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Increased TGF-β as a Biomarker of Connective Tissue Dysplasia in Cervical Artery Dissection

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Abstract

Introduction: The cause of cervical artery dissection (CeAD), one of the main causes of ischemic stroke (IS) at a young age, is the arterial wall weakness. Its morphological changes have been poorly studied, because the lethal outcome in these cases is rare. It is assumed that the weakness of the arterial wall is associated with connective tissue disorder. To assess this assumption the study of transforming growth factor beta (TGF-β), a cytokine that regulates the homeostasis of tissues, including connective tissue, can be helpful as its level increases in the hereditary connective tissue diseases.

Aim of the Study: The aim was to study TGF-β in patients with CeAD.

Materials and Methods: TGF-β in serum blood was studied by enzyme immunoassay in 74 of 336 patients with CeAD observed at the Research Center of Neurology (Moscow) from 2000 to 2021. The average patient's age at the time of the study was 41.6 ± 9.8 years; the proportion of women - 51%. TGF-β was studied in the first month of the disease (9 patients), for 2-3 months (12 patients) and during the late period (mean - 4.3 ± 5.03 years) (53 patients). The control group consisted of 20 healthy volunteers, comparable in gender and age. Dissection occurred in ICA (42 patients), VA (29 patients), ICA+VA (3 patients) and involved 1 artery (58 patients) or 2 - 3 arteries (16 patients). Clinical manifestations included IS (49 patients), isolated cervical-cephalic pain (23), lower cranial nerve palsy (2). Pathological CeAD tortuosity was detected by angiography in 13 patients, and a dissecting aneurysm in 15 patients.

Results: TGF-β1 and TGF-β2 were elevated in patients with CeAD compared with the control: TGF-β1 - 4990 [3950; 7900] pg/ml vs. 3645 [3230; 4250] pg/ml, p = 0.001; TGF-β2 - 6120 [4680; 7900] pg/ml vs. 3155 [2605; 4605] pg/ml, p = 0.001. The highest TGF-β1 and TGF-β2 levels were noted at 2-3 months of the disease. There was no correlation between the TGF-β level and various clinical and angiographic parameters.

Conclusion: Increased TGF-β level confirms that patients with CeAD have connective tissue disorder that underlies the arterial wall weakness. A higher TGF-β level at 2-3 months of CeAD seems to be connected with an active reparative process in arterial wall after dissection. TGF-β can be used as a biomarker of connective tissue dysplasia in patients with CeAD.

Keywords: *TGF-β; Connective Tissue Dysplasia; Dissection; Ischemic Stroke; Young Age*

Introduction

Cervical artery dissection (CeAD) is the main cause of ischemic stroke (IS) in young adults [1-7]. Dissection is the blood entering from the lumen into the artery wall through the intimal rupture, leading to an intramural hematoma, less often - to double lumen or aneurysm. The cause of dissection is arterial wall weakness [7-9]. Its morphological basis according to our studies is connective tissue dysplasia (CTD) similar to that in fibromuscular dysplasia (FMD) [10-13]. CTD in CeAD patients concerns not only the arterial wall, but bears wide-

spread character. This is indicated by connective tissue abnormalities revealed by electron microscopy of skin samples [14], as well as by various clinical signs of connective tissue weakness found in CeAD patients [15,16]. The hereditary connective tissue diseases (Marfan syndrome, Ehlers-Danlo, osteogenesis imperfecta, Loys-Didtz) are a very rare cause of CeAD [17] that allows one to consider CTD in CeAD patients as undifferentiated. According to our hypothesis based on histochemical and electron microscopic examination of muscle and skin samples in CeAD patients, mitochondrial cytopathy may be one of the causes of CTD [18-20]. For the first time, the association of mitochondrial disorders with large artery damage was noted by S.H. Tay and co-authors in 2006 [21]. They described a rupture of the aorta in a 15-year-old patient with MELAS and A3243G mutation. Histological and immunohistochemical studies of aorta found marked disruption of the smooth muscle and elastic layers of the tunica media and decreased cytochrome-C oxidase I activity in the endothelial and smooth muscle cell. They also demonstrated a high A3243G mutation load (85%) in the aorta.

