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Origional Research Article

Biochemical Diagnosis of Dupuytren's Disease

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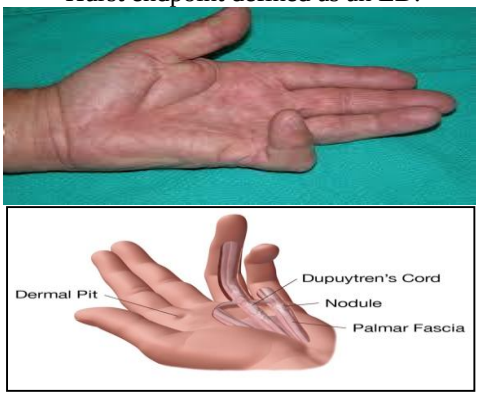
Abstract: Dupuytren's contracture is a chronic, progressive disease that involves abnormal thickening and tightening of the normally elastic tissue beneath the skin of the palm and fingers. This tissue is called fascia. The fascia contains strands of fibers, like cords, that run from the palm upward into the fingers. In Dupuytren's contracture, these cords tighten, or contract, causing the fingers to curl forward. In severe cases, it can lead to crippling hand deformities. As the disease progresses, rigid cords are formed in the palm of the hand causing flexion contractures primarily of the metacarpophalangeal (MCP) and proximal interphalangeal (PIP) joints. When compared to age-matched control aponeurosis, lesions of Dupuytren's disease contain higher contents of water, collagen and chondroitin-sulphate, as well as increased proportions of soluble collagens and of reducible cross-links; these indicate synthesis of new collagen. The lesions show also increased amounts of type III collagen and an increased hydroxylation and glycosylation of the reducible cross-links. All these parameters are characteristic of granulation and scar tissues. Type III collagen was located by means of immunofluorescence on thin argyrophilic fibres and also within the large fibre bundles which appeared to be disrupted into microbundles. The increase of type III collagen and the presence of myofibroblasts in the apparently unaffected aponeurosis show that the disease is widespread and suggest that it is initiated within the aponeurosis and propagated by the cells migrating along the collagen bundles Treatment of DC remains a challenge due to frequent recurrence. Surgery is the mainstay treatment, but is not without complications, and is associated with high recurrence rates. Minimal invasive procedures such as percutaneous needle fasciotomy (PNF) and Clostridium histolyticum collagenase. (Xiapex in Europe/Xiaflex in the US) have been increasingly used in recent years. However, both have recurrence rates that exceed those of surgery. Nevertheless, studies have shown that minimal invasive procedures are often preferred by the patient and the surgeon alike owing to the simplicity of the procedure and the patient's early return to activities of daily living after the procedure.

Keywords: Abnormal thickening, cords tighten, metacarpophalangeal, percutaneous needle fasciotomy, Minimal invasive.

INTRODUCTION

In 2009, *C. histolyticum* collagenase was introduced into clinical practice, with promising efficacy results compared with placebo (Urmaliya, V. B. et al 2010). In 2013, *C. histolyticum* collagenase treatment was introduced. The aim of this study was to evaluate the effectiveness of a single-shot *C. histolyticum* collagenase treatment at a regional hospital in non-selected patients with a least one year of follow-up (FU). The following outcomes were evaluated:

- Overall improvements MCP and PIP joints;
- Contraction recurrence – defined as extension deficit (ED) above 20°
- Hurst endpoint defined as an ED.



DUPUYTREN'S CONTRACTURE (A hand demonstrating Du)

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The first symptom for many patients is one or more lumps (nodules) under the skin in the palm of the hand. The lump may feel tender and sore at first, but this discomfort eventually goes away. The nodules cause tough bands of tissue to form under the skin in the palm. These inflexible bands cause the fingers to bend, or "curl," forward toward the wrist. As this curling gets worse, it becomes difficult, if not impossible, to straighten the fingers. The pinkie and ring fingers are most often affected, appearing clenched. Both hands are usually involved, although one may have worse symptoms than the other. People with Dupuytren's contracture often have a hard time picking up large objects, or placing their hands into their pockets, something you might do on an everyday basis to retrieve coins, cash, or your ID card. If you have this condition, you may also find it difficult to place your hand flat on the table, wear gloves, or shake hands, among other things. The cause of Dupuytren's contracture, also called Dupuytren's disease, is unknown, but certain biochemical factors that affect the palm's connective tissue may be involved. Injuries and overuse of the hand do not play a role. Tendons are not affected. However, certain things may make you more likely to develop Dupuytren's contracture. They include: Drinking a lot of alcohol, although most people with the disease do not have alcoholism, Diabetes, Seizures, such as those seen in people with epilepsy, Smoking, The condition usually runs in families, which means it is inherited. Chances of developing it increase as one gets older.

