DUPUYTREN'S CONTRACTURE; INCREASED CELLULARITY – PROLIFERATION, IS THERE EQUALITY?

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ABSTRACT

Background: **Dupuytren's disease is a chronic inflammatory process which causes contractures of the fingers by shortening and thickening the palmar fascia. During the proliferative phase, fibroblasts transform into myofibroblasts apparently under the influence of several different factors. The disease usually develops slowly, but in some patients it tends to develop aggressively. The pathogenesis of Dupuytren's disease remains unsolved. In this study, we analyzed some histological characteristics that seem to predict rapid recurrence.**

Material and Methods: **21 patients were divided into two groups. In 11 patients the disease was classified as aggressive because it had recurred within two years after an operation. In 10 cases it was non-aggressive, as no recurrence had been seen. Five control samples were taken from healthy palmar aponeurosis.**

The differences in cellularity, collagen, Ki-67, MSA, alpha-SMA and tenascin between the specimens were analyzed using immunohistochemistry.

Results: **Alpha-SMA and Ki-67 were present more often in the aggressive specimens. Immunohistochemical stainings for macrophages and lymphocytes were negative.**

Conclusion: **There may be differences in the histology and/or immunohistochemical appearance of pathological palmar connective tissue cords in aggressive and normal Dupuytren's disease. Further studies are needed to elucidate the pathogenesis of this disease.**

Key words: Dupuytren's disease; immunohistochemistry; recurrence

INTRODUCTION

Dupuytren's disease has been defined as a chronic inflammatory process which produces contractures of the fingers by shortening and thickening the palmar fascia. (1) Therefore, it is a deforming and pro-

gressive, irreversible condition that may affect palmar or plantar aponeurosis (Ledderhose's disease) or penile (Peyronie's disease) (2, 3). It is a disease characterised by fibroblast proliferation. The etiology of the fibrotic disease underlying Dupuytren's contracture remains unsolved despite research.

The pathogenesis of the disease has been widely explored (4, 5, 6, 7). The therapeutic practice is still to surgically excise the affected palmar fascia. Excision of palmar aponeurosis is a curative but not always permanently effective treatment. Dupuytren's contracture is known to recur occasionally. Clinically, in some patients, there is an aggressive tendency to develop recurrent contracture quite soon. The pur-

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pose of this study was to explore if there is some structural difference between the palmar fascias of patients who experience relapse and those who do not. This may help us to make a histological prognosis of whether the disease is likely to recur soon.

MATERIAL AND METHODS

Twenty-one patients with Dupuytren's contracture and 5 control patients were included in this study. The Dupuytren patients were further divided into two groups based on the recurrence of the disease. The disease was defined as "aggressive" if definite recurrence (recurrent mass, finger contracture and obvious need for a new operation) was diagnosed within two years after the previous aponeurectomy operation. There were 7 patients in this group.

The other group was defined as "non-aggressive". These patients did not have a previous history of Dupuytren's disease. At the beginning of the study, this group included 14 patients. During the follow-up, however, the disease of 4 patients recurred very soon and clearly fulfilled the criteria of "aggressive" disease. These patients were classified into the "aggressive" group on histological examination. Thus, there were altogether 11 patients in the "aggressive" group and 10 patients in the "non-aggressive" group. Histological samples of mature cords and nodules were examined. The control group consisted of 5 patients who underwent routine carpal tunnel release. The palmar fascia tissue samples of these patients were used as controls in a histological examination.

All the patients were examined clinically before the operation. After the follow-up period, all patients were interviewed by a hand surgeon over the telephone. This method was considered more reliable than a questionnaire. Eighteen of the patients were males and three were females. The operations were made under a tourniquet and brachial plexus block or general anaesthesia. The mean age in the non-aggressive group age was 63 years (range from 43 years to 81 years). No females were included in this group. In the aggressive group the mean age was 55 years (range from 37 years to 75 years). We determined the differences in cellularity, collagen, Ki-67, MSA, alpha-SMA and tenascin between the specimens.

The specimens were stained with haematoxylin-eosinophil. The cellularity of nodules and the amount of collagen were estimated from He stainings. Cellularity and the amount of collagen were categorized into three groups: + = minor, ++ = moderate and +++ = major.

