Ph.D. Dissertation of Medicine

Vitamin D status in Dupuytren's disease: Association with clinical status and vitamin D receptor expression

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Abstract

Dupuytren's disease(DD) is a progressive fibroproliferative condition involving contractures of the fascia of the palm. Up to now, there are no relevant investigations on patients with DD in case of serum vitamin D deficiency. We hypothesized that transforming growth factor-β1 (TGF-β1) is increased in patients with DD in consequence of vitamin D deficiency, thereby leading to myofibroblast differentiation and subsequent progression of contractures.

The study's aim was to analyze serum vitamin D levels and explore possible clinical and immunohistochemical correlates with vitamin D concentrations in a group of patients with DD. Vitamin D levels were measured in all DD patients and healthy controls. In the patient group, clinical characteristics were compared between vitamin D deficient and non-deficient subgroups. Diseased palmar fascia samples were obtained from 14 patients undergoing fasciectomy for DD. Correlations between vitamin D levels and vitamin D receptor(VDR), TGF-β1 expression levels in collected fascia samples were evaluated.

Vitamin D concentrations were significantly lower in patients than in healthy controls. In addition, total extension deficit of involved fingers was higher in vitamin D deficient patients. Moreover, a positive correlation was found between vitamin D levels and expression of VDR in pathologic fascia in patients undergoing fasciectomy for contracture. Serum vitamin D levels were found to be low in DD patients. Expression of VDR was lower in the vitamin D deficient group. The results suggest a potential link between vitamin D status and DD but causation is not yet established. The potential role of vitamin D and its interaction with VDR

and the TGF-β1 signaling pathway in the pathogenesis of DD needs to be explored further.

Keywords : Dupuytren's disease; Vitamin D; Vitamin D receptor; TGF-beta1; contracture

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Chapter 1. Introduction

1.1. Study Background

Dupuytren's disease(DD) is a late-onset benign fibroproliferative disease of the palmar aponeurosis that leads to irreversible flexion contracture of the fingers because of an increased deposition of collagen and physical shortening of the diseased fascia. The progression of DD is divided into three stages on the basis of the histological appearance of the affected palmar fascia.[1] In the first proliferative stage, fibroblasts proliferate and nodules develop. In the second involutional stage, myofibroblasts proliferate and align with lines of stress in the hand. Connective tissue begin to organize along these lines and develop into fibrotic cords, which then start to contract. In the final residual stage, the nodules regress and myofibroblasts are replaced by fibrocytes that progressively decrease in number, causing the cords to become hypocellular and scarlike.[2] Several exogenous factors and medical conditions are thought to be associated with the disease including manual work, alcoholism, smoking, diabetes mellitus and hypercholesterolemia. However, the role of these factors is not fully elucidated, and evidence is at times contradictory.

Numerous studies highlight the pivotal role of myofibroblasts in tissue contraction of DD.[3,4] Myofibroblasts originate as fibroblasts that make collagen and have intracytoplasmic myofibrillar bundles that express α-smooth muscle actin(α-SMA) and synthesize fibronectin with distinctive contractile forces and ability to create

cell-to-cell connections. Myofibroblasts form and deposit collagen type I and type III into the extracellular matrix(ECM). With continued collagen deposition, the ECM is progressively shortened.[5] The resulting cords are relatively acellular and show an increase in the ratio of type III collagen to type I collagen. The presence of myofibroblasts represents a key event during normal wound healing and tissue repair. Typically, myofibroblasts disappear as scarring matures or when tissue remodeling is complete, but it can also have negative effects on tissue function when they persist and become excessive.

The cause of myofibroblast proliferation is unclear. The development of myofibroblasts in general depends on several different environmental cues, including tension in the matrix and exposure to a variety of different mediators. The main factor that induces differentiation of original fibroblasts into myofibroblasts with contractile ability is transforming growth factor(TGF)-β1, a multifunctional cytokine involved in cell proliferation, differentiation, and ECM protein synthesis.[6] In canonical signaling, TGF-β receptor activation induces the phosphorylation of Smads which then form a complex with a co-mediator Smad, Smad4, that is translocated to the cell nucleus where it binds to gene promoters. These complexes then control the transcription of an extensive number of target genes, including pro-fibrotic genes.[7] TGF-β1 upregulates myofibroblast proliferation in DD,[8] and is a potent stimulator of collagen production in Dupuytren's fascia.[9] Using real-time polymerase chain reaction, Baird and colleagues found increased expression of TGF-β isoforms in DD.[10] In a later study, Badalamente et al. demonstrated TGF-β1 staining in fibroblasts, myofibroblasts, and capillary endothelial cells in DD samples using in situ hybridization.[11]

Interestingly, some of the causes suggested as candidates responsible for

individual development of DD are also associated with vitamin D deficiency.[12-14] While its role in calcium homeostasis is well known, there is increasing recognition that vitamin D regulates cell proliferation and differentiation and has anti-inflammatory and anti-fibrotic properties. It has been extensively studied as an anti-fibrotic agent in several chronic diseases.[15] Vitamin D deficiency is known in patients with liver cirrhosis, malabsorption and various autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, type 1 diabetes and multiple sclerosis.[16] The ability of vitamin D to regulate cellular processes is dependent on the vitamin D receptor (VDR), a member of the steroid nuclear receptor superfamily that is expressed in a wide variety of cells. When bound to the active form of vitamin D, 1,25-dihydroxyvitamin D3 (D3), the ligand-bound VDR acts as a transcription factor, binding to vitamin D response elements (VDREs) in the promoter regions of target genes to regulate their transcription.[17] Ding et al., using a mouse model of human liver fibrosis, reported that co-treatment with a synthetic VDR agonist resulted in a reduction in collagen deposition and decreased expression of genes involved in the process of fibrogenesis such as *COL1A1* and *TGF-β1*. The involvement of VDR in suppressing fibrogenesis was strengthened by the observation that VDR knockout mice spontaneously developed hepatic fibrosis.[18] The VDR has been demonstrated to be a negative regulator of TGF-β signaling. VDR interferes with TGF-β1/Smad transcriptional up-regulation of pro-fibrotic genes by antagonizing Smad3 binding to their cognate response elements. Impaired VDR signaling with reduced levels of vitamin D and decreased expression of VDR may contribute to uncontrolled activation of fibroblasts in systemic sclerosis.[19]

