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Expression of vitamin D receptor in the subsynovial connective tissue in women with carpal tunnel syndrome

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Abstract

Studies suggest that low vitamin D levels are associated with carpal tunnel syndrome. We aimed to evaluate whether level of vitamin D receptor expression in the endothelial cells of the subsynovial connective tissue is associated with clinical features of carpal tunnel syndrome. We obtained the subsynovial connective tissue from 52 women with carpal tunnel syndrome during surgery and performed immunohistochemical analysis of vitamin D receptors in the endothelial cells of the subsynovial connective tissue. We explored correlation of vitamin D receptor expression with clinical features of carpal tunnel syndrome, such as age, symptom duration, symptom severity and electrophysiological severity.

Diverse range of vitamin D receptor expression was observed. Vitamin D receptor expression was independently associated with distal motor latency. This suggests that vitamin D receptor expression may be associated with disease progression, as prolonged distal motor latency reflects severity of the disease. Further studies are necessary to explore the role of vitamin D and vitamin D receptors in patients with carpal tunnel syndrome.

Level of evidence: IV

Keywords

Vitamin D receptor, carpal tunnel syndrome, subsynovial connective tissue, distal motor latency

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Introduction

Carpal tunnel syndrome (CTS) is the most commonly encountered compressive neuropathy in the upper extremity (Bland, 2005). The mechanism of median nerve compression in CTS involves elevated pressure in the carpal tunnel due to reduction of its cross-sectional area or increased volume as the result of chronic tenosynovial thickening and fibrosis (Bickel, 2010; Dawson, 1993; Shum et al., 2002).

A number of studies have investigated the pathologic changes in subsynovial connective tissue (SSCT) with respect to its role in increasing the volume of contents in the carpal tunnel (Faithfull et al., 1986; Fuchs et al., 1991; Kerr et al., 1992; Lluch, 1992; Neal et al., 1987; Nakamichi and Tachibana, 1998). These studies have demonstrated that nonspecific fibrous as well as vascular changes, such as vessel wall thickening and intimal hyperplasia, are usually noted. These changes in the SSCT not only lead to an increase in the volume of the contents, but also alter its material properties, such as compliance and permeability, which sequentially predispose the SSCT to additional injury (Ettema et al., 2004).

Vitamin D has been shown to exert a multitude of effects on various systems, including neuroprotection, anti-inflammatory, anti-proliferative actions and pro-differentiation (Chen et al., 2008; Merke

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et al., 1989; Mitsuhashi et al., 1991). Vitamin D binds to intracellular vitamin D receptor (VDR), which regulates transcription of vitamin D-responsive genes. Studies reported that endothelial cells express VDR and possess the capacity to coordinate vitamin D-dependent regulatory activity (Haussler et al., 2008; Hirata et al., 2005; Merke et al., 1989; Mitsuhashi et al., 1991; Ni et al., 2014). With respect to CTS, a proteomic study reported alteration of vitamin D binding proteins in serum being down-regulated in patients with CTS (Oh et al., 2013). Other studies reported a potential link between low serum vitamin D levels and the occurrence of CTS (Gürsoy et al., 2016; Lee et al., 2016). However, VDR expression itself has not been identified in patients with CTS. Considering the potential role of the SSCT in the pathogenesis of CTS, we aimed to evaluate whether the level of VDR expression in the endothelial cells of the SSCT is associated with clinical features of CTS.

Patients and methods

Subjects

Our institutional review board approved this study, and informed consent for specimen collection was obtained from all participating patients. We included 52 consecutive women undergoing open carpal tunnel release after diagnosis of idiopathic CTS. The diagnosis was based on both clinical symptoms, such as paraesthesia or numbness over the median nerve territory and a positive electrophysiological study. A positive electrophysiologic result was defined as a median motor nerve distal onset latency of >4.0 ms or a median sensory nerve distal onset latency of >3.2 ms. These values were our electrodiagnostic laboratory's normal limits, in which a mean $+2$ standard deviation (SD) from 100 hands of 50 healthy subjects (25 women and 25 men with the mean age of 43 SD 12 years) was adopted. Patients were excluded who had rheumatoid arthritis or metabolic diseases, such as chronic kidney disease, hyperparathyroidism, malignancy, inflammatory arthritis and liver disease. Also excluded were patients with other nerve compressions, such as cubital tunnel syndrome or cervical radiculopathy, and those treated with osteoporosis medication or calcium/vitamin D supplementation. Men were excluded due to an insufficient number for the statistical analysis.

