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Original Article

Decreased serum periostin level is associated with carpal tunnel syndrome

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Abstract

Aim: Noninflammatory subsynovial connective tissue (SSCT) fibrosis with nerve compression is an obvious feature of carpal tunnel syndrome (CTS). We investigated serum levels of periostin, which is known to have an effect on fibrosis, in patients with CTS.

Material and Methods: A total of 39 healthy individuals and 32 patients who were newly diagnosed with CTS were included in this prospective clinical study. Duration of symptoms, severity, unilateral or bilateral side of CTS diagnosis of patients were recorded. Serum samples were received for the measurement of periostin levels from all participants and were analyzed using commercial enzyme-linked immunosorbent assay (ELISA) kits.

Results: Serum periostin levels were significantly lower in CTS patients than controls (p=0.030). Additionally, there was no correlation between serum periostin levels and CTS disease regarding the duration of symptoms and electrophysiological severity of symptoms (p > 0.05).

Conclusion: In the current study, it was evaluated that there is a significant relation between CTS and serum periostin levels. Periostin may have roles in the pathogenesis of the CTS. This is the first study to determine serum periostin levels in CTS patients.

Keywords: Carpal tunnel syndrome; ELISA; fibrosis; periostin; serum

INTRODUCTION

Neuropathies, which are characterized by pain, numbness or loss of function in peripheral nerves are entrapment neuropathies, caused by the compression of the surrounding anatomical structures. The most frequent entrapment neuropathy is the Carpal Tunnel Syndrome (CTS). Fibrosis, which is the most common histological finding in idiopathic CTS, is ascertained in the subsynovial connective tissue (SSCT) of the carpal tunnel, which is the vascular network and areolar connective tissue. Some researchers speculate that when SSCT is exposed to continuous stress and deformation, it performs a wound healing reaction that gradually increases the fibrosis as a response to this situation (1). In addition to this, even some studies argue that fibrosis may be the main cause of the disease rather than a result of the nerve compression (2,3). Yesil et al. reported that myofibroblasts are likely to activate in early stages of the disease, and thus, contribute to the onset of CTS (4). Many studies revealed that the up-regulation of the transforming growth factor β1 (TGF-β1) signaling and the cytokines associated with other fibrosis play important roles in SSCT fibrosis that is associated with idiopathic CTS (5,6).

Periostin, which is one of the extracellular matrix proteins, is known to be upregulated in many tissues in the development of fibrosis (7,8). Periostin, also known as the Osteoblast-specific Factor 2, is a molecule with a molecular weight of 93.3 kDA, released extracellularly, which was first detected in the osteoblast cells of mice. In addition to this, it is also expressed in mesenchymal tissues. It was found in previous studies that periostin acts as an adhesion molecule, and contributes to subepithelial fibrosis with extracellular matrix proteins like tenascin-c, fibronectin, and collagen V (9,10). It is already known that periostin has critical roles in cardiovascular and respiratory systems, oncology, tissue repair, and in various inflammatory diseases (11-13). In addition, this molecule is also known to be upregulated in tumor progression, cutaneous wound healing, and cutaneous fibrosis (14,15).

Periostin is expressed in tissues subjected to constant mechanical stress and it has an important role in fibrosis development (16). Fibrosis, which plays a role in the

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development of CTS, prevents the normal ulnodorsal movement of the median nerve while the wrist is bending, and therefore may cause nerve compression between the flexor tendons and the flexor retinaculum (17). In light of these data, the purpose of the study was to determine the serum levels of periostin, which is effective in the development of fibrosis, in patients with CTS.

MATERIAL and METHODS

Study Population

Patients diagnosed with 32 carpal tunnel syndrome between the ages of 20-65, who were admitted to Bozok University Hospital with complaints of numbness, pain, and tingling in their hands were randomly included in the study. Thirty nine individuals with no known disease and no CTS detected electrophysiologically were included in the control group.