1.2. Purpose of Research

Up to now, there are no relevant investigations on patients with DD in case of vitamin D deficiency in serum.[20] We hypothesized that TGF-β1 is increased in patients with DD in consequence of vitamin D deficiency, thereby leading to myofibroblast differentiation and subsequent progression of soft tissue and joint contractures. The study's aim was to analyze serum vitamin D levels and explore possible clinical and immunohistochemical correlates with vitamin D concentrations in a group of patients with DD.

Chapter 2. Body

2.1. Patients and Methods

We retrospectively reviewed all patients diagnosed with DD presenting to our outpatient clinic for whom serum vitamin D levels were measured from 2011 to 2019. We excluded patients treated with osteoporosis medication and/or calcium/vitamin D supplementation and those diagnosed with a disease influencing mineral metabolism such as chronic kidney disease, hyperparathyroidism, and liver disease. We excluded diabetic patients as low vitamin D status has been suggested to be associated with increased risk of developing type 1 or type 2 diabetes.[21,22] Women in the case group were excluded from the analysis due to low sample size and insufficient statistical power. A total of 32 men (mean age 69.6, range 58-80) with DD were recruited as cases.

For healthy control patients, we obtained the data of asymptomatic men who participated in the early medical diagnosis and disease prevention program at our institution's health care center over the same period. Among the 3,014 men, 64 age-matched healthy subjects (mean age 68.8, range 60-80) were recruited as controls for a 1:2 ratio.

From the case group, diseased palmar fascia samples were obtained from 14 patients undergoing open fasciectomy for DD. Four of the patients were being treated for a recurrence of the disease after having undergone a previous operation at another institution. This study was carried out under the approval of the hospital

Institutional Review Board (No. B-1606/352-304) and all tissues were collected after patients had given informed consent. Samples were submitted for vitamin D receptor and TGF-β1 immunohistochemical analysis.

2.1.1. Measurement of serum vitamin D levels

Serum 25(OH)D (25-hydroxyvitamin D) levels were measured in all DD patients and as one of the routine lab examinations for controls. We used Diels-Alder derivatization and ultrahigh-performance liquid chromatography-tandem mass spectrometry (Waters, Milford, MA, USA) for measurement, which is the reference standard for 25(OH)D measurement.[23] Vitamin D levels were categorized as deficient (<20 ng/mL) and non-deficient(≥20 ng/mL).

2.1.2. Evaluation of clinical features of DD

Clinical parameters including age, body mass index, duration of symptoms, past surgical history of the affected hand, age at disease onset, and total extension deficit at involved metacarpophalangeal(MP), proximal interphalangeal(PIP) and distal interphalangeal(DIP) joints were investigated.

We used the classification of Iselin[24] to assess the severity of the disease. This classification consists of four categories (Table 1).

Finger involvement was also classified according to Tubiana's staging[25] (Table 2).

2.1.3. Sample preparation

After surgical removal of diseased palmar fascia in 14 patients, the tissue samples were immediately fixed in 10% buffered formalin, embedded in paraffin, and sliced into 4-mm-thick blocks of tissue. 4-um-thick serial sections cut from these blocks underwent further staining to assess the histological features.

2.1.4. Immunohistochemical analysis

All immunohistochemical stains were performed on a Ventana BenchMark XT (Ventana Medical Systems, Tucson, AZ, USA) computerized automated stainer system.

Deparaffinized and hydrated 4-um-thick sections were mounted on charged slides. Antigen retrieval was achieved using a tris-based buffer, pH 8.4, (Cell Conditioning Solution CC1, Ventana Medical Systems) held at 100°C for 24 minutes. 3% hydrogen peroxide (H_2O_2) was used to block endogenous peroxidase activity at 37°C for 4 minutes.

Tissue sections were then incubated with rabbit polyclonal vitamin D receptor antibody (Cloud-Clone, product number PAA475Hu01, 1:500) or rabbit polyclonal TGF-β1 antibody (Cloud-Clone, product number PAA124Hu01, 1:200) at 37°C for 16 minutes.

Antigen-antibody reactions were then observed using the Ventana OptiView DAB IHC Detection Kit according to the manufacturer's recommendations. OptiView

HQ Universal Linker, which has numerous non-endogenous hydroxyquinoxaline haptens conjugated to a linker secondary antibody, binds the primary antibody at 37°C for 8 minutes. A tertiary antibody layer comprising horseradish peroxidase (HRP) multimer binds to the HQ haptens, also at 37°C for 8 minutes. The number of multimer molecules is exponentially multiplied in this way, resulting in increased staining intensity without increased background. DAB chromogen reacts with HRP and H_2O_2 to generate a clean, crisp brown signal that is readily detected by light microscopy.

Counterstaining was performed on the Ventana Benchmark XT using hematoxylin II for 8 minutes, followed by bluing reagent for 4 minutes.

The assessment of the degree of staining and distribution patterns of specific immunohistochemical staining were evaluated using a semi-quantitative assay as used for steroid receptors.[26,27] Two consultant histopathologists blinded to the clinical information independently examined each sample. The staining index (SI) was calculated by multiplication of the staining intensity and percentage of positively stained cells. Staining intensity was classified as follows: 0 = negative, 1 = weak, 2 = moderate, and 3 = strong staining. The percentage of positively stained cells was scored as follows: 0 = no staining, 1 = <10% of cells, 2 = 11% to 50% of cells, 3 = 51% to 80% of cells, and 4 = >81% of cells stained (Fig 1). The total SI per sample therefore ranged from 0 to 12; 0 to 1 indicates no staining (e.g., negative results), 2 to 4 indicates moderate staining, and 6 to 12 indicates high staining. This evaluation was based on the original Remmele and Stegner characterization for hormone receptors in breast cancer.[28]

2.1.5. Statistical analysis

We compared vitamin D levels between the DD group and the healthy control group using an independent samples t-test.

