doi: 10.21823/2311-2905-2020-26-1-147-152

Detection of Fungi in the Palmar Aponeurosis in Patients with Dupuytren's Contracture by Scanning Electron Microscopy

T.A. Stupina, N.S. Migalkin, N.A. Shchudlo, M.M. Shchudlo

Ilizarov National Medical Research Center of Traumatology and Orthopedics, Kurgan, Russian Federation

Abstract

The purpose of the study was to determine the capabilities of scanning electron and light microscopy in detecting fungi in surgical material from the patients with Dupuytren's contracture. ***Material and Methods.*** The fragments of palmar aponeurosis from 27 patients with Dupuytren's contracture were examined. Paraffin sections stained with hematoxylin-eosin and Periodic Schiff-Methenamine Silver were digitized using AxioScope.A1 microscope (Carl Zeiss Micro Imaging GmbH, Germany). For examination in JSM-840 scanning electron microscope (Jeol, Japan), the samples were dehydrated after fixation, soaked in camphene, dried, and silver sprayed in the IB-6 ionizer (Eiko, Japan). ***Results.*** In paraffin sections stained with methenamine-silver, the signs of mycotic microbiota were detected only in 2 (7.4%) of 27 patients, and with the use scanning electron microscopy — in 16 patients (59%). The morphological phenotypes and ultrastructure of intercellular contacts of yeast-like fungi were similar to Candida albicans. In all cases, the budding cells were detected. The destruction of the connective tissue of the palmar aponeurosis was observed in the form of loosening, stratification, and cavities formation (the zones of lysis). ***Conclusion.*** The scanning electron microscopy allowed to evaluate the ultrastructure of fungal cells and their interaction with surrounding tissues in the surgical material from the patients with Dupuytren's contracture. A higher percentage of detection of fungal invasion compared to the Periodic Schiff-Methenamine staining was associated with a higher resolution and the ability of a larger area of tissue samples analysis.

Keywords. Dupuytren's contracture, scanning electron microscopy, mycoses.

Cite as: Stupina T.A., Migalkin N.S., Shchudlo N.A., Shchudlo M.M. [Detection of Fungi in the Palmar Aponeurosis in Patients with Dupuytren's Contracture by Scanning Electron Microscopy]. *Travmatologiya i ortopediya Rossii* [Traumatology and Orthopedics of Russia]. 2020;26(1):147-152. doi: 10.21823/2311-2905-2020-26-1-147-152. (In Russian).

✉ *Tatiana A. Stupina*; e-mail: StupinaSTA@mail.ru

Received: 01.02.2019. Accepted for publication: 20.05.2019.

Introduction

Dupuytren's contracture (palmar fascial fibromatosis) is a fibrous proliferative disease characterized by an increased myofibroblasts content in the connective tissue of the palmar aponeurosis, hyperproduction of collagen type III and I, the formation of pathological chords [1], led to the restriction of fingers extension, and then progressive development of flexion contracture. It is assumed that this disease has no infectious nature, however, in the literature, there are isolated descriptions of clinical cases of phlegmon abscess infection in the initial stage of fibromatosis [2] and references to the cutaneous mycoses in the advanced contracture [3].

The laboratory methods for the mycoses diagnosis include light microscopy, luminescent analysis, immunohistochemistry, isolation of a pure culture by inoculation on a special nutrient media, and identification of the pathogen [4]. A histopathological examination is an important diagnostic tool, however, the morphological characteristics of the fungi are almost always nonspecific. Thus, a regular histopathological diagnosis includes a description of the fungus, a statement of tissue invasion presence or absence, and tissue response to infection.

