• Original Article in English •

# Plasma levels of tissue inhibitor of metalloproteinases-1 and transforming growth factor-β1 in patients with progressive muscular dystrophy

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**Abstract: Objective** Abnormal connective tissue proliferation in muscle following muscle fibre degeneration-regeneration is a feature of muscular dystrophy. Tissue inhibitors of metalloproteinases (TIMPs) are multifunctional proteins that can modify cellular activities and modulate matrix turnover. Transforming growth factor-β1 (TGF-β1) can promote tissue fibrosis. This paper studied the role of TIMP-1 and TGF-β1 in the pathogenesis of various muscular dystrophies. **Methods** Plasma TIMP-1 and TGF-β1 levels were measured by ELISA in patients with various muscular dystrophies. Forty-one patients with non-muscle disorders were used as the Control group. **Results** The plasma TIMP-1 level was significantly elevated in patients with Duchenne muscular dystrophy (DMD, 122.52±63.87 ng/ml) ($P<0.05$) and congenital muscular dystrophy (CMD, 124.87±63.14 ng/ml) ($P<0.05$) when compared with that of the Control group (85.71±29.13 ng/ml). Patients with Becker muscular dystrophy (BMD) had no significant elevation of the TIMP-1 level compared with the Control group. Compared with the Control group (6.24±1.12 ng/ml), the plasma TGF-β1 level was significantly elevated in patients with DMD (26.26±5.79 ng/ml) ($P<0.01$) and CMD (31.35±9.77 ng/ml) ($P<0.05$), but not in patients with BMD (3.46±1.38 ng/ml). There was a correlation between the concentrations of TIMP-1 and TGF-β1 ($r=0.6350$, $P<0.01$). **Conclusions** The plasma TIMP-1 and TGF-β1 levels were elevated in patients with DMD or CMD. This elevation suggests that TIMP-1 and TGF-β1 are correlated with the clinical severity of muscular dystrophy and suggests that they may play a role in the genesis of muscular dystrophy.

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**Key words:** Muscular dystrophy; Tissue inhibitor of metalloproteinases; Transforming growth factor-β1

## 进行性肌营养不良患者血浆金属蛋白酶组织抑制剂-1 和转化生长因子-β1 水平检测

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[**摘 要**] **目的** 伴随着肌纤维变性-再生的肌内异常结缔组织增生为肌营养不良的一个特征。金属蛋白酶组织抑制剂(TIMPs)是多功能蛋白，能改变细胞活性和调节基质转变。转化生长因子-β1(TGF-β1)也促进组织纤维化。该文探讨 TIMP-1 和 TGF-β1 在各种肌营养不良发病机制中的作用。**方法** 用 ELISA 法检测几种肌营养不良患者血浆 TIMP-1 和 TGF-β1 水平。**结果** Duchenne 肌营养不良(DMD)血浆 TIMP-1 水平为122.52±63.87 ng/ml，在先天性肌营养不良(CMD)为124.87±63.14 ng/ml，较对照组(85.71±29.13 ng/ml)明显升高(均 $P<0.05$)，但在 Becker 型肌营养不良(BMD)为86.93±48.93 ng/ml，与对照组比较无明显升高；血浆 TGF-β1 水平在 DMD 为26.26±5.79 ng/ml，CMD 为 31.35±9.77 ng/ml，也较对照组(6.24±1.12 ng/ml)明显升高，差异有显著性，均 $P<0.05$，但在 BMD 为3.46±1.38 ng/ml，无升高；而且 TIMP-1 和 TGF-β1 浓度有明显的相关性。**结论** 血浆 TIMP-1 和 TGF-β1 水平在 DMD 和 CMD 中明显升高，而 BMD 中未见明显升高，似乎与肌营养不良的临床严重性相