We divided our case population into two groups; vitamin D deficient (<20 ng/mL) and vitamin D non-deficient(≥20 ng/mL). We compared clinical characteristics between the two groups using an independent samples t-test for age, duration of symptoms, and body mass index (BMI). A Mann-Whitney U test was used for total extension deficit, Iselin staging, Tubiana total staging, and Tubiana staging for the worst affected finger. Pearson's chi-square test was run for early onset of disease and Fisher's exact test was run for past surgical history of the affected hand.

Correlations between vitamin D levels and VDR, TGF-β1 expression levels in collected palmar fascia samples were evaluated using the Spearman's rank correlation test.

All statistical analyses were performed using the SPSS software package (version 22.0; SPSS Inc., Chicago, IL, USA). A *p*-value of less than 0.05 was considered statistically significant.

2.2. Results

2.2.1. Serum vitamin D levels in patients and controls

Vitamin D concentrations were significantly lower (mean 19.33±6.28 ng/ml) in the patient group than the healthy control group (mean 22.89±7.89 ng/ml). (p=0.029)

2.2.2. Correlation between vitamin D levels and clinical features of DD

Thirty-two patients were classified according to serum vitamin D levels. Vitamin D <20 ng/ml was detected in 18 patients and ≥20 ng/ml in 14 patients. Overall, there was no significant difference in age, BMI, early onset of disease, duration of symptoms, past surgical history of the affected hand, Iselin staging, Tubiana total staging and Tubiana staging of the worst affected finger among the vitamin D status groups (Table 3). The total extension deficit of the affected fingers was significantly higher in those with vitamin D deficiency when compared to those who were vitamin D non-deficient. Although a Pearson correlation analysis for continuous variables showed a negative correlation between vitamin D levels and total extension deficit, it did not reach a level of statistical significance (r=-0.398, p=0.098).

2.2.3. Correlation between vitamin D levels and VDR, TGF-β1 expression in pathologic tissue

The mean SI of VDR and TGF-β1 was 3.1(SD 2.1, range 0-6) and 5.2(SD 2.8, range 0-9), respectively. Vitamin D levels and VDR expression were significantly and positively correlated (rho=0.65, *p*=0.012). Although vitamin D levels and TGF-β1 expression were negatively correlated, it did not reach a level of significance. (rho=-0.32, *p*=0.260)

Chapter 3. Discussion

In this study, we investigated serum vitamin D levels in DD patients. It was found that vitamin D concentrations were significantly lower than healthy controls. Moreover, a positive correlation was found between vitamin D levels and expression of VDR in pathologic fascia in a subset of patients undergoing fasciectomy for contracture. In addition, total extension deficit calculated at all joints of involved fingers was higher in vitamin D-deficient patients. Taken together, these results suggest that low vitamin D levels may lead to a decrease in vitamin D receptor-mediated anti-fibrotic effects. [29]

There is increasing evidence of vitamin D deficiency effects on a wide spectrum of chronic diseases, including nonalcoholic fatty liver disease, autoimmune conditions, cystic fibrosis and several forms of malignancy.[30-32] Studies *in vitro* have demonstrated $1,25(OH)_2D_3$ inhibits growth of murine fibroblasts and inhibits collagen type I and type III synthesis by fibroblasts grown from human tissues.[33,34] In mice, *in vivo* administration of $1,25(OH)_2D_3$ has been shown to reduce conversion of adipose tissue to fibrous tissue in mouse skin exposed to chronic UV irradiation.[35] Vitamin D has been implicated in genes involved in epithelial mesenchymal transition, a process implicated in fibrosis as well as keloid scarring.[36] The role of $1,25(OH)_2D_3$ in fibrosis has been largely demonstrated in particularly in the liver and kidney, the two organs where $1,25(OH)_2D_3$ is metabolized. As a proof of concept, oral supplementation of vitamin D analogues in chronic kidney disease patients resulted in amelioration of medical conditions.[37]

The diseased palmar fascia in DD is a useful model of fibrosis because it displays the entire set of cells, cytokines and extracellular matrix involved in

fibroproliferative diseases. A genetic predisposition is one of several causes that have been proposed for DD. In a genome-wide association study, nine chromosomal loci were found to be associated with susceptibility to DD.[38] Six of these loci contain genes involved in the Wnt signaling pathway. The Wnt gene family consists of structurally related genes that encode extracellular signaling molecules. Wnt signaling has been shown to promote cell proliferation and survival via β-catenin, and this pathway has been suggested as a primary cause of fibrosis in different organs. So far, pathologically activated canonical Wnt signaling has been implicated in the pathogenesis of various fibrotic conditions such as pulmonary, renal, dermal, and liver fibrosis.[39-41] Although the pathogenesis of DD is not fully understood, the cytokine TGF-β1 is believed to be the main growth factor involved in the disease process. Several studies have documented TGF-β1 expression in Dupuytren's palmar fascia using reverse transcriptase polymerase chain reaction, in-situ hybridization, and immunochemistry.[8,10,11] Multiple levels of cooperation between the TGF-β and Wnt signaling pathways in regulating gene expression have been documented. TGF-β1 operates in a canonical WNT/β-catenin pathway dependent manner. These two pathways stimulate each other through the Smad pathway or non-Smad pathways like PI3K/Akt pathway.[42] There has been some evidence of crosstalk between vitamin D and TGF-β in other diseases. Vitamin D was demonstrated to have a prophylactic effect on intestinal fibrosis through inhibition of TGF-β1/Smad3 pathway and upregulation of VDR in mice with chronic colitis.[43] It has been demonstrated that deficiency of vitamin D leads to upregulation of TGF-β1 in serum.[44,45] Although TGF-β1 expression in pathologic fascia was negatively correlated with vitamin D levels in our study, the association was not significant. The complexity of interactions between the TGF-β1 signaling

system and vitamin D is further highlighted by the observation that the cooperative actions of vitamin D and TGF-β1 can be synergistic or antagonistic in a cell-specific manner.[46,47] Tissue-specific gene expression patterns and amounts of Smad proteins, VDR and their receptors may be responsible for tissue-specific differences in cooperative actions between Smads and VDR.[48]