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Immunohistochemical stainings were performed on 3-m-thick, formalin-fixed, paraffin-embedded tissue sections. The tissue sections were prepared for antibody application by deparaffination and rehydration. For alpha-SMA antibody application, antigenity was retrieved by heating in a microwave oven in Sodium Citrate Buffer (0.01M, pH 6.0) for ten minutes, and for Ki-67 application, the specimens were kept in Tris/EDTA buffer (0.01 M, pH 9.0) for fifteen minutes. For Factor XIIIa application, the specimens were incubated in trypsin for 20 min at room temperature, and for MSA and tenascin antibody, they were incubated in pepsin (0.4 %) for 30 min at 37°C. The ultravision kit was used for anti-alpha-SMA (1A4 clone, DAKO Cytomation, 1:400 dilution, 60 minutes), anti-MSA (HHF35 clone, DAKO, 1:200 dilution, 60 minutes), anti-factor XIIIa (polyclonal, Biocare Medical, 1:500, 60 minutes incubation) and

tenascin (polyclonal, from Ismo Virtanen's laboratory, 1:200 dilution, 60 minutes incubation). Non-biotinylated Envision kit was used for Ki-67 (7B11 clone, Zymed, 1:300 dilution, 30 minutes incubation). For counterstaining, haematoxylin was used, and the primary antibody was replaced by PBS in the negative controls (Fig. 1). The immunohistochemical stainings were divided into three categories according to the intensity of the positive reaction classified as – = no positive reaction, + = weak and ++ = moderate and +++ = strong positive reaction.

RESULTS

HISTOLOGY

Dupuytren's contracture tissue is nodular, and the cellularity of the nodules was evaluated. Cellularity was scant in four aggressive cases, moderate in five cases and abundant in two cases. In one of the non-aggressive cases cellularity did not differ from normal, in eight cases it was scant, in one case moderate and in one case abundant. The amount of collagen was small in four aggressive cases, moderate in seven cases and large in none of the cases. The amount of collagen was small in two non-aggressive cases, moderate in six cases and large in two cases (Fig. 2). Neither macrophages nor lymphocytes were found in the specimen of Dupuytren's contracture.

There were no cells, nor any other findings to be found in the control specimens.

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The spindle-shaped cells of Dupuytren's tissue were alpha-SMA-positive. Alpha smooth muscle actin is a reliable marker of the myofibroblastic cellular phenotype. Reactions were weak in two aggressive cases and moderate in nine cases, and there were no strongly positive cases. The reaction was negative in three non-aggressive cases, weak in four cases and moderate in three cases, and there were no strong reactions.

MSA (= muscle-specific antigen) positivity was localised in the cellular areas. The reaction was weak in eight aggressive cases and moderate in three, and there were no strong reactions. In the non-aggressive cases, the reaction was negative in one case, weak in nine and moderate or strong in none.

Ki-67 is a monoclonal antibody, which is a widely applied cell cycle marker for proliferation. The staining showed scattered cells in cellular areas. The reaction was weak in seven aggressive cases. It was moderate in four cases and strong in none of the cases. The reaction was negative in four non-aggressive cases, weak in four and moderate in two, and there were no strong reactions.

Tenascin is an extracellular matrix protein. Its expression was located in the cellular areas. It was weak in five and moderate in six aggressive cases. There was no strong reaction. Expression was weak in nine and moderate in one non-aggressive case. There was no strong reaction.

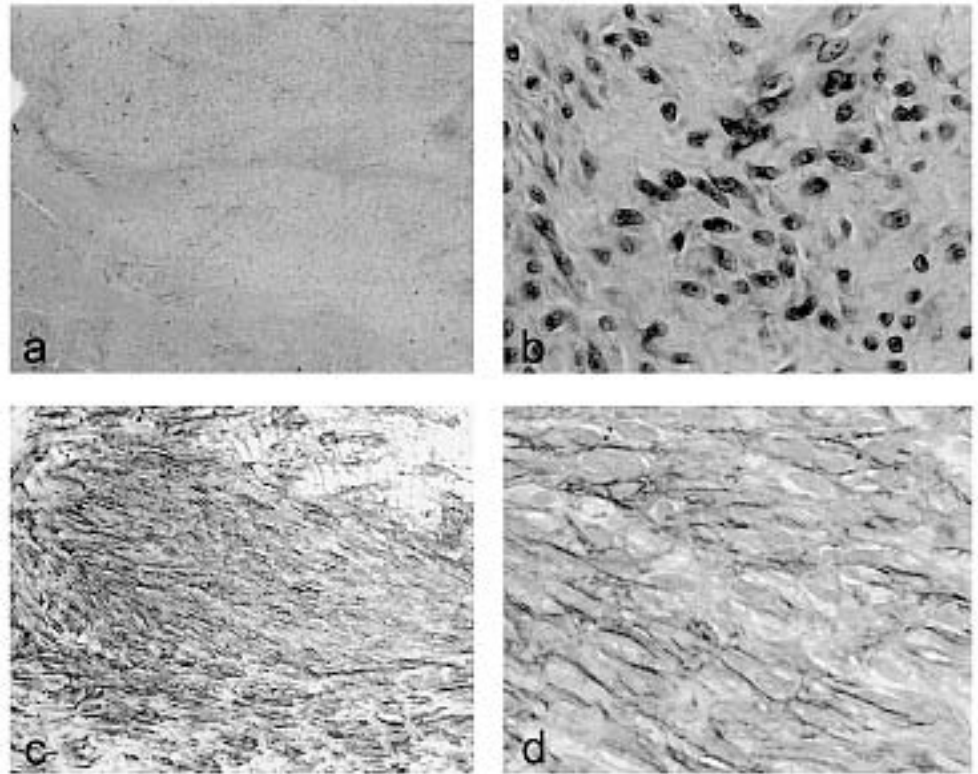The stellate dendrocytic cells in the areas surrounding the nodules were positive for factor XIIIa.