Electrodiagnostic studies were done in all patients. The diagnosis of CTS was based on the presence of at least one of the following symptoms: (1) pain in the median nerve distribution, numbness or paresthesia (2) these sensory complaints can be alleviated by resting, rubbing and shaking of the hands and sensory symptoms awaken from sleep at night (3). The diagnosis was generally supported by a positive Tinel or Phalen tests. All patients with CTS had median neuropathy, which was approved in the wrist by one or more of the following standard electrophysiological criteria: (1) prolonged distal motor latency (DML) in abductor pollicis brevis (APB) (abnormal ≥ 4.4 ms; wrist to APB, 7 cm); (2) antidromic distal onset sensory latency (DSOL) prolonged to second digit (abnormal ≥ 3.5 ms; wrist to index finger, 13 cm); and (3) the diagnosis was confirmed by the detection of abnormal sensory conduction velocity (SCV) <45 m / s. Nerve conduction studies were analyzed using standard techniques and guide-based bipolar superficial electrodes (21). Patients were categorized as follows: mild CTS (median sensory conduction velocity [SCV] slowed in the finger-wrist nerve segment with normal DML), moderate CTS (median SCV slowed in the finger-wrist nerve segment with increased DML), severe CTS (non SCV and abnormal DML).

The exclusion criteria were patients on dialysis, diabetes mellitus, pregnancy, coronary artery disease, liver failure, osteoporosis, malignancy, cerebrovascular disease, hypothyroidism, hyperparathyroidism, and/or vitamin B12 deficiency, cervical hernia, prior CTS surgery, or steroid injections.

Electrophysiological assessments

The median and ulnar nerves in both upper extremities, nerve conduction studies were evaluated utilizing a Medelec Synergy electromyography (EMG) machine (Medelec Synergy, Oxford Instruments, Surrey, UK). While filter settings were taken 20-2,000 Hz bandpass for sensory nerve studies, the motor used 2-10,000 Hz bandpass for nerve studies. The sweep speed was set at 1 ms per division. The skin temperature of the hand was around at 32°C. Records were made with supramaximal stimulation. All measurements were carried out in a warm room at a temperature kept at 26-28° C (19).

Biochemical Analysis

Blood samples from patients and the control group were taken to vacutainers without anticoagulant supplements. All blood samples were centrifuged for 3000 rpm for 10 min for serum isolation. The supernatant was quickly removed and was kept frozen at -80^{0}C until the assays to determine the patient's serum periostin levels. Serum samples were examined for human periostin using the Enzyme-linked Immuno Sorbent Assay (ELISA) kit (Bioassay Technology Laboratory, Shanghai, China). ELISA kit with standart curve range concentration of 0.5 ng/mL and 150 ng/mL was employed in determining the serum periostin levels. The sensitivity concentration of the kit was 0.251 ng/mL. The Spectramax ELISA reader (Molecular Devices) was used to determine the optical density values of the samples at 450 nm. The results are given as ng/mL.

Statistical analysis

The data analysis was carried out with the SPSS 18 Package Program (IBM Corp. Armonk, New York, USA). The graphic was drawn using GraphPad Prism 6 software (GraphPad Software, San Diego, California, USA). A Kolmogorov Smirnov test was utilized to analyze whether or not the data was normally distributed. The non-normally distributed data were compared by using the Mann-Whitney U test. Correlation analysis of normally distributed data was performed with Pearson Correlation Analysis, while correlation analysis of non-normally distributed data was performed with Spearman's Correlation Analysis. The main characteristics of patients were evaluated with descriptive and analytical statistics. For categorical variables chi-square test were used. Data were represented as mean±SD or median (25 percentile-75 percentile). For all tests, a P value <0.05 was considered statistically significant.

RESULTS

The clinical and demographic data for the groups are summarized in Table 1. No significant differences were found between the groups in the way of age or gender ($p > 0.05$), and no significant associations in the BMI in patients with CTS compared with controls ($p > 0.05$). While the median of the periostin level was 3.65 ng / mL interquartile range (3.12-4.45) in the patient group, the median was 3.94 ng / mL interquartile range (3.5-6.78) in the control group; Mean values were 3.40 ± 1.60 ng / mL in the patient group and 7.89 ± 9.62 ng / mL in the control group ($p=0.030$) (Figure 1). The serum periostin levels were low at a significant level in the CTS group compared to the control group. No correlations were detected between the periostin and CTS disease regarding the duration of symptoms and electrophysiological severity of symptoms (Table 2).