In recent years, the scanning electron microscopy (SEM) has been increasingly used in clinical and microbiological research practice [5, 6, 7, 8]. SEM allows to study the surface of fungal cells with high resolution, as well as visualize the interaction of the fungi with tissue structures [7]. In the suspicion for mycoses, and even in severe clinical manifestations, the microbiological culture often gives false negative results, while polymerase chain reaction (PCR) and immunohistochemical analysis are not available in most laboratories. Therefore, SEM is also considered as a diagnostic method [9] allows to distinguish among bacterial, fungal and parasitic lesion [10]. There are only few studies on the palmar aponeurosis pathomorphology in Dupuytren's contracture by SEM [11, 12, 13]. There is no information on the SEM detection of mycotic infection in the palmar aponeurosis of the patients with Dupuytren's contracture, as well as on the fungi detection by other laboratory methods. Since the detection and treatment of mycoses is important for the complete rehabilitation, improving the patients quality of life and life expectancy, the assessment of palmar aponeurosis mycotic lesions in the patients with Dupuytren's contracture is the relevant matter.

The purpose of the study was to determine the capabilities of light microscopy and SEM to detect the fungi in surgical material from patients with Dupuytren's contracture.

Material and Methods

The studied tissue samples consisted of pre-tendon chords of pathologically altered palmar aponeurosis from 27 patients (24 men and 3 women) aged 39–77 years (mean age 60.44±1.98) with Dupuytren's contracture. One patient had grade I Dupuytren's contracture by Tubiana [14], 3 patients — II degree, 19 patients — III degree, and 4 patients — IV degree. The disease duration ranged from 1 to 30 years (an average of 7.69±1.32).

The study of the surgically obtained samples was performed by two methods.

The preparation for the SEM study consisted of the following: tissue samples 3–5 mm in size after fixing in formalin were washed in distilled water, dehydrated in alcohols with an ascending concentration from 70 to 96%, and then soaked in camphene according to the original method (RF patent No. 2008150910/12) and dried in a thermostat at 37°C. Then, objects were installed using conductive glue on well-polished, clean aluminum disks, and silver sprayed in the IB-6 ionizer (Eiko, Japan). The charge was removed from the sprayed surface of the sample by means of a conductive paste. The studies were conducted on a JSM-840 scanning electron microscope (Jeol, Japan).

The light microscopy. For histological examination, the tissue samples were fixed in formalin and embedded into paraffin by the standard procedure. 5–7 mcm paraffin sections were made by the Reichert microtome (Austria) and stained with H&E and methenamine silver-Periodic acid-Schiff (MS PAS) to detect the fungi mycelium. The paraffin sections digital images were obtained using an AxioScope A1 microscope with an AxioCam digital camera and Zenblue software (Carl Zeiss Micro Imaging GmbH, Germany).

Results

The SEM revealed the presence of yeast-like fungi in 16 out of 27 patients (Fig. 1).

Of these 16, one patient had a grade I contracture with the duration of less than a year; 2 patients had grade II contracture with the duration from 1.5 years and 30 years; 10 patients had grade III contracture with the duration from 5 to 20 years; 3 patients had grade IV contracture with the duration of 8, 11 and 20 years. In 14 patients, the yeast form of fungi was revealed (Fig. 1 a, b, c), and in 2 patients — the blastospores form (Fig. 1 d). The yeast cells had a round, round-oval or pear-like shape. They were fastened into clusters, budding in several directions in the form of whorls or bushes (Fig. 1 a, b). Thin yeast cells of elongated cylindrical shape were met, both separately lying and bonded into clusters (Fig. 1 c). Cell sizes ranged from 2 to 7 mcm. A part of the cells showed a brightly contrasted area in the center with a diameter of about 25–30% of the cell. The areas of intercellular contact were less than 0.5 mcm. Both in the cases of intact and destroyed contacts, a ring-shaped girdle at one end of the cell and a bud at the other one were observable. The blastospores had a spherical shape with a diameter of about 1 mcm and were arranged in the form of bunch-like clusters (Fig. 1 d). Destruction of the palmar aponeurosis connective tissue appeared in the form of loosening, stratification and the formation of cavities (zones of lysis).

Light microscopy of paraffin sections in two of 27 patients revealed the areas of pseudomycelium accumulation of mycotic microbiota (Fig. 2).