OBSERVATION

Diagnosis of Dupuytren's contracture typically involves feeling the palm areas to check for nodules and recording how many nodules are found, grasp items with hands, pinch items with fingers, Measure the feeling in thumbs and fingers, range of motion in fingers, to see if one can straighten them all the way, These exams and tests will be repeated over time to determine if the condition is getting worse. Dupuytren's contracture is a deforming, fibrotic condition of the palmar fascia which has confounded clinicians and scientists since the early descriptions by Guillaume Dupuytren in 1831. It predominantly affects elderly, males, has a hereditary predisposition and has strong associations with diabetes, alcohol consumption, cigarette smoking and HIV infection. The major morphological features are an increase in fibroblasts, particularly around narrowed fibroblasts; a finding consistent with localized ischemia. During ischemia, adenosine triphosphate (ATP) is converted to hypoxanthine and xanthine, and endothelial xanthine dehydrogenase to xanthine oxidase (alcohol also mediates this change, a finding of particular relevance given the association of Dupuytren's contracture with alcohol intake). Xanthine oxidase catalyses the oxidation of hypoxanthine to xanthine and uric acid with the release of superoxide free radicals (O2-), hydrogen peroxide (H2O2) and hydroxyl radicals (OH.). These free radicals are highly reactive, with half-lives in the order of milliseconds and are toxic in high concentrations. A potential for free radical generation in Dupuytren's contracture was elicited by finding a sixfold increase in hypoxanthine concentrations in Dupuytren's contracture compared with control palmar fascia. In vitro studies affirmed the toxic effects of oxygen free radicals to Dupuytren's contracture fibroblasts, but also showed that, at lower concentrations (concentrations similar to those likely to occur in Dupuytren's contracture), free radicals had a stimulatory effect on fibroblast proliferation. Cultured fibroblasts were found to release their own O2-. These endogenously released free radicals were also found to be important in fibroblast proliferation. The collagen changes of Dupuytren's contracture were examined. The results established that fibroblast origin was unimportant, but that inhibition of type I collagen production at high fibroblast density accounted for the increase in type III/I collagen ratios observed by previous investigators. These biochemical and morphological observations throw new light on Dupuytren's contracture. They suggest that age, genetic and environmental factors may contribute to micro vessel narrowing with consequent localized ischemia and free radical generation. Endothelial xanthine oxidase derived free radicals may both damage the surrounding stroma and stimulate fibroblasts to proliferate. Proliferating fibroblasts lay down and contract collagen in lines of stress. Progressive fibroblast proliferation and deposition of collagen is likely to encourage further microvessel narrowing with a positive feedback effect consistent with the progressive nature of the condition. Biomechanical Analysis A tensile testing device was designed and built in our laboratory.[16] The apparatus consists of a spindle driven by a gear box motor, a load cell with a maximum load of 20 N and a resolution of 10 mN, a potentiometer to measure the deformation, specimen clamps to which abrasive paper (400 grit) was glued, and a bath containing phosphate-buffered saline. The temperature of the bath was controlled by a microprocessor-based thermostat. For tensile tests the temperature was kept constant at 258C. The reference length, ie, maximum length of a specimen at zero loads, was about 20 mm. We performed uniaxial strain-controlled tests. Strain values were calculated as deformation divided by reference length, i.e., relative deformation. Load deformation curves were continuously recorded and converted into load strain curves. The following test protocol was used. The specimens from the PA were strained at a rate of 5.0% per minute until a level of 2.5% was achieved and then kept constant for the relaxation test. During the relaxation phase, the load decreases following an exponential law. The time constant (t) is defined as the inverse of the initial slope. It is the tangent of the stress-relaxation graph at the beginning of the test. For viscoelastic materials, the load is composed of elastic and a viscous component. For the determination of the maximum Young modulus in DD samples and controls (carpal tunnel syndrome), the samples were loaded at a rate of 5.0% per minute until a strain level of 10% was achieved. Maximum

Young modulus is defined as the tangent in the steepest portion of the load strain curve. This tangent was normalized for the collagen content per unit length, representing a measure for the load-bearing quality of the cross-sectional area of a specimen. The relaxation experiments with rat skins were performed at 20% strain level, which was adjusted at a strain rate of 50% per minute.