Fig. 1. a) Haematoxylin- eosino- fil staining shows the nodular structure of dupuytren's tissue (HE, original magnification × 20). b) He-staining showing the my- ofibroblasts in nodules (HE, orig- inal magnification × 400). c) and alfa SMA (original magnification × 100). d) Spindle shaped myofi- broblasts expresses anti-α-SMA proteins in Dupuytren's contrac- ture (alfa SMA, original magni- fication × 400).

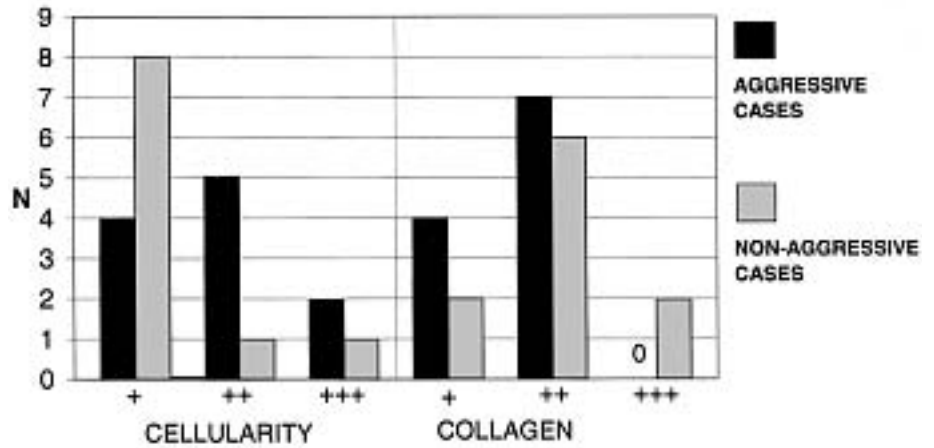## DISTRIBUTION OF COLLAGEN AND CELLULARITY

Fig. 2. N = number of cases
– cellularity means the amount of cells in highly cellular areas
– collagen means the amount and thickness of collagen bundles ac- cording to cellularity of the speci- men
– + low, ++ moderate and +++ high amount.

Dendrocyte is a factor XIIIa-positive cell, which has been found in, for example, some pathological con- ditions associated with fibrosis. The reaction was weak in four aggressive cases, moderate in seven and strong in none. There were eight weak and two mod- erate non-aggressive cases. There were no strong re- actions (Fig. 3).

DISCUSSION

The predisposition to develop Dupuytren's disease is associated with diabetes, alcoholism, cigarette smoking, epilepsy, gender, race, age, genetic back- ground, HIV and long-term manual labour (8). The disease is most severe and apparently affects mainly

Immunoreactivity and cellularity of Dupuytren's contracture

staining	Aggressive cases (N=11)				Non-aggressive cases (N=10)			
	−	+	++	+++	−	+	++	+++
cellularity	0	4	5	2	0	8	1	1
Alfa-SMA	0	2	9	0	3	4	3	0
MSA	0	8	3	0	1	9	0	0
Ki-67	0	7	4	0	4	4	2	0
Factor XIIIa	0	4	7	0	0	8	2	0
Tenascin	0	5	6	0	0	9	1	0

Fig. 3. –no reaction, + small cellularity/weak expression of protein, ++ moderate cellularity/strong protein expression, +++ high cellularity.

the Caucasian and Scandinavian races, especially older males (8, 9, 10, 11).

