HAND FASCIAE INNERVATION: THE PALMAR APONEUROSIS

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ABSTRACT

Introduction: There are few data in the scientific literature about the innervation of fasciae of the hand. The present study first elucidates the density and location of nervous structures in the palmar aponeurosis and, for comparison, in the flexor retinaculum (both can be considered specializations of the deep fascia of the upper limbs). Second, it compares non-pathological with pathological palmar aponeurosis.

Materials and Methods: Samples of non-pathological fascia were taken from the flexor retinaculum and palmar aponeurosis of 16 upper limbs of unembalmed cadavers. Samples of pathological palmar aponeurosis were taken from seven patients with Dupuytren's disease. All samples were stained immunohistochemically with anti-S100 and anti-tubulin antibodies, and analyzed quantitatively and qualitatively by microscopy.

Results: The palmar aponeurosis showed higher median density than the retinacula of free nerve endings (22 and 20 elements/cm^2, respectively), Pacinian corpuscles (2 and 0 elements/cm^2) and Golgi-Mazzoni corpuscles (1.0 and 0.5 element/cm^2). Some corpuscles were located at the intersections of the fibers in the three directions. Free nerve endings were denser in pathological palmar aponeurosis (38 elements/cm^2).

Conclusions: The results indicate that the palmar aponeurosis is central to proprioception in the hand and that surgeons should therefore avoid injuring it. The higher density of free nerve endings in pathological samples indicates that the nervous structures are implicated in the amplified fibrosis of Dupuytren's disease.

Keywords: Dupuytren's disease, fascia, proprioception, hand, mechanoreceptors, nerve endings

INTRODUCTION

The scientific literature contains much detail about the macroscopic and molecular anatomy of the palmar aponeurosis, thanks to studies of Dupuytren's disease, but to the best of our knowledge only one study has focused on the innervation of hand fasciae. Even this study reported only the free nerve endings in the palmar aponeurosis, without analyzing encapsulated receptors (Schubert et al., 2006). Innervation of fasciae has also been studied in other body regions: it has been demonstrated that the human thoracolumbar fascia is well innervated (Hirsch et al., 1963; Yahia et al., 1992), but there are variations in the degree of innervation in chronic back pain (Bednar et al., 1995), indicating that fasciae could be involved in proprioception and nociception. Pacini and Ruffini corpuscles are also found in fasciae of the forearm (Stecco et al., 2007) and in the plantar

fascia (Stecco et al., 2013). In murine models it has been shown that the thoracolumbar fasciae are innervated (Corey et al., 2011; Tesarz et al., 2011)I; the number of nociceptive receptors increases if these fasciae are inflamed, which could explain the pain (Hoheisel et al., 2015; Mense and Hoheisel, 2016). Similar studies have been conducted on crural fascia in the rat (Taguchi et al., 2013) and on the connective structures of the biceps brachii muscle in the horse (Palmieri et al., 1986).

The purpose of this study was quantitative and qualitative microscopic analysis of the innervation of the palmar aponeurosis, to determine whether it is involved in proprioception and nociception. Therefore, in addition to normal material from cadavers, we examined pathological samples, particularly from patients with Dupuytren's disease, who present with typical fascial fibrosis of the hand characterized by shortening, thickening, and fibrosis of the palmar fascia, causing flexive deformity of a finger (Flatt, 2001; Saar and Grothaus, 2000).

MATERIALS AND METHODS

This cross-sectional study was approved by the Ethical Review Board of our hospital. We followed its ethical regulations on research conducted on human tissues.

Samples of non-pathological fascia were obtained from 16 upper limbs of unembalmed cadavers that had been frozen and subsequently thawed (six females, ten males, range 60-92 years, mean age 78±11), thanks to the Body Donation Program of the Institute of Anatomy, University of Padua, Italy (Porzionato et al., 2012). Clinical data and objective examination of the bodies revealed no signs of upper limb surgery. To obtain non-pathological fascia samples a median longitudinal incision was made along the anterior skin of the forearm, followed by two transverse incisions in the elbow region and at the wrist. The skin was raised medially and laterally to expose the