Immunohistrochemical analysis of VDR in the SSCT

One surgeon, who is a highly experienced specialist (Tang and Giddins, 2016), performed standard open carpal tunnel release and a collection of small SSCT specimens, which is a meshwork of areolar connective tissue and its associated vasculature surrounding flexor tendon. The specimens were immediately fixed in formalin and were paraffin-embedded. Four-micrometer serial sections were cut and the slides were incubated with primary antibody (rat monoclonal antibody to vitamin D Receptor, Abcam, Cambridge, MA, USA), and the secondary antibody (OmniMap anti-rat HRP, Ventana Medical Systems, Tucson, AZ, USA). Slides were incubated in DAB Map Kit (Ventana Medical Systems, Tucson, AZ, USA) and H_2O_2 substrate followed by hematoxylin and bluing reagent for counterstain.

For quantitative analysis, tissue sections were evaluated for both staining intensity and percentage of VDR positive cells, according to the previously described scoring method (Remmele et al., 1986; Wang et al., 2015). Positive cells in at least four fields were counted using a $400\times$ objective, and the percentage of immunoreactive cells in each field were calculated. Staining intensity was classified as follows: 0 (negative), 1 (weak), 2 (moderate) or 3 (strong) (Figure 1). The percentage of positive cells was scored as 0 ($\leq$5%), 1 (6%–25%), 2 (26%–50%), 3 (51%–75%) and 4 (76%–100%). The staining intensity and percentage of stained cells were then multiplied to generate the staining index (SI) for each case, ranging from 0 to 12.

Two physicians, who were blinded to the clinical information, examined each sample. The whole scoring procedure was repeated three times and the mean SI value was used. We evaluated the inter-rater reliability using intraclass correlation coefficients and the value was 0.868, which indicates almost perfect agreement (Landis and Koch, 1977).

Measurement of serum vitamin D level

Serum 25(OH)D (25-hydroxyvitamin D) levels were measured in all patients preoperatively using Diels Alder derivatization and ultrahigh-performance liquid chromatography–tandem mass spectrometry (Waters, Milford, MA, USA), which is the reference standard for 25(OH)D measurement. Calibration was performed with standard reference material 972 from the National Institute of Standards and Technology; the intra-assay and inter-assay coefficients of variation at 29 ng/mL were 4.0% and 7.7%, respectively. Concentration of vitamin D below 20.0 ng/mL was viewed as 'deficient' and concentration equal to or above 20.0 ng/mL as 'non-deficient'. All the sampling was performed during daytime (Hollis, 2005).