Based on its predominant histological appearance, Dupuytren's disease is divided into three phases: proliferative, involutional and residual. During the proliferative phase, fibroblasts increase in number and transform into myofibroblasts, apparently under the influence of different factors (12). A myofibroblast is a cell that exhibits features of both smooth muscle cells and fibroblasts. Therefore, it has the ability to contract (13, 14).

The involutional phase is characterized by a loss of cellularity known as programmed cell death (apoptosis). Myofibroblasts decrease in number and tend to align parallel along lines of stress (1, 15). The residual phase lacks myofibroblasts. Mature fibroblasts and increased amounts of collagen are observed. Histologically, the tissue resembles the granulation tissue seen in wounds. The relative type III collagen content increases (16, 17, 18, 19, 20). Clinically, Dupuytren's nodules eventually form cords of mature scar tissue, contracting the fingers (21).

In Dupuytren's disease, the nodular tissue contains inflammatory cells, mostly macrophages and lymphocytes. There is a correlation between myofibroblasts and inflammatory cells (21, 22). Surprisingly, we did not here find either macrophages or lymphocytes in Dupuytren's contracture lesions. Immunohistochemical stainings for macrophages and lymphocytes were negative. Yet, it has been suggested that inflammatory cells have a great influence on developing the disease. This contrast with the present findings and some earlier studies warrants further research.

Macrophages release growth factors, which have a crucial role in the disease. Mainly TGF (transforming growth factor) and cytokines induce myofibroblast proliferation and transformation from fibroblasts while stimulating the production of collagen (21, 23, 24, 25). It is also assumed that matrix metalloproteinases (MMPs) and their inhibitors (TIMP) are involved in the development of Dupuytren's disease (26, 27, 28).

According to research concerning inoperable gastric carcinoma, patients who received Marimastat as a metalloproteinase inhibitor developed Dupuytren's contracture (28).

Local microvascular ischemia due to various reasons causing microvessel stenosis induces oxygen-free radicals, mostly endothelial xanthine oxidase-catalyzed reactions. Heavy alcohol consumption also generates free radicals. Furthermore, this process stimulates fibroblast proliferation (29, 30). Because Dupuytren's contracture is characterized by male predominance, it has been assumed that there is a hormonal effect involved. It has been presented that the affected palmar fascia in Dupuytren's disease expresses androgen receptors considerably more abundantly than normal palmar fascia (31).

In the light of the present findings, it seems that high cellularity predicts aggressiveness and recurrence of the disease. In the course of maturing disease, cellularity tends to decrease eventually, while the type III collagen ratio tends to rise. Especially myofibroblasts decrease and are replaced by fibroblasts in the residual phase. In our study, the patients with palmar contracture were operated in accordance with the normal practice of our hospital: the indication for surgery is a large annoying nodulus or cord or/and flexion contracture of 20–30 degrees of the MP joint(s) of the hand. Clinically, all patients were entitled to an operation, and the purpose of the study was to find a factor that would possibly predict the nature of the disease in normal practice. It could be assumed that, if Dupuytren's contracture is aggressive, the proliferative process is stronger and the maturation more time-consuming. Therefore, rich cellularity could predict recurrence of the disease. Vice versa, the collagen ratio remains low in aggressive cases.

This finding was confirmed tentatively by the fact that alpha-SMA, which is a marker of myofibroblasts, was also represented more frequently in aggressive specimens. The presence of myofibroblasts is considered to indicate the activity of the disease (22). There were, however, three non-aggressive cases where the disease was moderate, and there were three weak reactions in normal palmar fascia from control patients. Furthermore, is it possible that these control patients

may develop the disease in the future? As a marker of proliferation, Ki-67 also showed a tendency to be more widely represented in aggressive cases.

On the contrary, the collagen and MSA reactions did not differ significantly between the aggressive and non-aggressive cases. Tenascin and Factor XIIIa showed some difference between the aggressive and non-aggressive cases, with moderate expression in aggressive cases and weak reactions in non-aggressive cases.

The cellularity of specimens of Dupuytren's contracture as well as alpha-SMA as a marker of myofibroblasts might be used in clinical practice to predict the nature of the palmar disease.

Compared to some previous studies about Dupuytren's contracture and the results that suggest no difference or a correlation between the expression of connective tissue activation markers and recurrence, we were able to point out some differences. However, we did not investigate the substances covered in these previous studies (32). Because of the limited number of patients in our study, possible confounding factors, such as lifestyle or related diseases, were disregarded concerning the recurrence of Dupuytren's disease (32). In our study, the main concern was immunohistology.

Further studies are being carried out by our group to analyze the proteins of ECM and the molecular mechanisms underlying this disease.

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