antebrachial fascia (subcutaneous tissue and superficial fascia were left adhering to the overlying skin). The palm was dissected with two longitudinal incisions along the ulnar and radial borders and a transverse one along the palmar digital crease; the palmar skin was then raised, exposing the superficial layer of the palmar aponeurosis. The tendon of the palmaris longus muscle was isolated and cut. The vertical septa of Legueu and Juvara were cut to raise the superficial layer of the palmar aponeurosis. Samples from the flexor retinaculum, along the median line of the wrist, and the palmar aponeurosis (three samples at three different levels) along the ring finger ray of the hand, were taken from each cadaver. A previous study had revealed that the flexor retinaculum is innervated (Stecco et al., 2007), so samples of this fascial structure were also taken to compare them with the palmar aponeurosis. Full-thickness samples of the palmar aponeurosis were obtained from the skin to the vertical septa of Legueu and Juvara of four cadavers.

Samples of pathological palmar aponeurosis were taken along the ring finger ray of the hand from seven patients (six males and one female, age range 44-60 y, mean age 52±6), all affected by Dupuytren's disease, who had undergone aponeurectomy at our Clinic for Plastic and Reconstructive Surgery.

All samples were immediately mounted on cardboard, fixed in 10% formalin, embedded in paraffin, and then cut into sections 5μm thick and 1 cm^2 square. The sections were stained traditionally (hematoxylin-eosin, Azan-Mallory) and then immunohistochemically using anti-S100 (Polyclonal Rabbit Anti-S100, 1:5000, DAKO, Denmark) and anti-tubulin (Polyclonal Rabbit Anti-Tubulin β-3, 1:5000, BioLegend, San Diego, USA) antibodies (Menezes and Luskin, 1994; Kaemmer et al., 2010). All preparations were examined by optical microscopy (DM4500-B, Leica Microsystems, Wetzlar, Germany) and digital images were obtained with a camera connected to the microscope (DFC320, Leica Microsystems, Wetzlar, Germany). Microscopic observations allowed at least 10 sections per sample (5μm thick, 1 cm^2 square) to be analyzed quantitatively and the

innervation of the fascial structures of the hand to be described qualitatively. Mean values of all sections from the same individual were calculated, followed by the median value for all individuals.

Qualitatively, these structures were described according to the Freeman and Wyke classification (Ruffini, Pacini and Golgi-Mazzoni corpuscles, and free nerve endings) (Yeo et al., 2016). Anti-S100-stained sections discriminated all types of encapsulated receptors and free nerve endings according to morphology (Table 1). Anti-tubulin-stained sections confirmed the presence of non-myelinated free nerve endings, since anti-S100 antibodies target Schwann cells.

	Anti-S100
Ruffini corpuscles	Clusters of 3-6 ramifications
Pacini corpuscles	Smaller (cluster of 2-4) circular or ovoid structures
Golgi-Mazzoni corpuscles	stretched and irregularly shaped
Free nerve endings	Schwann cells

Table 1: Discrimination of encapsulated receptors (Ruffini, Pacini and Golgi-Mazzoni corpuscles) and free nerve endings by immunohistochemical examination: anti S-100 antibody allowed the three different encapsulated receptors to be stained and identified by morphology, and the Schwann cells were identified.

Quantitatively, the nervous structures of each type were counted in 1 cm^2 of fascia in each section (at least 10 sections per sample, considered approximately bidimensional) to determine their

density. The median density of each type of receptor in each fascial structure was then derived (as the number of nervous structures per cm^2 of fascia analyzed). Since most of these data were not normally distributed, descriptive statistics were expressed as median (Mdn) and interquartile range (IQR). Results were compared using a Mann-Whitney's two-tailed U test and values of $p< 0.05$were considered statistically significant.

RESULTS

Nerve elements were observed in all samples analyzed. Free nerve endings were the most frequent histological findings: they were visible only at high magnification (200x - 400x) and were ubiquitous among connective fibers throughout the fasciae examined (Fig.1). Pacini corpuscles among connective fibers appeared as circular or ovoid structures, smaller than the classical corpuscles seen in subcutaneous tissue, with nervous nuclei and a series of concentric connective laminae (Fig. 2). Golgi-Mazzoni corpuscles appeared as stretched, irregular nervous structures with multiple connective laminae surrounding the nerve fibers (Fig. 3). No Ruffini corpuscles were found in any of the samples.

