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The role of mast cells in the progression of Dupuytren's contracture

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Abstract

Introduction World literature data indicate the involvement of mast cells in the pathogenesis of Dupuytren's disease. There is no information on the content of mast cells in the pathologically altered palmar fascia of patients depending on the severity of the contracture. **Purpose** To study changes in the number, location and degranulation activity of mast cells in the palmar aponeurosis with Dupuytren's contracture of varying severity. **Material and methods** Histopathological study of surgical material from 52 patients (49 men and 3 women) with Dupuytren's contracture (age range 35-78 years, mean age 59.12 ± 1.25 years) using histomorphometry of epoxy semi-thin sections stained with methylene blue – the main fuchsin. Patients were divided into two groups: group 1 with 1-2 (n = 16) and group 2 with 3-4 (n = 36) grades of the contracture. **Results** In group 2, the location of mast cells among contractilely altered collagen fibers of dense connective tissue was recorded more often than in group 1. In group 2, compared with group 1, the following significantly increased: the median number density of mast cells – by 33.79 % (p = 0.0508), the median area of mast cells – by 48.40 % (p = 0.0008) and the index mast cell degranulation – by 94.68 % (p = 0.00000001). **Discussion** Along with a change in the typical location of mast cells, objective evidence of an increase in their number, activation and degranulation in patients with severe contractures was obtained. **Conclusion** The obtained results indicate the role of mast cells in the progression of Dupuytren's contracture and expand the understanding of potential therapeutic targets.

Keywords: palmar fascial fibromatosis, Dupuytren's contracture, histology, morphometry, mast cells

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INTRODUCTION

Dupuytren's contracture (palmar fascial fibromatosis) is a fibroproliferative disease that affects not only the palmar aponeurosis, but also all types of connective tissues of the palm [1]. Fibromatous nodes and chords [2] cause limitation of extensor movements, and then persistent flexion contractures of the finger joints, which impede delicate work and social activity of patients [3].

For hundreds of years, the disease has been well known to surgeons; numerous methods of its treatment have been proposed, each of which has strong and and weak sides [4, 5]. However, the problems of relapses [6, 7] and predicting the course of the disease in different categories of patients [8] have not been solved in order to adequately choose a treatment strategy. Despite numerous studies of genetic predisposition [9, 10] and epigenetic factors [11, 12], the etiopathogenesis of fibromatosis has not been fully elucidated up to the present time [13].

To date, the concept of fascial fibromatosis has been seen as a myofibroblastic disease [14, 15], but myofibroblasts are not specific to it [16]. Although the initiating factor of abnormal fibroblast proliferation and transdifferentiation into myofibroblasts observed in fibromatosis is unknown, current research focuses on TGF-β, IL-1β and VEGF as potential therapeutic targets in the treatment of Dupuytren's disease [17, 18]. Recognizing the leading role of TGF-β, other authors have demonstrated increased proliferation of myofibroblasts in tissue cultures from patients with Dupuytren's disease, stimulated by the secretion of IL-13 factor by mast cells [19]. Meanwhile, data on the amount of mast cells in the palmar fascia of patients with Dupuytren's contracture are scarce and contradictory. The comparison of 10 tissue samples of the palmar fascia from patients with idiopathic carpal syndrome with 20 similar samples from patients with Dupuytren's contracture found a 12-fold increase in the number of mast cells in the palmar fascia in Dupuytren's disease [20]. However, in a study of surgical material from 100 patients with Dupuytren's disease, mast cells were described as occurring sporadically near blood vessels [21]. Thus, in the available literature, we did not find data on the content and morphofunctional status of mast cells in the pathologically altered palmar fascia of patients in regard to Dupuytren's contracture grade.

Purpose To study changes in the number, location and degranulation activity of mast cells in the palmar aponeurosis in regard to Dupuytren's contracture severity.

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MATERIALS AND METHODS

A pathohistological study of the surgical material was performed: palmar aponeurosis in 52 patients (49 men and 3 women) with Dupuytren's contracture (age 35-78 years, mean age 59.12 ± 1.25 years).

Inclusion criteria were Dupuytren's contracture grades 1-2 (group 1, n = 16) and grades 3-4 (group 2, n = 36) based on Tubiana classification [22] with a histologically confirmed diagnosis of fascial fibromatosis. Exclusion criteria were hand injuries and history of polytrauma.