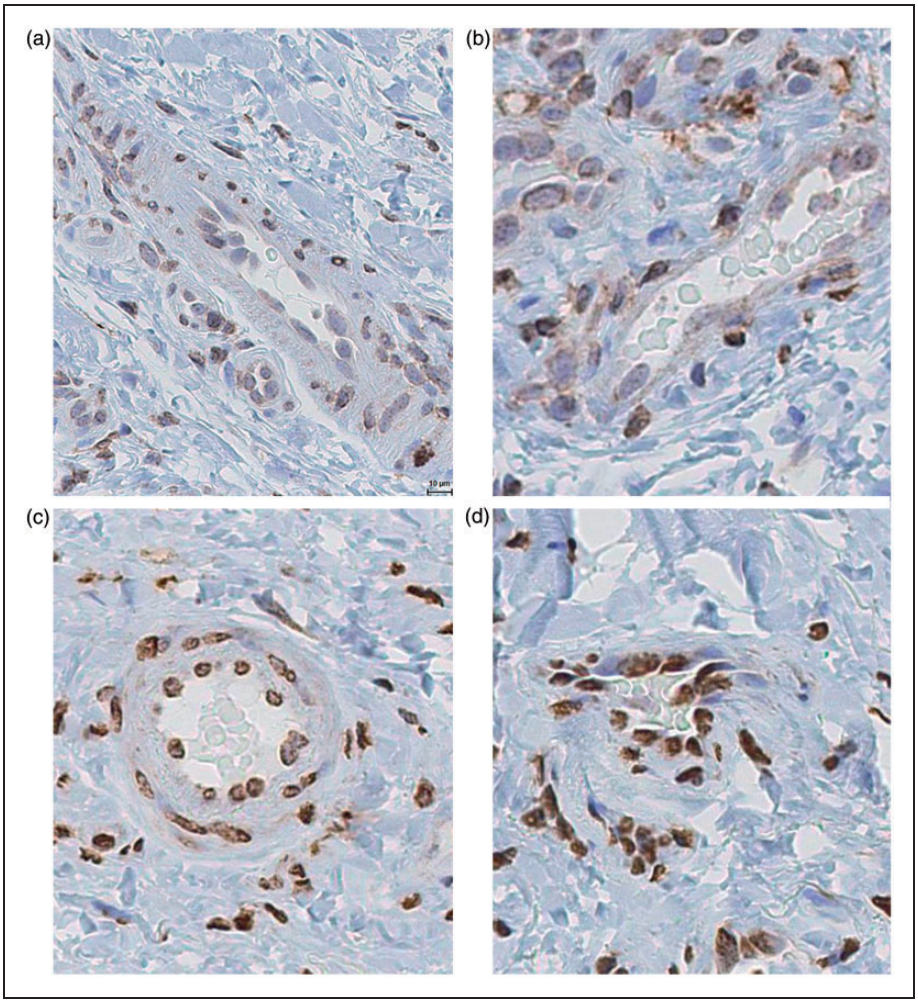

Figure 1. Representative cases showing varying degree of VDR staining index: (a) negative; (b) weak; (c) moderate; (d) strong (scale of 10 um is shown in (a)).

Evaluation of clinical features of CTS

Symptom severity was measured using the Disabilities of the Arm, Shoulder and Hand (DASH) questionnaire (Gummesson et al., 2003; Hudak et al., 1996; Jeon et al., 2011). A preoperative electrophysiologic examination was conducted in all patients. We recorded median nerve motor conduction velocity (MCV) and distal motor latency (DML), which correspond to neurophysiological severity of the disease (De Lean, 1988; Fox and Bangash, 1996; Havton et al., 2007; Kennedy et al., 2006; Uncini et al., 1993).

Statistical analysis

Results were reported as mean and SD unless otherwise indicated. Clinical features of patients with vitamin D deficiency (<20 ng/mL) were compared with the non-deficient group using independent samples *t*-test. Correlation of SI of VDR with serum vitamin D concentration and clinical features, such as age, body mass index, symptom duration, electrophysiologic variables (MCV and DML) and preoperative symptom severity (DASH), was analysed using the Pearson correlation coefficient. Variables with *P* values less than 0.1 by univariate analysis were included as independent variables in the multivariate analysis, which was performed using the stepwise elimination procedure. Goodness-of-fit was presented as adjusted R^2, which reflect the percentage of overall variability. All statistical tests were two-sided and *P* values of less than 0.05 were considered significant.

Results

Patient clinical characteristics

There were 52 women. The mean age 58 years (SD 10; range 40–83) and the mean symptom duration was 17 months (SD 25; range 1–120). The mean

Table 1. Clinical variable correlation with staining index.

	Univariate analysis		Multivariate analysis	
Variable	R	*P*-value	β	*P*-value
Serum vitamin D level (ng/ml)	0.273	0.051		
Age (years)	0.331	**0.017**		
Symptom duration (months)	0.187	0.185		
Body mass index (kg/m^2)	0.031	0.828		
MCV (m/s)	–0.187	0.185		
DML (ms)	0.361	**0.009**	0.596	**0.009**
DASH score	0.107	0.450		

MCV: motor conduction velocity; DML: distal motor latency; DASH: Disabilities of the Arm, Shoulder and Hand questionnaire.
Boldface indicates statistical significance.

preoperative DASH score was 38 (SD 20; range 4–79). The mean MCV was 54.0 m/s (SD 6.0; range 36.0–66.1) and the mean DML was 5.2 ms (SD 1.9; range 2.9–12.9). Mean serum vitamin D level was 14.9 ng/ml (SD 7.5; range 5.9–38.2). Forty-one patients were vitamin D deficient ($<$20 ng/ml), while 11 were vitamin D non-deficient ($\geq$20 ng/ml). Patients with vitamin D deficiency were found to present at a younger age ($P = 0.002$), with lower expression of VDR ($P = 0.045$) and with higher MCV ($P = 0.010$).