No statistically significant difference in the density of free nerve endings between the flexor retinaculum (Mdn = 20.0, IQR = 16.5-26.8) and the palmar aponeurosis (Mdn = 22.0, IQR = 17.3-27.8) were revealed using the anti-S100 antibodies (U by Mann-Whitney test = 127.0, p = 0.97) (Fig. 4).

There was a statistically significant difference (U = 62.5, $p <0.01$) in the density of Pacini corpuscles between the flexor retinaculum (Mdn = 0.0, IQR = 0.0-0.8) and the palmar aponeurosis (Mdn = 2.0, IQR = 0.0 - 3.0); the density was higher in the latter (Fig. 5).

The palmar aponeurosis also showed a higher density of Golgi-Mazzoni corpuscles (Mdn = 1.0, IQR = 0.0-2.8) than the flexor retinaculum (Mdn = 0.5, IQR = 0.0-1.0), though the difference was not statistically significant (U = 83.0, p = 0.07) (Fig. 6).

Anti-tubulin immunohistochemical staining confirmed the presence of free nerve endings in all sections of the flexor retinaculum (Mdn = 46.5, IQR = 37.0-54.5) and palmar aponeurosis (Mdn = 38.0, IQR = 30.5-43.5) studied (data not shown).

The macro-sections revealed some Pacinian corpuscles at the borders of fascial structures, at the intersections of longitudinal, transverse and vertical fibers (Fig. 7).

The density of free nerve endings in the palmar aponeurosis from patients with Dupuytren's disease (U = 3.5, $p < 0.01$) was higher in the pathological fasciae (Mdn = 38.0, IQR = 32.0-46.0) than the normal fasciae (Mdn = 22.0, IQR = 17.3-27.8) (Fig. 8).

DISCUSSION

This study shows that the palmar aponeurosis is innervated and has the typical features of a proprioceptive organ. It has high densities of Pacini corpuscles (median 2 elements/cm^2, compared with 0 elements/cm^2 in the flexor retinaculum) and Golgi-Mazzoni corpuscles (median 1.0 per cm^2 compared with 0.5 per cm^2 in the flexor retinaculum; a 100% difference). In contrast, the densities of free nerve endings differed by only 10%: the median values were 22 and 20 per cm^2 for the palmar aponeurosis and flexor retinaculum, respectively. These results are important when considered in terms of the specific functions of mechanoreceptors: Pacini corpuscles adapt rapidly to sudden changes in stress; Golgi-Mazzoni's corpuscles adapt slowly and are activated at extreme ranges of movements and when considerable stress is generated (Cavalcante et al., 2004). These findings are especially interesting in relation to the delicate motor functions of the hand, which is

capable of fine, precise movements. The complex tridimensionality of its fascial structures can perceive variations of tension in all directions, thus collaborating in proprioception. The Pacinian corpuscles at the borders of fasciae and intersections of fibers could be the main activators of this function because their strategic location enables them to detect stretches in all directions, integrating signals from the longitudinal, transverse and vertical axes. Another peculiarity of the palmar aponeurosis is its close connection to superficial integumentary structures and deep musculoskeletal structures by vertical fibers, i.e. the retinacula cutis and vertical septa of Legueu and Juvara (Stecco, 2015). Thus, proprioception from fascia can be integrated with exteroception from dermal receptors and proprioception from neuromuscular spindles and Golgi tendon organs (Stecco, 2015). This anatomical substrate explains the peculiar ability of the hand to adapt perfectly to the shapes of objects and thus achieve effective grip. The flexor retinaculum appears to have a lower density of encapsulated receptors. However, it is a complex structure with various layers (Stecco et al., 2010), so it is similar to the thoracolumbar fascia, which has different degrees of innervations to the different layers (Mense and Hoheisel, 2016). Therefore, as our results could have been affected by the small sample sizes, further analysis of the histology of the fascial planes of this region appears necessary.

The anti-tubulin antibodies revealed more elements than did the anti-S100 antibodies. This is because of the nature of free nerve endings: their terminal parts are not wrapped in myelin, so they are not always visible with anti-S100 immunohistochemical staining in microscopic sections (Standring, 2015).