A major limiting factor in investigating DD has been the lack of a well-described *in vivo* animal model that can capture its distinct biology, as DD is a condition unique to humans without a counterpart in animals. In contrast, various animal models of liver fibrosis have provided a means to study the cell and molecular mediators of fibrosis in a serial manner during progression and recovery.[49-51] Satish et al. developed an animal model of DD by orthotopic transplantation of human fibroblasts into the forepaw of athymic rats and found that DD-derived fibroblasts showed persistent expression of genes involved in fibrosis and contraction.[52] Future studies utilizing such animal models would help in understanding the kinetics of VDR and TGF-β1expression through different stages of the disease.

Epidemiological studies have shown that DD is more common in northern European populations and prevalence seems to be highest in Scandinavian countries.[53] However, the much-quoted concept of DD being labeled a "Viking" disease is probably only due to previous studies being limited to few geographic locations. In fact, increasing evidence shows a substantial prevalence of DD in countries in Asia and Africa.[54,55] A recent genome-based study from the United Kingdom has found no evidence for an excess of 'Viking origin in Dupuytren's disease'.[56] In northern latitudes, there is a strong seasonal variation in circulating levels of vitamin D levels.[57] In Norway, solar radiation is not enough to promote vitamin D synthesis during the six winter months.[58] A proposed protective role of

ultraviolet (UV) radiation for autoimmune diseases such as multiple sclerosis (MS) or type 1 diabetes has been suggested to act through vitamin D synthesis in the skin. The prevalence of these disorders has been shown to increase at higher latitudes.[59,60] An association between UV radiation, vitamin D intake and MS has been suggested by an epidemiological study.[61] The prevalence of MS is greatest in the northern latitudes of Europe and low in areas with at least 3000 hours of sunlight annually or with sufficient vitamin D intake.[62] To our knowledge, similar demographic studies exploring possible theories of influence for DD are lacking. The prevalence of DD in different geographical locations is extremely variable, and it is not clear whether this may be influenced by a genetic factor or environmental, or a combination of the two.[52] Future studies may be needed to find an association between latitude, vitamin D levels and DD.

Out of several clinical parameters, only total passive extension deficit was associated with low vitamin D status. Iselin's classification has flaws in that grading is solely based on which interphalangeal joints are involved in contracture; it does not discriminate between an extension deficit of a single MP joint of 15° or 80°, for instance. As only a single MP joint is involved, both deficits are equally classified as Iselin stage II, even though the difference in clinical appearance and impairment of hand function must be quite significant. Likewise, the total digital extension contracture used in the Tubiana classification could relate to two or three mildly contracted joints, although it could also equally apply to just one severely affected joint, with other joints unaffected. This may show the problems associated with this type of categorization. However, the total extension deficit of all involved fingers is a continuous variable which may not be associated with these shortcomings and is a better indicator of impaired hand function.

There are several limitations to this study. Women in the case group were excluded from the analysis due to low sample size; therefore, the present study may not represent the general population with DD. Epidemiological studies have shown that DD is much less common in women, with reported overall male-to-female ratios ranging between 3.5:1 to 9:1,[63-64] with the frequency in women catching up to that in men by the eighth or ninth decades of life.[65] It remains unclear why men are affected more frequently; although Pagnotta et al.[66] found that the expression of androgen receptors in DD is considerably higher than in normal palmar fascia, further studies will be needed to evaluate whether this is related to the high incidence of DD in the male sex. Vitamin D deficiency was assumed to be binary (either present or absent) and this binary expression, although commonly used in related studies, might obscure important relationships that might exist across a continuous spectrum of values. Interpretation of any causal estimate between vitamin D and disease severity such as total extension deficit may be limited because of this dichotomization of vitamin D levels. Familial predisposition is frequent in DD, suggesting an autosomal dominant inheritance pattern with variable penetrance. The ratio of cases with positive family history range between 12.5% and 44% in other studies.[67-69] A study has found that individuals with positive family history require earlier surgery for DD.[70] There is also a genetic background to vitamin D concentrations, as twin and familial studies from past decades have demonstrated a substantial quantity of heritability.[71-73] Unfortunately, family history was not collected at initial visit for the majority of patients in our population and thus could not be analyzed. Only immunohistochemistry could be performed on biopsy samples, thus offering a limited view of protein expression. The study would be greatly strengthened with additional data such as reverse

transcriptase PCR or mRNA evaluation. VDR and TGF-β1 expression was not evaluated in a disease-free control group because of the practical difficulties in obtaining palmar fascia in healthy individuals. Thus, it cannot be determined how VDR and TGF-β1 contribute to the occurrence of DD. In the future, a more robust patient population with appropriate diversity measuring vitamin D and VDR levels with a matching control patient population would greatly enhance the results of this study.