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关，表明 TIMP-1 和 TGF-β1 在肌营养不良的发病中可能起作用。 [中国当代儿科杂志，2004，6(4)：256－260]
[关 键 词] 肌营养不良；金属蛋白酶组织抑制剂；转化生长因子-β1
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Transforming growth factor-β1 (TGF-β1) is a 25-KD homodimeric protein secreted by platelets, macrophages, epithelial cells, fibroblasts and many other cells. TGF-β1 not only stimulates the synthesis of many extracellular matrix (ECM) molecules, but also decreases matrix degradation via differential effects on the expression of proteases and their inhibitors, strongly promoting generation of ECM[1]. Some research has shown that TGF-β1 can induce the synthesis and accumulation of components of the ECM in fibrotic disorders such as liver cirrhosis, glomerulonephritis, lung fibrosis, keloids and chronic pancreatitis[1－6].

Tissue inhibitors of metalloproteinases (TIMPs) are a family of secreted proteins that play central roles in regulating ECM metabolism[7]. By virtue of their abilities to inhibit the matrix metalloproteinase (MMP) family of degradative enzymes that includes collagenases, stromelysins, gelatinases and membrane-type MMPs[8,9], they act to limit the extent of ECM degradation during normal tissue-remodeling processes. So far, four mammalian TIMPs have been identified (TIMP-1, -2, -3 and -4)[7]. Appropriate regulation of MMPs and their inhibitors TIMPs during the tissue repair processes is important for tissue remodeling, because any imbalance in favour of inhibitors can lead to fibrotic processes, where the increased enzymatic activity will result in tissue destruction or cell invasion[8]. TIMPs may be involved in the pathogenesis of pulmonary fibrosis, human renal allograft interstitial fibrosis, hepatic fibrosis, dermatomyositis and valvular disease of the heart[10－14]. Given these actions, it is conceivable that TIMPs may play a pathogenic role in the genesis of progressive muscular dystrophy (PMD).

Abnormal connective tissue proliferation in muscle following muscle fibre degeneration is a feature of PMD[15], while pathogenesis of interstitial fibrosis is still unknown. The clinical and histopathological features of PMD suggest that an imbalance between the synthesis and degradation of ECM molecules in the diseased muscle appears to be important in pathogenesis of interstitial fibrosis. The studies have reported that MMP-2 and MMP-9 are involved in skeletal muscle degeneration and regeneration[16]. TGF-β is thought to be a key regulator of muscle regeneration. Variations in the amounts or locations of the TGF-β within a lesion may thus profoundly influence the balance between regeneration of muscle fibers and the production of excessive fibrosis[17]. However, the plasma TIMP-1 concentration and the relationship between TGF-β1 and TIMP-1 in PMD have not been examined in human PMD. Therefore, the TIMP-1 expression in plasma obtained from patients with various kinds of muscular dystrophy was measured by ELISA. Moreover, the relationship between TIMP-1 and TGF-β1 in PMD was studied.

## Materials and methods

### Subjects

Forty-four patients with Duchenne muscular dystrophy (DMD, 1－30 years old), 10 with Fukuyama-type congenital muscular dystrophy (FCMD, 1－13.8 years old), 8 with merosin-positive CMD (0.5－12.7 years old), and 14 with Becker muscular dystrophy (BMD, 4－15 years old) who were admitted to the Department of Pediatrics of Tohoku University School of Medicine were enrolled in the research. The course of disease was different from 6 months to 25 years. The diagnosis was all based on clinical, laboratory, muscle biopsy histochemistry, and dystrophin and merosin immunohistochemistry, and multiplex PCR for the dystrophin gene. Forty-one patients with non-muscle disorders, including 35 with epilepsy (0.5－20 years old), 4 with mental retardation (2.5－20.8 years old), and 2 with tic, cranial nerve palsy were used as the Control group.

Two ml blood samples were collected in tubes containing EDTA-2Na using standard venepuncture techniques, after obtaining informed consent from the patients' families. After being centrifuged (3 000 r/min, 10 min, 4℃), the plasma was stored at below −20℃ until detection. In patients with muscular dystrophy, the serum creatine kinase (CK) level was determined simultaneously.