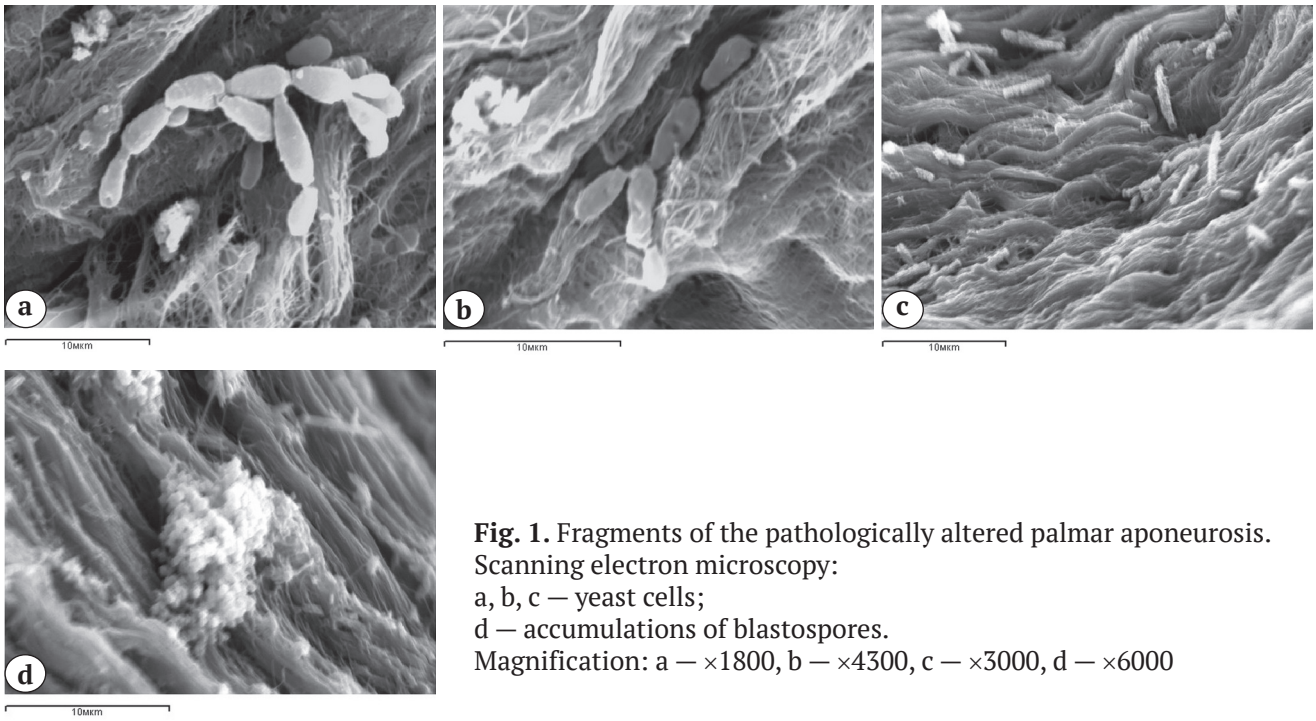

Fig. 1. Fragments of the pathologically altered palmar aponeurosis. Scanning electron microscopy:
a, b, c — yeast cells;
d — accumulations of blastospores.
Magnification: a — ×1800, b — ×4300, c — ×3000, d — ×6000