Chapter 4. Conclusion

As far as we know, this is the first study about associating the levels of serum vitamin D in patients with DD. The results suggest a potential link between vitamin D status and DD but the current study does not definitely demonstrate a true causation between these findings. The potential role of vitamin D and its interaction with VDR and the TGF-β1 signaling pathway in the pathogenesis of DD needs to be explored further. Care must be taken in drawing conclusions because expression of VDR is regulated in a tissue-specific manner. Future research should be designed to overcome the limitations this study presents and should aim to characterize the role of VDR in fibroblast activation in DD. mRNA and protein levels of VDR should be examined in human fibroblasts exposed to chronically increased levels of TGF-β1 expression. For *in vivo* studies, levels of VDR may be analyzed in the skin of mice overexpressing for TGF-β1.

Conflict of interest statement

None.

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Bibliography

1. Luck JV. Dupuytren's contracture: a new concept of the pathogenesis correlated with surgical management. *J Bone Joint Surg Am* 1959;41-A(4):635–64.

2. Schurch W, Skali I, Gabbiania G. Cellular biology. In: McFarlane RM, McGrouther DA, Flint MH, eds. Dupuytren's Disease. New York: Churchill Livingstone, 1990:31-34.

3. Tomasek JJ, Gabbiani G, Hinz B, Chaponnier C, Brown RA. Myofibroblasts and mechano-regulation of connective tissue remodelling. *Nat Rev Mol Cell Biol* 2002;3(5):349-363.

4. Badalamente MA, Stern L, Hurst LC. The pathogenesis of Dupuytren's contracture: contractile mechanisms of the myofibroblasts. *J Hand Surg Am* 1983;8(3):235-243.

5. Bisson MA, Mudera V, McGrouther DA, Grobbelaar AO. The contractile properties and responses to tensional loading of Dupuytren's disease-derived fibroblasts are altered: a cause of the contracture? *Plast Reconstr Surg* 2004;113(2):611-621.

6. Hinz B, Celetta G, Tomasek JJ, Gabbiani G, Chaponnier C. Alpha-smooth muscle actin expression upregulates fibroblast contractile activity. *Mol Biol Cell* 2001;12(9):2730-2741.

7. Clayton SW, Ban GI, Liu C, Serra R. Canonical and noncanonical TGF-β signaling regulate fibrous tissue differentiation in the axial skeleton. *Sci Rep* 2020;10(1):21364.

8. Berndt A, Kosmehl H, Mandel U, et al. TGF beta and bFGF synthesis and localization in Dupuytren's disease (nodular palmar fibromatosis) relative to cellular activity, myofibroblast phenotype and oncofetal variants of fibronectin. *Histochem J* 1995;27(12):1014-1020.

9. Alioto RJ, Rosier RN, Burton RI, Puzas ZE. Comparative effects of growth factors on fibroblasts of Dupuytren's tissue and normal palmar fascia. *J Hand Surg Am* 1994;19(3):442-452.

10. Baird KS, Crossan JF, Ralston SH. Abnormal growth factor and cytokine expression in Dupuytren's contracture. *J Clin Pathol* 1993;46(5):425-428.

11. Badalamente MA, Sampson SP, Hurst LC, Dowd A, Miyasaka K. The role of transforming growth factor beta in Dupuytren's disease. *J Hand Surg Am* 1996;21(2):210-215.

12. Tardelli VS, Lago MPPD, Silveira DXD, Fidalgo TM. Vitamin D and alcohol: a review of the current literature. *Psychiatry Res* 2017;248:83-86.

13. Brot C, Jorgensen NR, Sorensen OH. The influence of smoking on vitamin D status and calcium metabolism. *Eur J Clin Nutr* 1999;53(12):920-926.

14. Berridge MJ. Vitamin D deficiency and diabetes. *Biochem J* 2017;474(8):1321-1332.

15. Kitson MT, Roberts SK. D-livering the message: the importance of vitamin D status in chronic liver disease. *J Hepatol* 2012;57(4):897-909.

16. Cutolo M. Further emergent evidence for the vitamin D endocrine system involvement in autoimmune rheumatic disease risk and prognosis. *Ann Rheum Dis* 2013;72(4):473-475.

17. Saccone D, Asani F, Bornman L. Regulation of the vitamin D receptor gene by environment, genetics and epigenetics. *Gene* 2015;561:171–80.

18. Ding N, Yu RT, Subramaniam N, et al. A Vitamin D receptor/SMAD genomic circuit gates hepatic fibrotic response. *Cell* 2013;153: 601-613.

19. Zerr P, Vollath S, Palumbo-Zerr K, et al. Vitamin D receptor regulates TGF-β signalling in systemic sclerosis. *Ann Rheum Dis* 2015;74(3):e20.

20. Seyhan H, Stromps JP, Demir E, Fuchs PC, Kopp J. Vitamin D deficiency may stimulate fibroblasts in Dupuytren's disease via mitochondrial increased reactive oxygen species through upregulating transforming growth factor-β1. *Med Hypotheses* 2018;116:40-41.

21. Dong JY, Zhang WG, Chen JJ, et al. Vitamin D intake and risk of type 1 diabetes: a meta-analysis of observational studies. *Nutrients* 2013;5(9):3551-3562.

22. Song Y, Wang L, Pittas AG, et al. Blood 25-hydroxy vitamin D levels and incident type 2 diabetes: a meta-analysis of prospective studies. *Diabetes Care* 2013;36(5):1422-1428.

23. Holick MF. Vitamin D status: measurement, interpretation, and clinical application. *Ann Epidemiol* 2009;19(2):73-78.

24. Iselin M, Iselin F. Maladie de Dupuytren. In: Traité de chirurgie de la main. Paris: Flammarion, 1967:676-678.

25. Tubiana R. Evaluation des déformations dans la maladie de Dupuytren. In: Tubiana R, Hueston JT, eds. la maladie de Dupuytren. Paris: Expansion Scientifique Française, 1986:111-114.

26. Jeschke U, Bischof A, Speer R, et al. Development of monoclonal and polyclonal antibodies and an ELISA for the determination of glycodelin in human serum, amniotic fluid and cystic fluid of benign and malignant ovarian tumors. *Anticancer Res* 2005;25(3A):1581-1589.