**Methods**

The concentrations of TIMP-1 (Fuji Chemical Industries, Ltd) and TGF-β1 (Cambridge, MA, USA) were determined with commercial sandwich-type ELISA kits. These assays were carried out according to the supplier's instructions. Sample values were determined from a standard curve.

**Statistical analysis**

Results are presented as the mean ± standard deviation ($\bar{x} \pm s$). Data were analyzed using a unpaired 2-sided student's $t$ test. A level of $P < 0.05$ was considered statistically significant. A linear regression was used to examine the correlation between TIMP-1 and TGF-β1. Statistical analysis was performed using StatView J 5.0 (SAS Inc., NC, USA).

## Results

**Detection of plasma TGF-β1 and TIMP-1 levels**

As shown in Table 1, the plasma TIMP-1 level was significantly elevated in patients with DMD ($P < 0.05$) or CMD ($P < 0.05$), including FCMD and merosin-positive CMD patients, compared with the Control group. However, the plasma TIMP-1 level in patients with BMD was not significantly elevated ($P > 0.05$).

The plasma TGF-β1 level was also significantly elevated in patients with DMD ($P < 0.01$) or CMD ($P < 0.05$), compared with that of the controls. The plasma TGF-β1 level in patients with BMD was not different from that of the controls ($P > 0.05$).

**Table 1** Plasma levels of TIMP-1 and TGF-β1 in patients with various muscular dystrophies and the Control group (ng/ml)

| Group | TIMP-1 | | TGF-β1 | |
|---|---|---|---|---|
| | n | $\bar{x} \pm s$ | n | $\bar{x} \pm s$ |
| Control | 18 | 85.71±29.13 | 41 | 6.24±1.12 |
| DMD | 44 | 122.52±63.87[a] | 26 | 26.26±5.79[b] |
| BMD | 14 | 86.93±48.93 | 8 | 3.46±1.38 |
| CMD | 18 | 124.78±63.14[a] | 15 | 31.35±9.77[a] |

Note: a vs the Control group $P < 0.05$; b vs the Control group $P < 0.01$

**The comparison of plasma levels of TIMP-1 and TGF-β1 in patients with merosin-positive CMD and FCMD**

As shown in Table 2, the TIMP-1 level in the patients with FCMD was higher than that in the patients with merosin-positive CMD ($P < 0.01$) and that in the controls ($P < 0.01$). There was a significant difference in the plasma TGF-β1 level between FCMD and merosin-positive CMD patients ($P < 0.05$). The TGF-β1 level in the patients with FCMD was also significantly higher than that of the Control group ($P < 0.05$).

**Table 2** Plasma levels of TIMP-1 and TGF-β1 in patients with CMD and FCMD (ng/ml)

| Group | TIMP-1 | | TGF-β1 | |
|---|---|---|---|---|
| | n | $\bar{x} \pm s$ | n | $\bar{x} \pm s$ |
| Control | 18 | 85.71±29.13 | 41 | 6.24±1.12 |
| CMD | 8 | 83.38±18.12 | 6 | 10.40±3.26 |
| FCMD | 10 | 157.9±67.23[a] | 7 | 49.31±15.18[b] |

Note: a vs the CMD and Control groups $P < 0.01$; b vs the CMD and Control groups $P < 0.05$

**The correlation among the TIMP-1, TGF-β1 and serum CK levels**

The TIMP-1 level was distinctly correlated with the TGF-β1 level in patients with DMD ($r = 0.6350$, $P < 0.01$); but there was no correlation between serum CK level and TIMP-1 level in those patients ($r = 0.0083$, $P = 0.5566$).