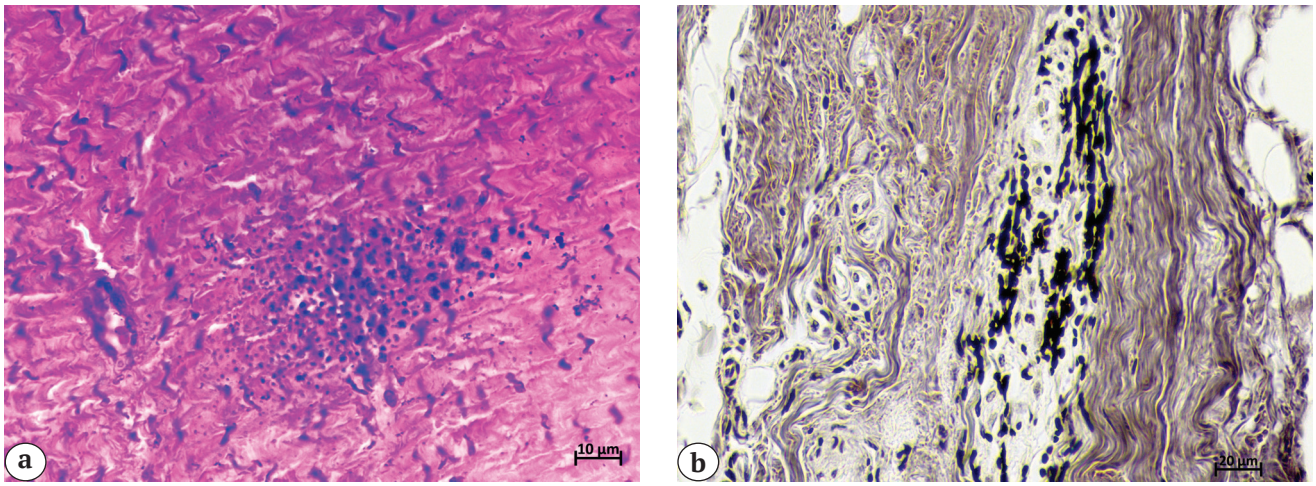

Fig. 2. A fragment of the palmar aponeurosis strand in Dupuytren's contracture with signs of fungal invasion:
a — the paraffin section stained with hematoxylin and eosin;
b — Periodic Schiff-Methenamine silver, the fungi are stained black.
Magnification: a — ×600, b — ×400

Discussion

On histological examination, the detection of fungi is not always possible due to the small volume of the biopsy specimen, especially because of their low concentration in the tissues and the focal character of the mycotic process. It is not surprising that on the light microscopy, the signs of mycotic microbiota were detected only in two (7.4%) of 27 patients, and on the SEM — in 16 (59%). The advantages of SEM are the ability to analyze a large area of a tissue sample (up to 15 mm^2 in our study) and a much higher resolution [15].

The revealed fungal invasion of the palmar aponeurosis in Dupuytren's contracture is pathogenic, because the detection of the budding cells. It is known that budding cells are an infectious form of the fungus, and pseudomycelium is the form in which the fungus exists in tissues [16]. Morphologically, the revealed yeast-like fungi were similar to the fungal genus Candida. They had an elongated cylindrical or oval pear-like shape and under certain conditions could form a primitive mycelium. Also, they possessed a characteristic property called dimorphism. That meant they occurred both in the form of blastospores and in the form of short or long threads [17].

The study limitation was the absence of immunohistochemical analysis, PCR, and immunohybridization. However, the fungi were detected by two methods, one of which (SEM) made it possible to identify their taxonomic affiliation. This was the characteristic form of intercellular contacts, considered as a specific ultrastructural feature of Candida albicans, despite the pronounced pleomorphism of this species [18].

The most obvious factor predisposing to candida infection in patients with Dupuytren's contracture is the problem of skin folds hygiene because of persistent deformity of the hand, advanced age and comorbid status. However, as our pilot study showed, the mycotic infection of the palmar aponeurosis occurred even in the grade I contracture (actually in the absence of contracture), as well as in young patients who did not have concomitant diseases.

The fungal aponeurosis lesion could be the result of the disruption of the skin barrier properties in dermal fibromatosis [19],

as well as the skin blood supply and innervation disturbances [20, 21]. The weakening of the immune reactivity and antioxidant status in Dupuytren's disease should be considered as systemic predisposing factors to fungal infections [22].

Complex additional studies are needed to comprehensively evaluate the clinical significance of opportunistic invasive fungal infection [23] in Dupuytren's contracture, given that these patients are characterized by earlier mortality [24].

The SEM allows to visualize and evaluate the fungal cells morphology, their shape, size, relative position and can be used in diagnostic technologies of clinical microbiology. The performed study allows to conclude that the frequency of mycotic lesions of the palmar aponeurosis in the patients with Dupuytren's contracture is high. This must be taken into account in the examination and treatment protocols.

Publication ethics

Patients gave voluntary informed consent to participate in the research study.