Immunohistrochemical analysis of VDR

The diverse expression level of VDR was observed in endothelial cells of SSCT. The mean SI of VDR was 5.2 (SD 3.1; range 0.5–11.5). Univariate analysis indicated that VDR expression significantly correlated with age and DML, but not with serum vitamin D level, symptom duration, body mass index, MCV and preoperative DASH scores (Table 1). These variables and serum vitamin D level were analysed for multivariate analysis, which showed that only DML was independently associated with VDR expression in endothelial cells of the SSCT (adjusted $R^2 = 0.113$, $P = 0.009$).

Discussion

In this study, we demonstrated the expression of VDR in endothelial cells of the SSCT in patients with CTS. We correlated VDR with clinical features of CTS and found that DML was independently associated with VDR expression. This suggests that VDR expression may be associated with disease progression, as prolonged DML possibly reflects severity of the disease.

We focused on the endothelial cells of the SSCT. The typical histologic findings of CTS reveal a non-inflammatory fibrous connective tissue with oedema, thickening of vessel walls, vessel hypertrophy and increased vascularity, and tenosynovial thickening in the SSCT (Fuchs et al., 1991; Neal et al., 1987). Ischemia-reperfusion injury has also been proposed to be the cause of CTS (Freeland et al., 2002; Hirata et al., 2005; Oh et al., 2004). These studies imply that vascular property of the SSCT could play a role in the pathogenesis or progression of CTS. The current study suggests that VDR in the endothelial cells of the SSCT might play a role in regulating regional vascular properties.

Previous studies provide evidence that hypovitaminosis D is associated with several neurodegenerative disorders, such as multiple sclerosis, Parkinson's disease and motor neuron disease (Garcion et al., 1997, 1998; Glass et al., 2010). The administration of vitamin D has been shown to reduce neurological injury in a variety of animal systems (Garcion et al., 1998). A study on multiple sclerosis reported increased VDR and 1α-hydroxylase mRNA expression in active brain lesions (Smolders et al., 2013). An animal study reported VDR expression is increased in dorsal root ganglion neurons in diabetes mellitus (Filipovic et al., 2013). In our study, however, we did not examine VDR expression in the neural tissue of the median nerve due to the obvious risk of nerve damage. Further studies on animal model of CTS may reveal the effect of vitamin D and VDR in neural tissue.

The relationship between VDR expression and serum vitamin D level is unclear. Bischoff-Ferrari et al. reported no relationship between serum vitamin D and VDR expression in human muscle (Bischoff-Ferrai et al., 2004). Kinyamu et al. also did not find a relationship between serum vitamin D and mucosal VDR in the intestine (Kinyamu et al., 1997). In the current study, the correlation between serum vitamin D level and VDR expression in the SSCT was not significant. However, the group of patients with vitamin D deficiency showed significantly lower VDR expression compared with the non-deficient group. Further studies are necessary to determine whether vitamin D supplementation could affect VDR expression in the endothelial cells and the vascular changes in the SSCT and thereby could have a therapeutic effect on CTS.

There are several limitations to this study. All included patients had open carpal tunnel release, which may have resulted in exclusion of patients in early stage CTS. Men were excluded, as their number was insufficient for statistical analysis. Therefore, the present study may not represent the general

population with CTS. Evaluation of the VDR was cross-sectional, so the causal relationship between VDR and pathologic vascular changes of the SSCT cannot be implicated. Furthermore, VDR expression was not evaluated in a disease-free control group due to the limitations of obtaining the tissue in healthy individuals. Thus, the exact role of VDR in the occurrence of CTS cannot be determined. The results of this study could serve as a foundation for future studies, including a healthy control group, to further investigate the possible role of VDR in development of CTS.

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