Morphological verification of CTD by skin biopsy in CeAD patients is limited and cervical artery biopsy is impossible. This raises the question of finding laboratory CTD markers. A potential marker may be transforming growth factor-β (TGF-β). It is a multifunctional cytokine that regulates cell growth, differentiation, adhesion, migration and death dependent on cell type, developmental stage, or tissue conditions [22,23]. In the arterial wall, TGF-β regulates various cellular functions, modulates the production of extracellular matrix, ultimately playing an essential role in arterial remodeling [24]. Due to its multifunctionality, the TGF-β signaling pathway is involved in the pathogenesis of various diseases and biological process: the connective tissue diseases, chondro-and osteogenesis, myogenic differentiation, the control of cellular immunity, wound healing, the development of fibrosis [25,26]. TGF-β is increased in patients with hereditary connective tissue diseases that may manifest by aortic dissection [27-30]. Ganesh., *et al*. (2014) found the increased TGF-β level in patients with fibromuscular dysplasia (FMD) and showed that clinical features of this disease extend beyond arterial pathology and include different mild connective tissue features. They suggested that FMD is a systemic disease with altered TGF-β expression and connective tissue features [31].

In patients with CeAD TGF-β has not been studied. A. Pezzini and co-authors [32] investigated mutations in the TGF-β receptor gene in 56 patients with CeAD and found the TGF-β2 mutation in 2 patients (3.6%). Morphological and clinical signs of CTD in CeAD patients, as well as increased TGF-β in hereditary connective tissue diseases with vascular manifestations and in FMD allowed us to assume that TGF-β may be increased in CeAD patient.

Aim of the Study

The aim of present work was to determine TGF-β1 and TGF-β2 in patients with CeAD.

Materials and Methods

74 out of 336 CeAD patients studied at the Research Center of Neurology (Moscow) in the period from 2000 to 2021 entered to this study. Mean age - 41.6 ± 9.8 years; men proportion - 49%. In all patients, dissection was verified by angiography (MRA, CTA, conventional angiography) and in most patients by MRI of the neck arteries (T1-fat-sat mode). Dissection localized in internal carotid artery (ICA, 42 patients), vertebral artery (VA, 29 patients) and ICA+VA (3 patients) and manifested by ischemic stroke (49 patients, 35%), isolated cervicocephalic pain (23 patients), lower cranial nerve palsy (2 patients). Dissection involved one artery (58 patients, 78%) and 2-3 arteries (16 patients, 22%). Pathological tortuosity of the ICA or VA was found in 13 patients (17.5%), dissecting aneurysm - in 15 patients (20%).

TGF-β1 and TGF-β2 in blood serum were studied by the enzyme immunoassay. Reagent kits of Vector-Best JSC (Russia), R&D Systems (USA) were used. The laboratory staff had no information about the clinical and neuroimaging data of CeAD. In the first month of the disease, 9 patients were examined, within 2 - 3 months - 12 patients, after 3 months (mean - 4,3 ± 5,03 years) - 53 patients. The control group consisted of 20 healthy volunteers, comparable in gender and age (average age - 38.3 ± 11.6 years; men - 48%).

All patients and volunteers of the control group gave informed consent to participate in the study and to the processing of personal data. The protocol of the study was approved by the local Ethics committee of Research center of Neurology.

Statistical analysis

Statistical analysis was performed using the software packages SPSS Statistics version 25.0 (IBM, USA). Frequency and percentage (%) were used as descriptive statistics for categorical and ordinal variables, respectively, and median and quartiles were used for quantitative variables. In all cases, two-way versions of the statistical criteria were used. The null hypothesis was rejected at a significance level of $p < 0.05$. The Shapiro-Wilk test was used to determine the normal distribution of the quantitative variable. Mann-Whitney and Kruskal-Wallis criteria with pairwise comparisons by the Mann-Whitney criterion (using Bonferroni multiplicity adjustment) were used if the distribution of the variable did not match the normal one. ANOVA was performed for scale dependent variables to test hypotheses about the difference.