Table 1 Collagen Biosynthesis In Differently Involved Dupuytren's Disease Tissues

Tissues No. DNA, mg/g of Dry Weight Collagen Biosynthesis, % Collagen Biosynthesis, dpm/mg of DNA 3 1022 Noncollagen Protein Biosynthesis, dpm/mg of DNA 3 1023					
Tissues	No.	DNAmg/g of Dry Weight Collagen Biosynthesis	% Collagen Biosynthesis	Collagen Biosynthesis, dpm/mg of DNA 3 1022	Non collagen protein dpm/mg of DNA 3 1023
NPA	5	1.10 6 ±0.12	1.24 ±0.7	0.40±0.3	0.590±0.123
ANPA	7	1.77± 0.43†	1.87 6±0.9	0.78±0.3	0.759±298
TFB	12	2.33± 0.27‡	2.64±87	2.15±0.65	1.462±0.765
ACP CB	6	5.36 6 ±1.98§	3.70±	3.71±1.029	1.788±0.651

Results are expressed as mean ± SD. dpm indicates disintegrations per minute; NPA, normal palmar aponeurosis; ANPA, apparently normal palmar aponeurosis; TFB, thickened fiber bundles; ACP-CB, active cell proliferation–contracture bands; and RS-CB, residual stage–contracture bands. P <, .05. P<.01. and P < .001 vs NPA.

TABLE 2 Biophysical and Biochemical Properties of Rat Skins from Animals of Different Ages*

Table 3. Biophysical and Biochemical Properties of Rat Skins From Animals of Different Ages*				
Age TD	$T_m{}^{0C}$	T_m	Viscous Fraction, %	
Newborn	57.0 6 ±1.0	61.0±0.6	70.6±2.5	17.3±3.4
2 months	59.5±0.3	62.0±0.3	52.4±6.2	12.5±6.2
2 months 6	61.5±0.3	65.5±0.3	33.3±1.5	8.97±1.6
18 months	61.8 ± 0.4	64.9±0.5	27.9±2.5	8.7±2.3

DISCUSSION

Dupuytren's contracture is a deforming, fibrotic condition of the palmar fascia which has confounded clinicians and scientists since the early descriptions by Guillaume Dupuytren's in 1831. It predominantly affects elderly, males who have hereditary predisposition and has strong associations with diabetes, alcohol consumption, cigarette smoking and HIV infection. The major morphological features are an increase in fibroblasts, particularly around narrowed fibroblasts; a finding consistent with localized ischemia. During ischemia, adenosine triphosphate (ATP) is converted to hypoxanthine and xanthine, and endothelial xanthine dehydrogenase to xanthine oxidase (alcohol also mediates this change, a finding of particular relevance given the association of Dupuytren's contracture with alcohol intake). Xanthine oxidase catalyses the oxidation of hypoxanthine to xanthine and uric acid with the release of superoxide free radicals (O2-), hydrogen peroxide (H2O2) and hydroxyl radicals (OH.). These free radicals are highly reactive, with half-lives in the order of milliseconds and are toxic in high concentrations. A potential for free radical generation in Dupuytren's contracture was elicited by finding a sixfold increase in hypoxanthine concentrations in Dupuytren's contracture compared with control palmar fascia. In vitro studies affirmed the toxic effects of oxygen free radicals to Dupuytren's contracture fibroblasts, but also showed that, at lower concentrations (concentrations similar to those likely to occur in Dupuytren's contracture), free radicals had a stimulatory effect on fibroblast proliferation. Cultured fibroblasts were found to release their own O2-. These endogenously released free radicals were also found to be important in fibroblast proliferation. The collagen changes of Dupuytren's contracture were examined. The results established that fibroblast origin was unimportant, but that inhibition of type I collagen production at high fibroblast density accounted for the increase in type III/I collagen ratios observed by previous investigators. These biochemical and morphological observations throw new light on Dupuytren's contracture. They suggest that age, genetic and environmental factors may contribute to micro vessel narrowing with consequent localized ischemia and free radical generation. Endothelial xanthine oxidase derived free radicals may both damage the surrounding stroma and stimulate fibroblasts to proliferate. Proliferating fibroblasts lay down and contract collagen in lines of stress. Progressive fibroblast proliferation and deposition of collagen is likely to encourage further microvessel narrowing with a positive feedback effect consistent with the progressive nature of the condition.