The study was performed in accordance with the ethical standards of the Declaration of Helsinki (revised in October 2013), approved by the ethics committee (protocol No. 4 (68) of 11/11/2020). Voluntary informed consent was obtained from all patients to publish the results of the study without disclosing their identity.

The surgical material was fragments of a pathologically altered palmar aponeurosis, fixed in a mixture of solutions of glutaraldehyde and paraformaldehyde on a phosphate buffer with the addition of picric acid, post-fixed in a 1 % solution of osmium oxide (VIII) and embedded in araldite. Semi-thin sections were produced with an ultratome "Nova" LKB (Sweden). Due to the polyanionic properties of the heparin molecule to bind to cationic dyes, semi-thin sections were stained with a polychrome dye, methylene blue and basic magenta to visualize mast cells [23]. Light-optical examination and digitization of micropreparations were carried out using an AxioScope.A1 microscope and an AxioCam digital camera (Carl Zeiss MicroImaging GmbH, Germany). Quantitative data were obtained using the Zenblue software (Carl Zeiss MicroImaging GmbH, Germany). The numerical density of mast cells (N) in the field of view and their area (S, μm^2) were determined in full-color images of semi-thin sections at a magnification of 1000x; an average of 20 fields of view from each case were analyzed. Degranulation (secretory activity) of mast cells was determined by a semi-quantitative method proposed by Lindner et al (1980), by calculating the degranulation index (DGI):

$$DGI = A \times 0 + B \times 1 + C \times 2 + D \times 3/n,$$

where A – are inactive cells; B – weakly degranulated cells; C – mildly degranualated cells; D – severely degranulated cells; n – total number of cells [24].

Statistical data processing was performed using the Attestat software, version 9.3.1 (registration certificate with Rospatent No. 2002611109). Quantitative data were presented as medians and quartiles (Me (P25-P75)), since for some samples the hypothesis of normality was rejected. The Mann-Whitney test was used to test statistical hypotheses about differences in pairwise comparison of groups with each other.

RESULTS

The light-optical study of semi-thin sections revealed mast cells in both groups mainly in the loose connective tissue near the vessels, as well as in the nerve fascicles of the fibrously altered hypodermis adjacent to the palmar fascia (Fig. 1 and Fig. 2 a, b).

In group 2, more often than in group 1, the location of mast cells was away from blood vessels in the intercellular substance among altered contracted collagen fibers of the dense connective tissue (Fig. 2 c, d).

Histomorphometric study (Table 1) revealed that in group 2, compared with group 1, the median of the numerical density of mast cells was increased by 33.79 %, the median of the area of mast cells – by 48.40 % and the degranulation index of mast cells – by 94.68 %.

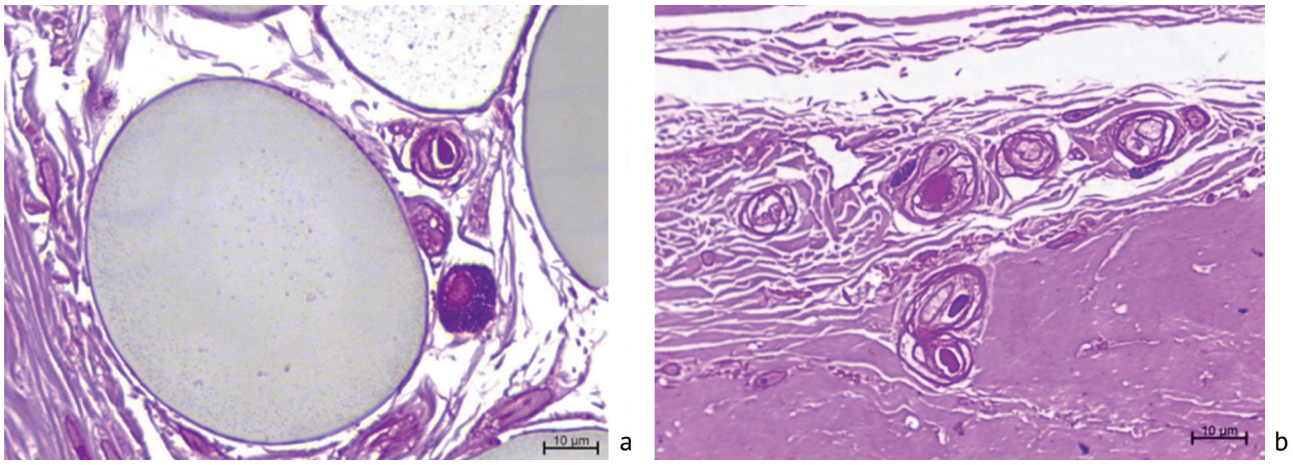

Fig. 1 Fragments of semi-thin sections of tissue samples from patients with Dupuytren's contracture of grades 1-2. Location of mast cells in loose connective tissue near the vessels. Staining: methylene blue – basic magenta. Magnification – ×1000