Results

Results of TGF-β1 and TGF-β2 study in 74 CeAD patients are presented in table 1 and 2. TGF-β1 and TGF-β2 were statistically significant increased in CeAD patients compared to the control: TGF-β1 - 4990 [3950; 7900] pg/ml vs. 3645 [3230; 4250] pg/ml in control ($p = 0.001$); TGF-β2 - 6120 [4680; 7900] pg/ml vs. 3155 [2605; 4605] pg/ml in control ($p = 0.001$). The highest levels of TGF-β1 (6835 [5240; 8760] pg/ml, $p = 0,001$) and TGF-β2 (7950 [5325; 9965] pg/ml, $p = 0,001$) were observed during 2-3 months of the disease. At a later period (mean - 4.3 ± 5.03 years) their levels were lower, but remained elevated compared to the control, being more significant for TGF-β2 (5635 [4325; 7750] pg/ml, $p = 0.001$) than TGF-β1 (4755 [3915; 7390] pg/ml, $p = 0.01$). During the first month of CeAD, TGF-β levels were also increased, but statistically significant differences concerned only TGF- β2 (6120 [5250; 6300] pg/ml, $p = 0.019$), but not TGF-β1 (4700 [3870; 5410] pg/ml, $p > 0.05$).

TGF-β1 and TGF-β2 levels	CeAD patients n = 74	Control n = 20	p
TGF-β1, pg/ml, Me [Q25%, Q75%]	4990 [3950; 7900]	3645 [3230; 4250]	0.001
TGF-β2, pg/ml, Me [Q25%, Q75%]	6120 [4680; 7900]	3155 [2605; 4605]	0.001

Table 1: *TGF-β1 and TGF-β2 levels in CeAD patients and in control (Mann-Whitney criterion, $p < 0.05$). Me - Median, Q - Quartile, p - Significance Level.*

	1	2	3	4		
	1 month n = 9	2-3 months n = 12	>3 months* n = 53	Control n = 20	p	post-hoc
TGF-β 1, pg/ml Me [Q25%, Q75%]	4700 [3870; 5410]	6835 [5240; 8760]	4755 [3915; 7390]	3645 [3230; 4250]	0,001	$P_{2-4} = 0.001$, $P_{3-4} = 0.01$, $P_{1-3, 1-2, 3-2, 4-1,} > 0.05$
TGF-β 2, pg/ml Me [Q25%, Q75%]	6120 [5250; 6300]	7950 [5325; 9965]	5635 [4325; 7750]	3155 [2605; 4605]	0,001	$P_{1-4} = 0.019$, $P_{2-4} = 0.001$, $P_{3-4} = 0.001$, $P_{1-2, 1-3, 3-2,} > 0.05$

Table 2: *TGF-β1 and TGF-β2 levels in different CeAD stage and in control (Kruskal-Wallis criteria, $p < 0,05$). *4 month - 20.5 years, mean - 4.3 ± 5.03 years.*

Using a single-factor analysis of variance with repeated measurements, a comparison of the TGF-β level and some clinical parameters (clinical CeAD manifestations, past history headache) and angiographic parameters (single or multiple dissections, pathological tortuosity or dissecting aneurysms) was carried out. No correlation between TGF-β levels and clinical/angiography parameters was found.

Discussion

The present study showed that of the TGF-β level in CeAD patients elevated in comparison with normal control. TGF-β regulates the homeostasis of various tissues, including the arterial wall, where it controls cell proliferation, differentiation, and their relationship with the extracellular matrix [22-24]. Its level increased in patients with hereditary connective tissue diseases and FMD, which manifest by dissection of various arteries and aorta and various mild clinical signs of CTD [27-31]. This allows one to consider an elevated TGF-β level as a biomarker of CTD especially of arterial wall in patients with CeAD. The search of CTD biomarkers in CeAD patients is very important because morphological examination of the arterial wall is available only in cases with a fatal outcome, which is very rare in this disease [1]. According to most authors, CeAD is a multifactorial disease leading to the weakness of the arterial wall, the rupture of which can be triggered by various factors [7,33]. Our morphological studies, carried out in 4 cases with a fatal outcome and in 1 case of ICA sample taken during the surgery to correct the pathological ICA tortuosity that complicated by dissection, found dysplastic changes in arterial wall. Moreover, they were found not only in the dissected ICA, but also in the opposite ICA, as well as in both VA and their branches [10-13].