TABLE 3. Biomechanical Properties and Type III Collagen Content of Tendons, Control Palmar Aponeurosis, and Differently Involved Palmar Aponeurosis From Patients With Dupuytren's Disease

Tissue No. Type III Collagen Proportion	Number	Type III Collagen Proportion, %	Viscous Fraction%	% Young Modulus	Time constant
NT	7	1.6 6 ±0.4	1.9 ± 0.3	3100 ±184	8.6 ±+ 1.8
NPA	8	2.8 ±0.2	5.1 ± 1.8	1500 ±330	12.2 ± 2.1
ANPA	12	3.7 6+ 0.8	10.6 + 1.8	1200 ± 280	28.1 ± 2.8
TFB	17	15.8 ±2.9	13.4 ±6 1.8	1100 ±70	49.1 ±3.4
ACP-CB	11	14.9 ±6 2	15.7 + 1.9	1350 ± 190	88.0 6±13.5
RS-CB	20	19.7± 6 2	24.2 ± 1.7	1500 ±210	RS-CB

BIOCHEMICAL ANALYSIS

A tensile testing device was designed and built in our laboratory.16 The apparatus consists of a spindle driven by a gear box motor, a load cell with a maximum load of 20 N and a resolution of 10 mN, a potentiometer to measure the deformation, specimen clamps to which abrasive paper (400 grit) was glued, and a bath containing phosphate-buffered saline. The temperature of the bath was controlled by a microprocessor-based thermostat. For tensile tests the temperature was kept constant at 258C. The reference length, i.e., maximum length of a specimen at zero load, was about 20 mm. We performed uniaxial strain-controlled tests. Strain values were calculated as deformation divided by reference length, ie, relative deformation. Load deformation curves were continuously recorded and converted into load strain curves. The following test protocol was used. The specimens from the PA were strained at a rate of 5.0% per minute until a level of 2.5% was achieved and then kept constant for the relaxation test. During the relaxation phase, the load decreases following an exponential law. The time constant t, which is defined as the inverse of the initial slope, i.e., the tangent of the stress-relaxation graph at the beginning of the test. For viscoelastic materials, the load is composed of elastic and a viscous component. The elastic fraction, ie, final (equilibrium) load divided by initial load, represents a dimension-free parameter. The viscous fraction is given as 1 minus the elastic fraction. For the determination of the maximum Young modulus in DD samples and controls (carpal tunnel syndrome), the samples were loaded at a rate of 5.0% per minute until a strain level of 10% was achieved. Maximum Young modulus is defined as the tangent in the steepest portion of the load strain curve. This tangent was normalized for the collagen content per unit length, representing a measure for the load-bearing quality of the cross-sectional area of a specimen. The relaxation experiments with rat skins were performed at 20% strain level, which was adjusted at a strain rate of 50% per minute. The skins were strained in the direction of the ''body axis'' of the animals.

Table 4 Relationship between Clinical Classification and Type III Collagen Proportion

Table 5. Relationship Between Clinical Classification and Type III Collagen Proportion*		
Dupuytren's Disease Stage No.	No.	Type III Collagen, %
0	9	4.7 ± 0.9
I	11	9.0± + 1.7
II	12	14.7 6± 2.8
III	20	18.5 ± 4.3
IV	12	22.2 ± 5.1

SUMMARY AND CNCLUSION

As yet, the potential modifications of biophysical properties by type III collagen and its impact on clinical parameters, such as the degree of contracture, have not been evaluated in DD. Similar interrelations were observed between type III collagen content and biophysical parameters as in rat skin (viscous fraction of stress, Tm, TD), with the exception of the Young modulus and the time constant of stress relaxation (t), which increased with advancing contracture, i.e., in the direction of increasing type III collagen content. This divergence may be explained by the massive structural changes encountered when comparing the different stages of DD from ANPA to CB.Furthermore, the Young modulus is a clear-cut function of the number and nature of crosslinks41 and less dependent on the collagen type distribution. In DD, the redistribution of type III collagen fibers is closely associated with a relative increase in type III collagen.3 The concomitant structural changes that lead to contracture might even result from the altered collagen type spectrum. Thus, in our hands, type III collagen content in DD tissue correlated closely with clinical stages of contracture (evaluated for each finger involved) as defined by Tubiana.21 Furthermore, type III collagen proportion increases parallel to increasing involvement of the tissue, as defined by macroscopic clinical appearance supported by histologic inspection.

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