27. Mylonas I, Makovitzky J, Jeschke U, et al. Expression of Her2/neu, steroid

receptors (ER and PR), Ki67 and p53 in invasive mammary ductal carcinoma associated with ductal carcinoma in situ (DCIS) versus invasive breast cancer alone. *Anticancer Res* 2005;25(3A):1719-1723.

28. Remmele W, Stegner HE. Recommendation for uniform definition of an immunoreactive score (IRS) for immunohistochemical estrogen receptor detection (ER-ICA) in breast cancer tissue. *Pathologe* 1987;8(3):138-140.

29. Park JW, Kim ST, Lee KS, Gong HS. Vitamin D status in Dupuytren's disease: Association with clinical status and vitamin D receptor expression. *J Plast Reconstr Aesthet Surg* 2022;75(6):1916-1922.

30. Chesdachai S, Tangpricha V. Treatment of vitamin D deficiency in cystic fibrosis. *J Steroid Biochem Mol Biol* 2016;164:36-39.

31. Peterlik M, Cross HS. Vitamin D and calcium deficits predispose for multiple chronic diseases. *Eur J Clin Invest* 2005;35(5):290-304.

32. Liu T, Xu L, Chen F-H, Zhou Y-B. Association of serum vitamin D level and nonalcoholic fatty liver disease: a meta-analysis. *Eur J Gastroenterol Hepatol* 2020;32(2):140-147.

33. Dosquet-Bernard C, Wilhelm F, Lomri N, Tobelem G, Caen JP. 1 alpha, 25-dihydroxyvitamin D3 modulates the growth of 3T3 cells and human skin fibroblasts stimulated by platelet-derived growth factor. *Cell Biol Int Rep* 1986;10(12):931-938.

34. Ramirez AM, Wongtrakool C, Welch T, et al. Vitamin D inhibition of pro-fibrotic effects of transforming growth factor beta1 in lung fibroblasts and epithelial cells. *J Steroid Biochem Mol Biol* 2010;118(3):142-150.

35. Koshiishi I, Mitani H, Sumita T, Imanari T. 1,25-dihydroxyvitamin D(3) prevents the conversion of adipose tissue into fibrous tissue in skin exposed to

chronic UV irradiation. *Toxicol Appl Pharmacol* 2001;173(2):99-104.

36. Hahn JM, McFarland KL, Combs KA, Supp DM. Partial epithelial-mesenchymal transition in keloid scars: regulation of keloid keratinocyte gene expression by transforming growth factor-β1. *Burns Trauma* 2016;4(1):30.

37. Kovesdy CP, Ahmadzadeh S, Anderson JE, Kalantar-Zadeh K. Association of activated vitamin D treatment and mortality in chronic kidney disease. *Arch Intern Med* 2008;168(4):397-403.

38. Dolmans GH, Werker PM, Hennies C, et al. Wnt signaling and Dupuytren's disease. *N Engl J Med* 2011;**365**, 307–317.

39. Chilosi M, Poletti V, Zamo A, et al. Aberrant Wnt/beta-catenin pathway activation in idiopathic pulmonary fibrosis. *Am J Pathol* 2003;162:1495–1502.

40. He W, Dai C, Li Y, Zeng G, Monga SP, Liu Y. Wnt/beta-catenin signaling promotes renal interstitial fibrosis. *J Am Soc Nephrol* 2009;20:765–776.

41. Wei J, Melichian D, Komura K, et al. Canonical Wnt signaling induces skin fibrosis and subcutaneous lipoatrophy: a novel mouse model for scleroderma? *Arthritis Rheum* 2011;63:1707–1717.

42. Akhmetshina A, Palumbo K, Dees C, et al. Activation of canonical Wnt signalling is required for TGF-β-mediated fibrosis. *Nat Commun* 2012;3:735.

43. Tao Q, Wang B, Zheng Y, et al. Vitamin D prevents the intestinal fibrosis via induction of vitamin D receptor and inhibition of transforming growth factor-beta1/Smad3 pathway. *Dig Dis Sci* 2015;60(4):868-875.

44. Isik S, Ozuguz U, Tutuncu YA, et al. Serum transforming growth factor-beta levels in patients with vitamin D deficiency. *Eur J Intern Med* 2012;23(1):93-97.

45. Irani M, Seifer DB, Grazi RV, et al. Vitamin D supplementation decreases TGF-β1 bioavailability in PCOS: a randomized placebo-controlled trial. *J Clin*

Endocrinol Metab 2015;100(11):4307-4314.

46. Nagel D, Kumar R. 1 alpha,25-dihydroxyvitamin D3 increases TGF beta 1 binding to human osteoblasts. *Biochem Biophys Res Commun* 2002;290(5):1558-1563.

47. Subramaniam N, Leong GM, Cock TA, et al. Cross-talk between 1,25-dihydroxyvitamin D3 and transforming growth factor-beta signaling requires binding of VDR and Smad3 proteins to their cognate DNA recognition elements. *J Biol Chem* 2001;276(19):15741-15746.

48. Ding N, Yu RT, Subramaniam N, et al. A vitamin D receptor/SMAD genomic circuit gates hepatic fibrotic response. *Cell* 2013;153:601–613.

49. Ala-Kokko, L, Stenback F, Ryhanen, L. Preventative effect of malotilate on dimethylnitrosamina-induced liver fibrosis in rats. *J Lab Clin Med* 1989:113: 177–183.

50. Madden JW, Gertman PM, Peacock EE. Dimethylnitrosamine induced hepatic cirrhosis – a new canine model of an ancient human disease. *Surgery* 1970; 68:260–268.

51. Zengdun S, Wakil AE, Rockey DC. Strain specific differences in mouse hepatic wound healing are mediated by divergent T. helper cytokine responses. *Proc Natl Acad Sci* 1994;94(20):10663–10668.