Table 1. Clinical and demographic characteristics of the participants

	Patient (n= 32)	Control (n= 39)	U	P
Age (years)	49.62 ±11.95 50.5 (45-55.75)	46.35 ±10.82 47 (35-56)	532	0.287
Gender Female/male (%)	25 (78.1%) 7 (21.9%)	30 (76.9%) 9 (23.1%)	-	0.904**
BMI (kg/m²)	29.87 ± 2.64 30 (29-30.26)	29.30 ± 1.66 29 (28-30.24)	506	0.167*
Serum level of periostin ng/mL	3.40 ± 1.60 3.65 (3.12-4.39)	7.89 ± 9.62 3.94 (3.5-6.78)	421.5	**0.030***
Symptoms duration (months)	4.5 ± 4.30 3 (2-6)	-	-	-
Diagnosis of CTS Unilateral/Bilateral	14 unilateral (43.8 %) 18 bilateral (56.2%)	-	-	-
Degree of CTS	Mild 11 (34.4%) Mild-moderate 4 (12.5%) Moderate 9 (28.1%) Severe 8 (25%)	-	-	-

CTS: Carpal Tunnel Syndrome Continuous variables are expressed as median (25th-75th percentile), mean± SD, *Mann-Whitney U Test (U); ** Chi square test; P < 0.05 Bold value statistical significant

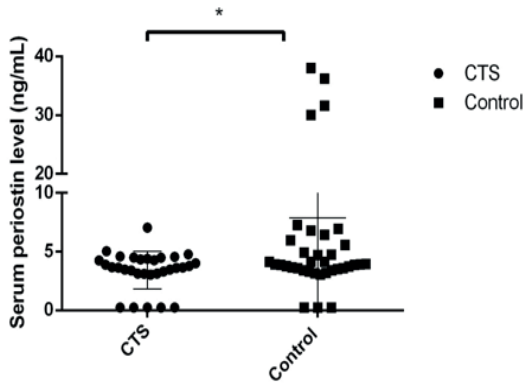

Figure 1. Scatter plot graph of serum levels of periostin between control subjects and CTS patients. The data presented are the mean ± SD of an experiment performed. Asterisks (*) denote significant differences (p<0.05). CTS: Carpal Tunnel Syndrome

Table 2. Clinical and demographic characteristics of the participants

			Serum periostin levels
Spearman's rho	**Symptoms Duration**	Correlation Coefficient	0.032
		Sig. (2-tailed) P=	0.866
		N	32
	Age	Correlation Coefficient	0.194
		Sig. (2-tailed) P=	0.295
		N	32
	Electrophysiological severity of symptoms	Correlation Coefficient	0.053
		Sig. (2-tailed) P=	0.766
		N	32

There is no significant p value here

DISCUSSION

Periostin was examined in previous studies as a ligand for integrins like αvβ3 and αvβ5, and it was shown to play roles in fibroblast cell proliferation, survival and cell migration through phosphoinositide-3-kinase–protein kinase B/Akt (PI3K/Akt) signal pathway (20). It is found in collagen-rich fibrous connective tissues including the, tendons, corneas, heart valves, perichondrium, and periodontal ligaments that are exposed to stable mechanical stresses during embryonic development or pathogenesis. Periostin belongs to the matricellular proteins group, which is characterized as the modulators of cell-matrix interactions and cellular function (21). These proteins play roles in the activation of tissue enzymes and protease during tissue remodeling, rather than ensuring the structural integrity of tissues (22). Moreover, they might represent intriguing targets to treat chronic connective tissue diseases (23). Studies show that periostin also plays roles in the pathobiology of various inflammatory diseases, including tissue damage, fibrosis, arthritis, and atherosclerosis (24).