The study was supported by the Ministry of of Healthcare of the Russian Federation as the state assignment to the Ilizarov National Medical Research Center of Traumatology and Orthopedics, the scientific research plan for 2018–2020.

Competing interests: The authors declare that there are no competing interests.

Funding: State budgetary funding.

Authors' contributions

T.A. Stupina — research concept and design, SEM examination, data analysis, text preparation, editing

N.S. Migalkin — pathohistological examination, data analysis, text preparation

N.A. Shchudlo — research concept and design, data analysis, text preparation

M.M. Shchudlo — research concept and design, data analysis, text preparation

References

1. Ng M., Thakkar D., Southam L., Werker P., Ophoff R., Becker K., Nothnagel M. et al. A Genome-wide Association Study of Dupuytren Disease Reveals 17 Additional Variants Implicated in Fibrosis. *Am J Hum Genet.* 2017;101(3):417-427. doi: 10.1016/j.ajhg.2017.08.006.
2. Unglaub F., Loos B., Schwarz S., Kneser U., Dragu A., Horch R.E. Phlegmonous-infection in first degree Dupuytren's disease. *Arch Orthop Trauma Surg.* 2009;129(4):445-448. doi: 10.1007/s00402-008-0589-7.
3. Dumitrescu-Ionescu D. A New Therapeutic Approach to Dupuytren's Contracture / Disease (DD). *Adv Plast Reconstr Surg.* 2017;1(5):129-136.
4. Guarner J., Brandt M.E. Histopathologic diagnosis of fungal infections in the 21st century. *Clin Microbiol Rev.* 2011;24(2):247-280. doi: 10.1128/CMR.00053-10.
5. Ensikat H.-J., Weigend M. Creating internal conductivity in dry biological SEM samples by a simple vapour treatment. *J Microsc.* 2014;256(3):226-230. doi: 10.1111/jmi.12177.
6. Sugimoto S., Okuda K., Miyakawa R., Sato M., Arita-Morioka K., Chiba A. et al. Imaging of bacterial multicellular behaviour in biofilms in liquid by atmospheric scanning electron microscopy. *Sci Rep.* 2016;6:25889. Available from: https://www.nature.com/articles/srep25889. doi: 10.1038/srep25889.
7. Chebotar I.V., Novikov I.A., Subbot A.M., Mayansky N.A. Lanthanoid staining as a fast technology of preparing microbiological specimens for scanning electron microscopy. *CTM.* 2017;9(3):23-29. doi: 10.17691/stm2017.9.3.03.
8. Raab N., Bachelet I. Resolving biofilm topography by native scanning electron microscopy. *J Biol Methods.* 2017;4(2):e70. doi: 10.14440/jbm.2017.173.
9. Yue X., Wang A., Li Q. The Role of Scanning Electron Microscopy in the Direct Diagnosis of Onychomycosis. *Scanning.* 2018:1581495. doi: 10.1155/2018/1581495.
10. Yuping R., Kaiwen Z., Wenying H., Jinghong H., Xiaowei F., Shuang C. et al. Observation of Fungi, Bacteria, and Parasites in Clinical Skin Samples Using Scanning Electron Microscopy. In: *Modern Electron Microscopy in Physical and Life Sciences.* 2016. Available from: https://www.intechopen.com/books/modern-electron-microscopy-in-physical-and-life-sciences/observation-of-fungi-bacteria-and-parasites-in-clinical-skin-samples-using-scanning-electron-microsc. doi: 10.5772/61850.
11. Legge J.W., Finlay J.B., McFarlane R.M. A study of Dupuytren's tissue with the scanning electron microscope. *J Hand Surg Am.* 1981;6(5):482-492. doi: 10.1016/s0363-5023(81)80109-8.

12. Józsa L., Salamon A., Réffy A., Renner A., Demel S., Donhöffer A., Pintér T., Thöring J. Fine structural alterations of the palmar aponeurosis in Dupuytren's contracture. A combined scanning and transmission electronmicroscopic examination. *Zentralbl Allg Pathol.* 1988;134(1):15-25.