52. Satish L, Palmer B, Liu F. Developing an animal model of Dupuytren's disease by orthotopic transplantation of human fibroblasts into athymic rat. *BMC Musculoskelet Disord* 2015;16:138.

53. Hindocha S, McGrouther DA, Bayat A. Epidemiological evaluation of Dupuytren's disease incidence and prevalence rates in relation to etiology. *Hand (N.Y)* 2009;4:256-269.

54. Egawa T, Senuri S, Horiki A, Egawa M. Epidemiology of the Oriental patient. In: Mcfarlane RM, Flint MH, eds. Dupuytren's disease. Edinburgh: Churchill Livingstone, 1990: 239-245.

55. Gebereegziabher A, Baraki A, Kebede Y, Mohammed I, Finsen V. Dupuytren's contracture in Ethiopia. *J Hand Surg Eur* 2017;42(1):26-28.

56. Ng M, Lawson DJ, Winney B, Furniss D. Is Dupuytren's disease really a 'disease of the Vikings'? *J Hand Surg Eur* 2020;45(3):273-279.

57. Andersen R, Mølgaard C, Skovgaard LT, et al. Teenage girls and elderly women living in northern Europe have low winter vitamin D status. *Eur J Clin Nutr* 2005;59(4):533-541.

58. Brustad M, Alsaker E, Engelsen O, Aksnes L, Lund E. Vitamin D status of middle-aged women at 65–71°N in relation to dietary intake and exposure to ultraviolet radiation. *Public Health Nutr* 2004;7(2):327-335.

59. Cantorna MT. Vitamin D and autoimmunity: is vitamin D status an environmental factor affecting autoimmune disease prevalence? *Proc Soc Exp Biol Med* 2000;223(3):230-233.

60. Hayes CE, Cantorna MT, DeLuca HF. Vitamin D and multiple sclerosis. *Proc Soc Exp Biol Med* 1997;216(1):21-27.

61. VanAmerongen BM, Dijkstra CD, Lips P, Polman CH. Multiple sclerosis and vitamin D: an update. *Eur J Clin Nutr* 2004;58:1095-1109.

62. Goldberg P. Multiple sclerosis: vitamin D and calcium as environmental determinants of prevalence (a viewpoint). Part I: sunlight, dietary factors and epidemiology. *Int J Environ Studies* 1974;6(1):19-27.

63. Ross DC. Epidemiology of Dupuytren's disease. *Hand Clin* 1999;15(1):53-62.

64. Zemel NP. Dupuytren's contracture in women. *Hand Clin* 1991;7(4):707-711.

65. Wilbrand S, Ekbom A, Gerdin B. The sex ratio and rate of reoperation for Dupuytren's contracture in men and women. *J Hand Surg [Br]* 1999;24(4):456-459.

66. Pagnotta A, Specchia N, Greco F. Androgen receptors in Dupuytren's contracture. *J Orthop Res* 2002;20(1):163-168.

67. Coert JH, Nérin JPB, Meek MF. Results of partial fasciectomy for Dupuytren disease in 261 consecutive patients. *Ann Plast Surg* 2006;57(1):13-17.

68. Hindocha S, John S, Stanley JK, Watson SJ, Bayat A. The heritability of Dupuytren's disease: familial aggregation and its clinical significance. *J Hand Surg Am* 2006;31(2):204-210.

69. Lanting R, van den Heuvel ER, Westerink B, Werker PMN. Prevalence of Dupuytren disease in The Netherlands. *Plast Reconstr Surg* 2013;132(2):394-403.

70. Becker K, Tinschert S, Lienert A et al. The importance of genetic susceptibility in Dupuytren's disease. *Clin Genet* 2015;87(5):483-487.

71. Hunter D, De Lange M, Snieder H, et al. Genetic contribution to bone metabolism, calcium excretion, and vitamin D and parathyroid hormone regulation. *J Bone Miner Res* 2001;16(2):371-378.

72. Shea MK, Benjamin EJ, Dupuis J, et al. Genetic and non-genetic correlates of vitamins K and D. *Eur J Clin Nutr* 2009;63(4):458-464.

73. Karole C, Su S, Kumari M, et al. Heritability and seasonal variability of vitamin D concentrations in male twins. *Am J Clin Nutr* 2010;92(6):1393-1398.

초록

듀피트렌 구축의 병적 조직에서 비타민D와 비타민D 수용체의 평가

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듀피트렌 질환은 수장 및 수지건막의 진행성 증식성 섬유 형성으로, 소결절이나 섬유대를 형성하여 중수지 관절과 지관절에 굴곡 변형 및 수지 기능의 장애를 초래하는 질환이다. 현재까지 비타민D 결핍과 듀피트렌 질환과의 연관성에 대한 연구는 전무하다. 비타민D 결핍시 TGF-β1의 발현이 상향 조절되어 이를 매개로 근섬유아세포 증식이 촉진되며 손의 구축이 진행된다는 가설 아래 연구를 진행하였다.

듀피트렌 질환을 앓고 있는 환자군에서 혈청 비타민D 농도를 측정하고 혈청농도와 임상양상, 그리고 면역조직화학적 특성과의 관계를 규명하고자 하였다. 혈청 비타민D 농도는 환자군과 건강한 대조군 모두에서 측정하였다. 환자군은 비타민D 결핍군과 충분군으로 나누어 임상 양상을 비교하였다. 진행성 구축으로 수장막 절제술을 시행 받은 14명의 환자에서 병적 건막조직을 채취하였고 이 건막조직 내 비타민D 수용체와 TGF-β1 단백질의 발현정도와 혈청 비타민D 농도 간의 관계를 조사하였다.