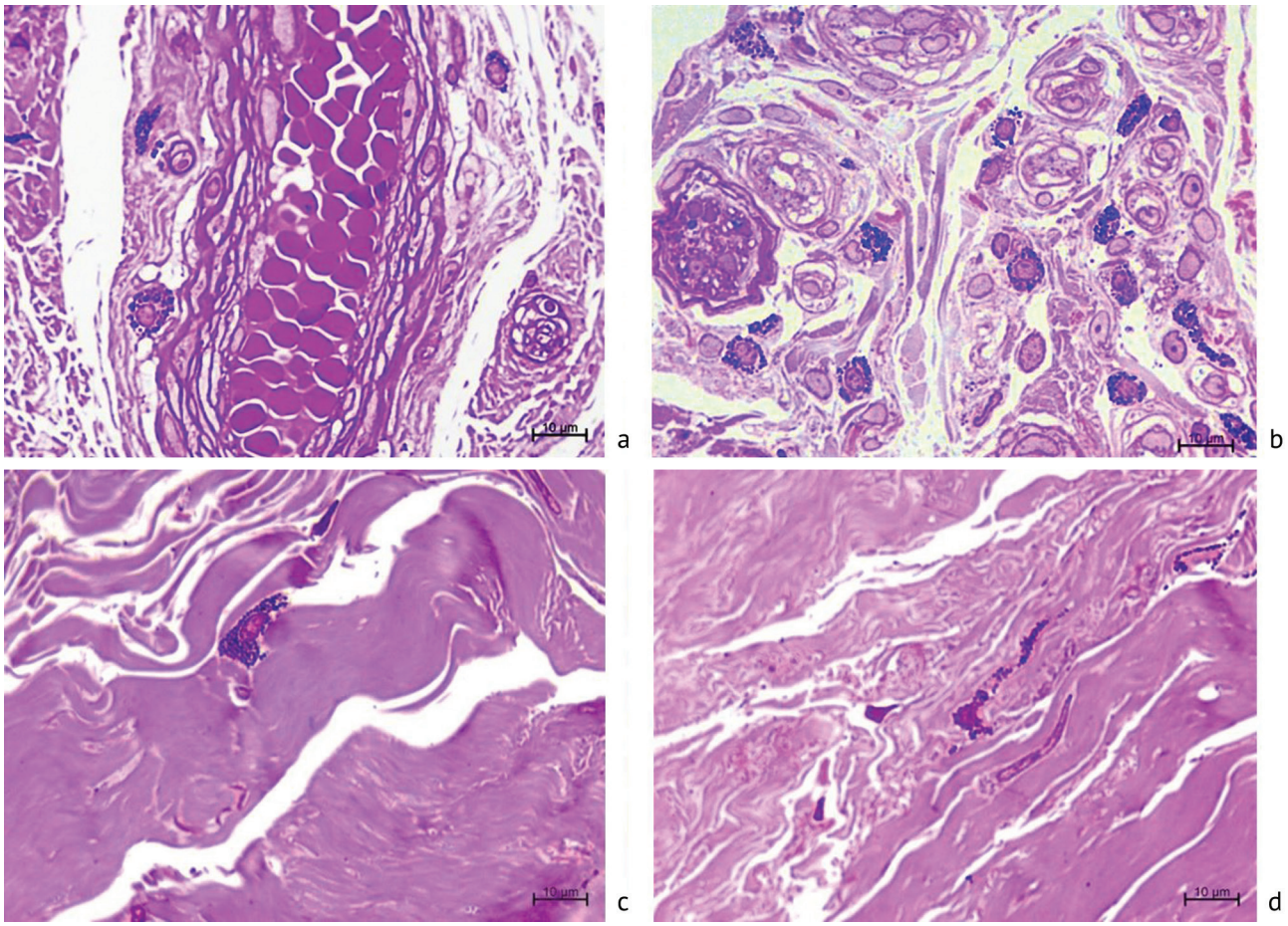

Fig. 2 Fragments of semi-thin sections of tissue samples from patients with Dupuytren's contracture grade 3-4; location of mast cells in the loose connective tissue near the vessels and nerve fascicules (*a, b*), and among the contractilely altered collagen fibers of the dense connective tissue (*c, d*). Staining: methylene blue – basic magenta. Magnification – ×1000