Periostin initiates several pathological events, which include wound repair inflammation, tissue formation, and tissue remodeling (25). Periostin exists under epidermal

wound edges or at the dermal-epidermal connection areas of the granulation tissue and supports cutaneous wound healing by increasing the proliferation and migration of dermal fibroblasts. Periostin regulates myofibroblast differentiation and collagen matrix contraction, and animal models that have periostin deficiency exhibit delayed wound healing (26). It also involves collagen production by fibroblasts after acute myocardial infarctions (27). Normal regulation of periostin might be needed during the repair process, and the dysregulation of periostin can lead the balance to excessive repair, which can cause keloid formation and other fibroproliferative disorders. Periostin is an extracellular matrix (ECM) protein that has minimal expression in adult ventricles; however, it is largely re-expressed by cardiac fibroblasts following a pressure load or myocardial infarction. Periostin, which is a very important adhesion molecule, is important in regulating the ECM integrity and modulate hypertrophy with its ability to connect to multiple ECM components and integrins (28). It modulates cell-to-extracellular matrix interactions and can bind to collagen, fibronectin, tenascin-C and several integrins (16). CTS is characterized by the compression and irritation of the median nerve that passes through the carpal tunnel in the wrist (29). Pain, numbness, thumb paranesthesia, index finger, middle finger and radial half (thumb side) of the ring finger are among the symptoms of CTS. The etiology of idiopathic CTS is not yet understood in full. In our study, it was found that serum periostin levels decreased in the patient group when compared to the control group. Periostin is considered to be an important structural means performing appropriate balance against appropriate and inappropriate tissue adaptation in response to damage and injury. A recent study also reported that periostin is a catabolic factor enhancing collagen and proteoglycan degradation (30). The exact pathophysiology of CTS is not yet known in full; however, an increase in SSCT and in fibrosis remains to be decisive in the development and progress of CTS (31). Non-inflammatory fibrosis in flexor tendons surrounding SSCT (i.e. the tissues controlling hand movements) is the main histomorphological change seen in CTS patients (6). In previous studies, increased activation of TGF-β second messenger Smads in SSCT fibrosis (6), increase in downstream fibrotic agents like Connective Tissue Growth Factor (CTGF) (6,31), increased collagen II, III and IV (2,32), and decreased matrix metalloproteinases were reported (6,32). Additionally, various studies have revealed that the up-regulation of TGF-β1 signaling and other fibrosis-related cytokines have a critical role in the SSCT fibrosis associated with idiopathic CTS (31,33). Periostin modulates TGF-β signalization with cell-matrix interactions. TGF-β plays a vital role in regulating cell proliferation, differentiation, inflammation, angiogenesis, and tissue repair (34,35). TGF-β1 modulates the expression of many extracellular matrix proteins, such as fibronectin, collagen, and elastin, as well as down-regulating matrix metalloproteases. In this way, the TGF-β1 stimulation causes vicious cycle of fibrosis that will negatively affect the normal organ function (35,36). In a previous study, it was examined that the therapies that target the TGF-β signal could be used as potential local antifibrosis therapies in patients who have CTS (37).

Regarding fibrotic conditions, periostin has a role in bone marrow fibrosis and determines the biomechanical properties of connective tissues by regulating collagen fibrillogenesis (21). It has been emphasized that periostin plays various functions in tissues under pathological conditions or mechanical stress (38). Especially in periodontal diseases with inflammatory activity progressing with tissue damage, the decrease in periostin level has been associated with the failure of damaged tissues to produce sufficient periostin. Additionally, the researchers claimed that increasing periostin levels or preventing periostin reduction may result in faster tissue repair and more attachment gain (39). Furthermore, in another study suggest that a primary aberration in response to mechanical stress within the nitrofen-exposed fetal lung tissue, characterized by a downregulation in periostin in a model of congenital diaphragmatic hernia lung hypoplasia (40).

CONCLUSION

When these studies are evaluated, it may be speculated that periostin, which plays roles in collagen destruction and modulation of TGF-β signaling, may be decreased in serum due to the lack of sufficient secretion owing to tissue damage caused by CTS. This is the first study that determines the serum level of periostin in CTS patients. It is necessary to validate these findings with a larger cohort to reach a more decisive conclusion. Our results suggest the need for further studies to elucidate the role of periostin in CTS pathogenesis in vivo studies and with clinical tissue samples.

Competing interests: The authors declare that they have no competing interest.

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Ethical approval: The present study was conducted in accordance with the protocol approved by Bozok University Local Ethics Committee (2017-KAEK-189_2019.01.02_02).

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