환자군의 혈청 비타민D 농도는 대조군에 비해 유의하게 낮게 측정되었으며, 비타민D 결핍군에서 손의 구축 각도가 유의하게 높게 나타났다. 아울러 병적 건막조직 내 비타민D 수용체 발현정도와 혈청

비타민D 농도는 양의 상관관계를 나타내었으며, 비타민D 결핍군에서 비타민D 수용체 발현이 낮게 측정되었다.
위 연구 결과 비타민D는 듀피트렌 질환의 발생에 있어 잠재적 역할을 수행할 것으로 생각되나, 비타민D 수용체의 발현 또는 TGF-β1을 매개로 한 신호전달체계에 있어 역할 규명이 추가적으로 연구되어야 할 것이다.

주요어 : 듀피트렌 질환, 비타민D, 비타민D 수용체, TGF-β1, 구축

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Table 1.

Grade	Deformity
I	Palmar nodules and small cords without signs of contracture
II	Contracture of the MP joint
III	Contracture of the MP and PIP joint
IV	Severe contracture of the MP and PIP joint with hyperextension deformity of the distal interphalangeal (DIP) joint

Staging of Dupytren's disease according to the Iselin classification. Expressed by the grade of the worst affected finger.

Table 2.

Stage	Deformity
0	No lesion
N	Nodular presence without finger contraction
1	Total extension deficit between 0°and 45°
2	Total extension deficit between 45°and 90°
3	Total extension deficit between 90°and 135°
4	Total extension deficit superior to 135°

Staging of Dupytren's disease according to the Tubiana classification. Total passive extension deficit of each involved finger is calculated using a goniometer adding the extension deficit at MP, PIP, and DIP joints. For scoring purposes, the nodular stage (N) is graded at 0.5 point. The number of each other stage determines the points, e.g. stage 2 scores 2 points.

Table 3.

Patient characteristics	Vitamin D deficient (<20 ng/mL) (n=18)	Vitamin D non-deficient (≥20 ng/mL) (n=14)	*p* value
Age (years)	70	68	0.454[a]
Onset of disease < 60 yr	9(50%)	6(42.9%)	0.688[b]
Duration of DD symptoms (years)	7.2	5.9	0.952[a]
BMI	24.2	23.4	0.233[a]
Past surgical history	8(44.4%)	3(21.4%)	0.266[c]
Total extension deficit	72.5 (50)	50(61.25)	**0.034[d]**
Iselin staging	3 (1)	3(1)	0.925[d]
Tubiana total staging	2 (1.25)	2(1.625)	0.985[d]
Tubiana staging of worst affected finger	2(1)	1(1)	0.220[d]

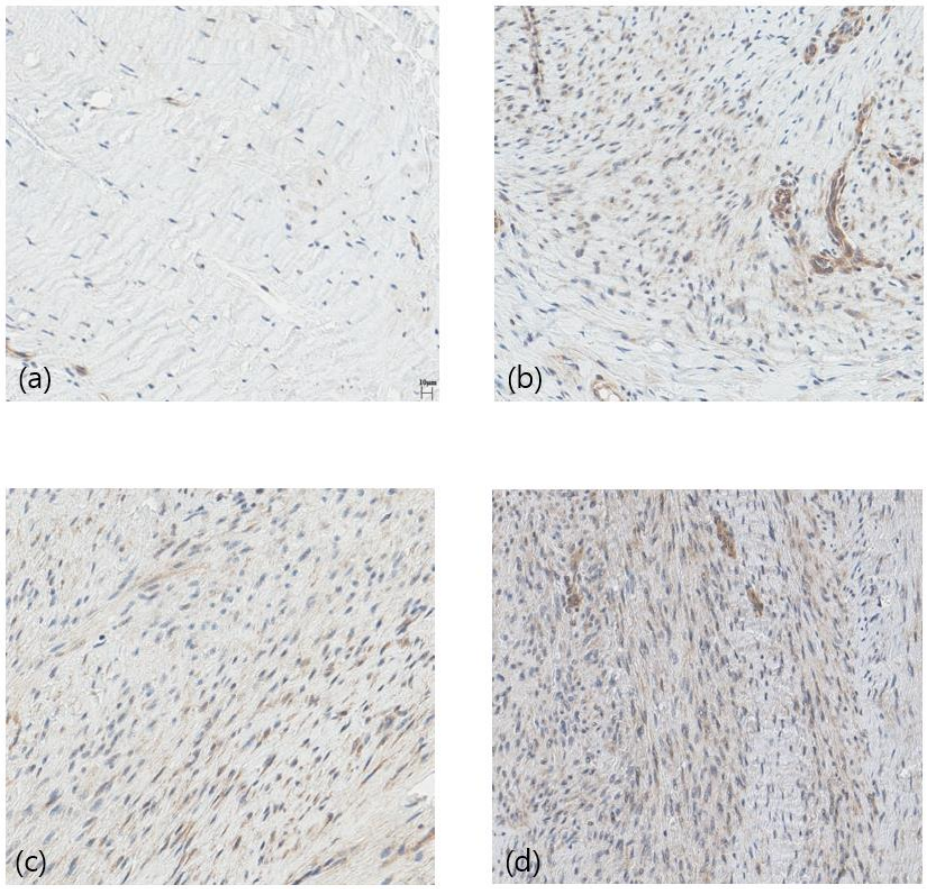
Clinical characteristics of patients classified by vitamin D status. Mean value is indicated when T-test was used, median value and interquartile range is

indicated when Mann-Whitney U test was used and incidence when Pearson's chi-squared test or Fisher's exact test was used.

[a]T-test, [b]Pearson's chi-square test, [c]Fisher's exact test, [d]Mann-Whitney U test

Iselin staging : worst affected finger

Figure 1. Representative cases showing varying degrees of VDR staining index: (a) negative (b) weak (c) moderate (d) strong.[①]



① Figures 1. (a)~(d) : ㈜수퍼바이오칩에서 슬라이드 사진 제공