Table 1

Morphometric characteristics of mast cells in the palmar aponeurosis in Dupuytren's contracture

Parameter	Group 1 (n = 16)	Group 2 (n = 36)	U, p
	Me (P25-P75)		
Numeric density (N, mm^{-2})	1.45 (1.25-2.16)	1.95 (1.5-2.57)	U = 386.5; p = 0.05
Area (S, mcm^2)	28.16 (19.57-35.52)	41.79 (31.28-50.94)	U = 456; p = 8×10-4
Degranulation index (DGI)	0.94 (0.63-1.16)	1.83 (1.64-2.15)	U = 571.5; p = 1×10-8

Note: Me – median; P25-P75 – procentile; U – values of the Mann-Whitney test, differences significant at $P \leq 0.05$

DISCUSSION

Numerous animal and human studies have shown that mast cells promote scarring. In fetal tissues, the number and activation of mast cells is decreased; accordingly, wounds heal without scar formation [25, 26]. A significant increase in the content of myofibroblasts, mast cells, and neuropeptides in the joint capsule was found when post-traumatic contractures were modeled in experimental animals [27]. Like skin wound healing, connective tissue diseases such as Dupuytren's disease or Peyronie's disease can be affected by inflammation [28, 29], but the contribution of mast cells to the process of connective tissue degeneration in these diseases is poorly understood.

Schubert et al. [20] found a 12-fold increase in the number of mast cells in Dupuytren's contracture compared to normal fascia tissue; their association with sprouting of nociceptive fibers was noted. However, Mayerl et al. revealed sporadic mast cells near blood vessels in the material from 100 patients [21].

Significant qualitative and quantitative differences in mast cells that depend on the grade of Dupuytren's contracture were established on sufficient clinical material in our study for the first time. The typical location of mast cells near blood vessels reflects their participation in the regulation of vascular homeostasis and angiogenesis in normal conditions [30] and

determines the vasogenic factors in the pathogenesis of palmar fascial fibromatosis [31]. For patients with severe Dupuytren's contracture (grades 3-4), in addition to the paravasal location of mast cells, they locate far from the nerves of the hypodermis and blood vessels in the intercellular substance of dense connective tissue among collagen fibers altered by contraction what indicates their participation in the remodeling of the extracellular matrix. At the same time, the numerical density, area and degranulation index of mast cells are significantly increased compared with similar histomorphometric parameters of the group with grades 1-2 of the contracture. Thus, along with a change in the typical location of mast cells in patients with severe contractures, objective evidence of their increased number, activation, and degranulation was obtained.

It is known that activation and degranulation of mast cells promote experimental renal fibrosis [32]; a correlation between the number of mast cells and the severity of pulmonary fibrosis has been clinically revealed [33], and their role in the pathogenesis of liver fibrosis has been established [34].

Preclinical studies revealed a favorable dose-dependent effect of the mast cell membrane stabilizer ketotifen on the severity of joint capsule fibrosis and biomechanical parameters of post-traumatic contracture, mediated by a decrease in the content of myofibroblasts, mast cells, and P-reactive nerve fibers in the joint capsule [35, 36]. Since this drug has been used in the treatment of allergic diseases for more than 40 years, clinical studies of its effect on the course of Dupuytren's contracture in patients with concomitant allergic diseases seem promising.

CONCLUSION

Our results indicate the role of mast cells in the progression of Dupuytren's contracture and expand the understanding of potential therapeutic targets.

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***Ethical expertise** The study was performed in accordance with the ethical standards of the Declaration of Helsinki (revised in October 2013), approved by the ethics committee (protocol No. 4 (68) of 11/11/2020).*

***Informed consent** Voluntary informed consent was obtained from all patients to publish the results of the study without disclosing their identity.*

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Shchudlo N.A.: development of the concept and design of the study, writing the text;
Stupina T.A.: development of the concept and design of the study; collection, analysis and interpretation of data, writing the text;
Varsegova T.N.: collection, analysis and interpretation of data, writing the text;
Ostanina D.A.: data collection, writing a fragment of the text.