The cause of TGF-β increasing in hereditary connective tissue diseases has not been definitively established. It is assumed that in Marfan syndrome TGF-β increases compensatory, due to its inability to bind to fibrillin, the structure of which is changed due to a mutation of the corresponding gene [27]. In the extracellular matrix of the aorta TGF-β activates proinflammatory factors that increase expression of matrix metalloproteinases and cytokines, causes the accumulation of reactive oxygen species leading eventually to further degradation of elastin fibers [28]. Unlike this Z. Mallat and co-authors (2017) [29], consider that an elevated TGF-β has no pathogenetic significance in the aortic wall damage in hereditary diseases characterized by arterial dissection and the aneurysm development.

In CeAD patients the increase of TGF-β level seems to be compensatory due to impaired TGF-β binding to myocytes and fibroblasts of the arterial wall, which leads to a secondary change in the extracellular matrix. We assume that impaired binding of TGF-β may be due to mitochondrial cytopathy, the presence of which was demonstrated by histochemical and electron microscopic examination of skin and muscle biopsies in CeAD patients [12,18-20].

Our study showed that TGF-β elevation in CeAD patients is detected both at the first 3 months of the disease and in several years. This indicates that arteriopathy underlying dissection is not transient, although its relapses after 1 - 2 months are very rare [34-36]. Our morphological study of cerebral and cervical arteries found myocyte necrosis, areas of fibrosis, incorrect orientation of myocytes, a decreased number of elastic fibers in media, thinning and splitting of the internal elastic membrane that is in accordance with chronic character of arteriopathy [11-13]. The highest level of TGF-β within 2 - 3 months of the disease probably is due to active reparative process at this period, whereas during the first month IMH resolution and inflammatory reaction are predominant. Such sequence of events is characteristic of wound healing [37]. The TGF-β elevation concerned its both isoforms (TGF-β1 and TGF-β2); however, the TGF-β2 increasing was statistically more significant. Evidently it plays a leading role in regulating the interaction of cells and extracellular matrix in the arterial wall. In this regard, it is interesting to note that the mutation in the TGF-β gene identified by A. Pezzini and co-authors in 3.6% of patients with CeAD concerned specifically TGF-β2 [32].

TGF-β elevation in patients with FMD, the clinical and morphological manifestations of which are similar to those in CeAD [31,38] raises the question of the proximity of these diseases. One can assume that arteriopathy underlying FMD and arteriopathy underlying CeAD represent the same pathology - connective tissue dysplasia, the severity of which and the predominant localization within the arterial bed have some differences. Close biological relationship between FMD and CeAD recently discussed in the work of S. Bonacina and co-authors (2021) [39]. The authors hypothesized that CeAD occurring in the context of an underlying FMD represent a specific non-atherosclerotic, non-inflammatory arteriopathy which should be considered as a distinct nosographic entity. According to our hypothesis, an undifferenti-

ated connective tissue dysplasia probably similar to those in FMD underlies the arteriopathy, leading CeAD. The increased TGF-β may be the biomarker of CTD in CeAD patients.

Conclusion

TGF-β regulates various cellular functions, plays an essential role in arterial wall remodeling and its level elevates in hereditary connective tissue disease. TGF-β increasing in CeAD patients assumes that they also have connective tissue disorder that underlies the arterial wall weakness and predispose to dissection. A higher TGF-β level at 2-3 months of CeAD compared to the first month seems to be connected with an active reparative process in arterial wall after dissection. TGF-β can be used as a biomarker of connective tissue dysplasia in patients with CeAD.

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