## Discussion

TIMPs are multifunctional proteins that have the capacity to modify cellular activities and to modulate matrix turnover. TIMPs are presumed to be important regulators of the ECM, and their ex-

pressions are correlated with the development of fibrosis. In addition, TIMPs have been shown to possess biologic functions that are independent of MMP-inhibitory activity, including stimulation of cell proliferation in autocrine or paracrine fashion, inhibition of apoptosis, and inhibition of angiogenesis[18-20]. Previous studies have shown that TGF-β1 is more significantly expressed in the muscle of DMD patients than controls and that its expression is related to the degree of muscle fibrosis and the patient's age[21]. There are high TGF-β1 immunoreactivity expressions in muscle fibers and extracellular space in DMD patients and most of BMD patients. This suggests that TGF-β1 may play an important role in the synthesis and accumulation of ECM in PMD[22].

In this study, it was found that the plasma levels of TIMP-1 and TGF-β1 increased in patients with DMD and CMD, but not in the BMD patients. It is well known that clinical symptoms of BMD are mild. There was also a significant difference in the plasma TIMP-1 and TGF-β1 levels between FCMD and merosin-positive CMD patients. Clinical symptoms are more severe in patients with FCMD than those in patients with merosin-positive CMD. Although there are atypical cases in the two types of CMD, none of the patients with FCMD can learn to walk alone, while 92% of patients with merosin-positive CMD can learn to walk alone[23]. These findings suggest that the differences in plasma TIMP-1 and TGF-β1 levels among various kinds of PMD seem to reflect the clinical severity of muscle dystrophy.

The present study has shown the absence of a correlation between CK and TIMP-1, which suggests that TIMP-1 is not necessarily secreted from necrotic fibers alone. The TIMP-1 concentration was distinctly correlated with the TGF-β1 concentration. The growth factor TGF-β1 was identified as a important regulator of MMP and TIMP expression in hepatic stellate cells (HSC) in vitro and was profoundly involved in hepatic tissue repair reactions[24]. The finding of a positive correlation between the expression of protein for TGF-β and TIMPs also suggests that TGF-β has a fibrogenic action by indirectly inhibiting matrix degradation via up-regulation of the TIMPs[11].

To sum up, the production of TIMP-1 and TGF-β1 increased in patients with DMD or CMD, but not in the patients with BMD, which suggests that TIMP-1 and TGF-β1 may play a role in the genesis of muscular dystrophy, and that they may be correlated with the clinical severity of muscular dystrophy. Because of the limitation of the small sample in this study, the function of TIMP-1 in muscle remains to be elucidated further.

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## · 消息 ·

# 《新生儿机械通气治疗学》已由人民卫生出版社出版发行

由广州医学院附二院周晓光副教授、暨南大学附一院肖昕副教授和广东省人民医院农绍汉副主任医师主编、中国医科大学附二院韩玉昆教授主审的《新生儿机械通气治疗学》已由人民卫生出版社出版发行。本书包括新生儿机械通气的医学基础，新生儿机械通气治疗，常见新生儿疾病的机械通气治疗，新生儿机械通气有关的临床研究，新生儿常用呼吸机简介、消毒、保养与维护等 5 个篇章，全面、系统地阐述新生儿机械通气的主要基础理论和临床问题，如新生儿呼吸生理、血液气体、呼吸衰竭、呼吸监护、持续气道正压通气、常规机械通气、高频通气、体外膜肺、液体通气和负压通气等；重点介绍了新生儿常见疾病和危重症如呼吸暂停、呼吸窘迫综合征、胎粪吸入综合征、感染性肺炎、缺氧缺血性脑病、肺出血、休克、多器官功能衰竭等的机械通气治疗；还介绍了新生儿复苏、氧气疗法、湿化疗法、肺表面活性物质替代疗法、一氧化氮吸入疗法、胸部物理疗法、新生儿气道护理等临床治疗方法。适合儿科、新生儿科医务人员以及医学院校高年级本科生、研究生阅读。全书共 5 篇，48 章，94 万字，大 16 开本，定价 70 元。全国新华书店有售，也可与广州市黄埔大道西 613 号暨南大学附一院围产医学中心肖昕主任联系邮购。邮政编码：510632，电话：020－38688706；