Although encapsulated receptors function as proprioceptors, free nerve endings can provide proprioceptive or nociceptive stimuli and are related to the transmission of pain (Hoheisel et al., 2015; Standring, 2015). Concerning nociception, this analysis confirms the previous study by Schubert et al. (2006), which showed there were more receptors in fascia affected by Dupuytren's

disease, considered an inflammatory disorder (Baird et al., 1993; Meek et al., 1999; Qureshi et al., 2001). In some similar connective tissue diseases such as Achilles tendinosis (Schubert et al., 2005), patellar tendinopathy (Schwartz et al., 2015; Fu et al., 2002) and thoracolumbar inflammation (Bednar et al., 1995; Hoheisel et al., 2015; Mense and Hoheisel, 2016), substance P-positive nerve fibers have been seen to sprout: substance P is a neurotransmitter involved in both fibroblast stimulation (Lai et al., 2003) and nociception (Mashaghi et al., 2016). The effect could be attributable to increased secretion of nerve growth factor (Lubahn et al., 2007), which acts on both nerve fibers and fibroblasts, causing differentiation into myofibroblasts. These results implicate pathological innervation in amplifying fibrosis and this could be the main cause of pain onset, indicating a connection between the fibrotic and painful forms of the disorder.

The concept that palmar fascia possesses sensory innervation implies that improper and inaccurate surgical procedures can impair proprioception and cause pain. It is therefore imperative to act accordingly on fascial structures, to minimize their involvement, and to reconstitute them as far as possible to a physiological state.

This work contributes to our understanding of the possible role of hand fasciae in proprioception and nociception. It has some limitations, which could be overcome in the future: first, the number of samples, especially from patients with Dupuytren's disease; second, the difference in mean age between cadavers and living patients, making comparisons less reliable. In fact, the quantitative results of this study could have been affected by the age of the cadavers examined, because aging could lead to alterations in the number of nerves in body tissues (Peters, 2002; Verdù et al., 2000). In addition, the methods used for sampling and preparation of sections did not allow us to establish with absolute confidence that multiple observed nervous structures, and particularly free nerve endings, were not in fact the same nervous fiber crossing the same section two or more times. Nevertheless, from a statistical viewpoint, such events could have affected all measurements, so

they have little influence on the comparisons. In any case, a more meticulous analysis, with the production and study of serial sections of the same sample, could yield more detailed and accurate results. Increasing the number of samples will make it possible to establish correlations between innervation and the symptoms of Dupuytren's disease and also connect density data with patient age and gender. Further studies examining the above-mentioned clinical implications will be necessary if these results are to be used in therapy for painful and inflammatory diseases of the hand and to improve surgery.

CONCLUSIONS

This work indicates that the palmar aponeurosis has the typical features of a proprioceptive organ and emphasizes the importance of fascial innervation in the perception of pain. This evidence indicates that surgical access to fascia should avoid injuring nerve terminations so pain is not caused. Lastly, the higher nerve density in Dupuytren's disease could explain the painful symptoms reported by some patients.

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LEGENDS

Figure 1. Free nerve endings observed after IHC staining with anti-S100 antibodies. (A) Flexor retinaculum, 200x. (B) Palmar aponeurosis, 200x.

Figure 2. Pacini corpuscles observed after IHC staining with anti-S100 antibodies. (A) Flexor retinaculum, 200x. (B) Palmar aponeurosis, 400x.

Figure 3. Golgi-Mazzoni corpuscles observed after IHC staining with anti-S100 antibodies. (A) Flexor retinaculum, 400x. (B) Palmar aponeurosis, 400x.

Figure 4. Boxplot showing the density of free nerve endings in fascial structures observed after IHC staining with anti-S100 antibodies.

Figure 5. Boxplot showing the density of Pacinian corpuscles in fascial structures observed after IHC staining with anti-S100 antibodies.

Figure 6. Boxplot showing the density of Golgi-Mazzoni corpuscles in fascial structures observed after IHC staining with anti-S100 antibodies.

Figure 7. Pacinian corpuscles at the intersection of longitudinal, transverse and vertical fibers of the palmar aponeurosis. (A) 12.5x magnification, Azan-Mallory staining. (B) 12.5x magnification, hematoxylin-eosin staining.

Figure 8. Boxplot showing the density of free nerve endings in normal and pathological palmar aponeurosis observed after IHC staining with anti-S100 antibodies.