13. Shchudlo N.A., Stupina T.A., Shchudlo M.M. [Scanning Electron Microscopy of Palmar Fascia in Dupuytren's Disease of Advanced Stage]. *Travmatologiya i ortopediya Rossii* [Traumatology and Orthopedics of Russia]. 2019;25(2):150-156. doi: 10.21823/2311-2905-2019-25-2-150-156. (In Russian).

14. Tubiana R. Dupuytren's disease of the radial side of the hand. *Hand Clin.* 1999;15(1):149-159.

15. Scherer W.P., Scherer M.D. Scanning electron microscope imaging of onychomycosis. *J Am Podiatr Med Assoc.* 2004;94(4):356-362. doi: 10.7547/0940356.

16. Kubota T. Chronic and recurrent vulvovaginal candidiasis. *Anippon Isbink Gakkai Zassibi.* 1998;(39)4: 213-218. doi: 10.3314/jjmm.39.213.

17. Hmel'nickij O.K., Bykov V.L., Hmel'nickaya N.M. [Pathomorphological diagnosis of Pathomorphological diagnosis of mycosis caused by opportunistic fungi]. Available from: http://www.rusmedserv.com/mycology/html/labora15.htm (In Russian).

18. Staniszewska M., Bondaryk M., Swoboda-Kopec E., Siennicka K., Sygitowicz G., Kurzatkowski W. Candida albicans morphologies revealed by scanning electron microscopy analysis. *Braz J Microbiol.* 2013;44(3):813-821. doi: 10.1590/S1517-83822013005000056.

19. Wade R., Igali L., Figus A. Skin involvement in Dupuytren's disease. *J Hand Surg Eur.* 2016;41(6):600-608. doi: 10.1177/1753193415601353.

20. Shchudlo N.A., Kostin V.V. [Pathogenesis of neuropathy in Dupuytren's contracture]. *Genij ortopedii*. 2019;25(1):58-64. doi: 10.18019/1028-4427-2019-25-1-58-64. (In Russian).

21. Dolganova T.I., Shchudlo N.A., Shabalin D.A., Kostin V.V. [Assessment of hemodynamics of the hand arteries and skin microcirculation in Dupuytren's contracture stages 3 to 4 of before and after surgical treatment with the use of Ilizarov transosseous fixation]. *Genij ortopedii* 2019; 25(1):86-92. doi: 10.18019/1028-4427-2019-25-1-86-92. (In Russian).

22. Rehman S., Goodacre R., Day P.J., Bayat A., Westerhoff H.V. Dupuytren's: a systems biology disease. *Arthritis Res Ther.* 2011;13(5):238. doi: 10.1186/ar3438.

23. Badiee P., Hashemizadeh Z. Opportunistic invasive fungal infections: diagnosis & clinical management. *Indian J Med Res.* 2014;139(2):195-204.

24. Gudmundsson K.G., Arngrímsson R., Sigfússon N., Jónsson T. Increased total mortality and cancer mortality in men with Dupuytren's disease: a 15-year follow-up study. *J Clin Epidemiol.* 2002;55(1):5-10. doi: 10.1016/s0895-4356(01)00413-9.

AUTHORS' INFORMATION:

Tatiana A. Stupina — Dr. Sci. (Biol.), Senior Researcher, Laboratory of Morphology, Ilizarov National Medical Research Center of Traumatology and Orthopedics, Kurgan, Russian Federation

Nikolai S. Migalkin — Researcher, Laboratory of Morphology, Ilizarov National Medical Research Center of Traumatology and Orthopedics, Kurgan, Russian Federation

Natalia A. Shchudlo — Dr. Sci. (Med.), Head, Clinical and Experimental Laboratory of Reconstructive-Plastic Microsurgery and Hand Surgery, Ilizarov National Medical Research Center of Traumatology and Orthopedics, Kurgan, Russian Federation

Mikhail M. Shchudlo — Dr. Sci. (Med.), Senior Researcher, Laboratory Clinical and Experimental Laboratory of Reconstructive and Plastic Microsurgery and Hand Surgery, Ilizarov National Medical Research Center of Traumatology and Orthopedics, Kurgan, Russian Federation