to controls. Our result is consistent with scientific literature indicating that the protein and mRNA levels of TIMP-2 were decreased in HM reported on animals[6,8,15]. Form the other hand different animal model investigators has been reporting an increased about the protein and mRNA levels of MMP-2 in myopic eyes[6,8,15,19-23], and an increased[20-21] or unchanged[15] expression of TIMP-2. The study by Jia *et al*[4] revealed that in human subjects with HM vitreous and aqueous humor MMP-2 and TIMP-2 levels were significantly increased comparative to controls[9]. In addition, our results show that the serum TIMP-2 concentration was positively correlated with the MMP-2, which be similar to the finding that the vitreous level of MMP-2 was correlated strongly and positively with TIMP-2 level[9]. We speculate that the decreased TIMP-2 level was a normal biochemical response accompanied with decreased MMP-2 level in human serum. However, in humans the ratio TIMP2/MMP2 were not correlated with AL. It can be explained that concentrations of MMP-2 in human serum may be not only associated with high myopia, but also affected by other factors.

Our findings suggest that serum levels of MMP-2 and TIMP-2 are negatively correlated with AL. In contrast, a literature revealed that the levels of MMP-2 and TIMP-2 in the aqueous humor were positively correlated with AL, and the differences of the ratio MMPs/TIMPs among different axial length groups were statistically significant[4].

The difference between the results of our research and an existing scientific literature can be explained by following assumptions.

Firstly, as the result of the blood-aqueous barrier MMP-2 and TIMP-2 concentration in human serum are different from aqueous humor and vitreous body. In high myopia , axial length changes potentially influence choroidal blood flow[24]. So far, little is known about the influence of high myopia on the blood-aqueous barrier. It is important to simultaneously evaluate serum, aqueous and vitreous body samples from patients with high myopia, which is related to an impaired blood-aqueous barrier.

Secondly, although some studies have shown that high myopia is associated with the expression of MMP-2 and TIMP-2[17-18,25], but recent researches suggest that MMP-2 and TIMP-2 genes do not have a critical role in the development of high myopia[26-28]. Thus, the view that MMP-2 and TIPM-2 genes play a role in the pathogenesis of high myopia is controversial. At the same time, some investigators advised that the role of MMP is not driven by single nucleotide polymorphisms, but is influenced by other genetic changes which may lead to different expression[29].

Thirdly, MMPs, a group of proteolytic enzymes, which not only degradate proteins that made up of ECM and the membranes of vessels[30], but also take part in the progress of many neoplasmatic diseases and fibrous tissue disease. The levels of serum MMP-2 and TIMP-2 were lower in tumor patients than in healthy controls[31-34]. The tendency of serum MMP-2 and TIMP-2 is similar to our results. At the same time, levels of MMP-2 and TIMP-2 in the blood of the patients with direct and recurrent hernias were increased, which can suggest that ECM defects may as a basis of pathogenesis of this disease[30]. Serum levels of MMP-2 in patients with PEX syndrome that is characterized by excessive synthesis and progressive accumulation of a fibrillar material were higher than in patients with cataract[35]. Hence, we speculate that high myopia may be associated with tumor disease or desmosis, or alternatively, is a clinical manifestation of tumor disease or desmosis in eyes.

Finally, the major limitation of our study is the relatively small sample size. In addition, case-control study methods imply certain selection bias, and in actual clinical work, selection bias maybe relatively large because of some potential diseases has not yet been found, so statistical credibility is affected.

In conclusion,we found a decreased serum levels of MMP-2 and TIMP-2 in eyes with elongated axial length, and that the levels of serum MMP-2 and TIMP-2 were negatively correlated with AL. Large prospective studies are necessary to reveal the exact relationship between the levels of MMP-2 and TIMP-2 in serum, aqueous humor and vitreous body and their correlation with the pathogenesis of high myopia in humans.

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