THE DIABETIC FOOT SYNDROME CLINICAL MORPHOLOGY

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Introduction

The number of diabetes mellitus (DM) patients worldwide is growing sustainably, making it one of the biggest contemporary healthcare challenges worldwide, with the global prevalence of diabetic foot syndrome (DFS) varying from 3% in Oceania to 13% in North America, with a global average of about 6.4%. The annual incidence of DFS complicating DM is known to be about 2-5% with the lifetime risk ranging from 15-20%. It is worth mentioning that peripheral arterial disease (PAD) is an important risk factor associated with a twentyfold higher prevalence in diabetic patients with the probability of amputation within one year after the first ulcer or gangrene over 34% and the mortality rate over 5%. Approximately 20-33% of all costs related to DM are expenditures related to treatment of DFS, with the cost of medical care for DFS increasing. The steady growth in the number of diabetic patients has become an important issue in the decision of the health and welfare budget worldwide. In addition, the increase in complications due to diabetes is a burden, not only for patients, but also for the national healthcare systems and economies. Furthermore, DFS determines huge portion of public health loses making it essential for public health policy makers to advocate for implementation of prevention and treatment recommendations.

Pathogenesis of DFS involves both systemic and local factors. Major influence on metabolic disorders, endothelial dysfunction and atherosclerosis

is thought to have strong genetic background with multiple genetic polymorphisms involved. Local factors are generally more likely linked to microbiota, biofilm formation and impaired local resistance not to mention neurological and vascular factors, etc.

Treatment of diabetic foot is complex and often unsuccessful. Most recent IWGDF Guidelines indicate that "Multiple disciplines are involved in the management of diabetic foot disease, and interdisciplinary treatment is the cornerstone of its management and prevention". Moreover, common combination of systemic effects of diabetes and comorbidities like hypertension and target-organ damage add complexity to this task. While the term "chronic diabetic wound" to some extend corresponds to diabetic foot syndrome, there are several pathogenetic mechanisms involved in the condition making it distinguishable from other chronic wounds and skin defects. Moreover, surprisingly few papers are dedicated to morphological studies of the DFS, stimulating current research interest.

The aim of the study

The objective of the study was to evaluate the morphological changes in diabetic foot syndrome emphasizing the ultrastructural cellular changes.

Material and methods

The comprehensive pathomorphological study of biopsy material of 120 cases of surgical treatment of DFS was performed. Biopsies of the chronic skin wounds in DFS patients were taken intraoperatively or during non-operative procedures (debridement, necrectomies, tissue grafting, etc). The study was conducted in full accordance with the principles of the Council of Europe Convention on Human Rights and Biomedicine, Declaration of Helsinki on the ethical principles for medical research involving human

subjects, and other acting international and national legislations in bioethics (including GCP, EU directives, etc). All patients signed an informed consent prior to participating in the study. The study protocol was fully approved by the institutional ethics committee. All patients underwent treatment conducted in accordance with the state-of-the-art guidelines and recommendations.

Bioptates sampling, preparation and staining

The bioptates material underwent standard care and passed all necessary preparatory stages (Fixation, Embedding, Sectioning, Staining, and Mounting). Samples were fixed for 48 hours in a neutral buffered 10% formalin solution, then dehydrated in an ascending battery of alcohols and embedded into paraffin medium. Serial histological sections 5 μm thick were produced on a sled microtome. Several approaches were combined for optical analysis: review histological methods (hematoxylin and eosin staining, van Gieson picric acid and acid fuchsia staining); histochemical methods (Gömöri trichrome stain, Slinchenko stain, and Weighert staining for detection of connective tissue components, fibrin; PAS-reaction; toluidine blue for visualization of tissue basophils and large granular lymphocytes).

Electron microscopy

For electron microscopy, bioptates samples were fixed in 2.5% phosphate buffered glutaraldehyde solution (pH 7.2-7.4) and fixed in 1% osmium tetroxide solution. The material was dehydrated in alcohols of increasing concentration and embedded in Araldite (HAM, USA). Morphological structures were contrasted in the process of dehydration of the material with a saturated solution of uranyl acetate, and in sections with lead citrate. Sections with a thickness of 40-60 nm were analyzed in the TESLA BS-500 electron microscope.

Results

Destruction and inflammatory infiltration involving all layers of skin and soft tissues of the marginal area of skin wounds were observed during morphological examination. The epidermis (absent in ulcers) was characterized by a decrease in thickness towards the edge of the wound, the presence of a large number of cells with the phenomena of vacuolation, apoptosis or karyolysis; tissue edema, diffuse infiltration by neutrophils, vascular plethora with stasis and thrombosis, necrosis. The lumen of dilated blood vessels was filled with clearly contoured blood elements. Hyalinosis of the microcirculatory tract vascular wall was present in all tissues studied. In the presence of phlegmon or abscess, the vascular wall was infiltrated with leukocytes and subjected to lysis, which led to small hemorrhages.

The microcirculatory tract thrombosis exacerbated tissue ischemia, contributing to the progression of pathologic processes. Destruction of collagen fibers (fibrinoid edema and necrosis) with infiltration of the surrounding area by neutrophils and macrophages was observed in the wound edges. Against the background of necrotic changes, elements of granulation tissue in the form of short thin strands of proliferating spindle-shaped fibroblasts accompanied by capillaries were revealed; at some patients such phenomenon was absent. At a distance of 0.1-0.3 mm from the edges of the wound, we observed the regeneration of the epidermis in the form of small thin layers of newly formed keratinocytes.

Masses of chaotically located high electron density fibrillary structures, occasionally were found in the cytoplasm of macrophages. In microfibrillar intracellular masses, tufts of more or less scattered fibrils with fuzzy contours

were observed. The fibrous fibrillary structures or their fragments were partially dissolved in the cytosol mass, especially near the nucleus.

The excrescences located on the cell surface were represented by large fields of peripheral areas of the cytoplasm. The granular endoplasmic reticulum was moderately developed and represented by intracellular channels or cisterns. The cytoplasm contained a small number of membrane-bound ribosomes. There was mainly peripheral localization of the granular endoplasmic reticulum and vacuolar formations. The latter are represented by clusters of rounded or irregularly shaped vacuoles. In addition, the endoplasmic reticulum had the appearance of oblong cisterns and accumulations of rounded or elongated vesicles.

A small number of pinocytic edged vesicles, as well as homogeneous small electron-dense granules in the cytoplasm were observed in some macrophages. The cytoplasmic matrix had a moderate electron density, on outside of the cell, insignificant digit-like processes or pseudopodia were observed. In some vesicles there was a fine electron density of fine-grained material. Larger vacuoles contained membrane fragments and myelin figures. The extracellular material included preserved and cleaved collagen fibrils, granular matter, electron-dense homogeneous formations. The cytoplasm between the zones of the lamellar complex was occupied by small mitochondria, a small number of polysomal rosettes, tanks of granular endoplasmic reticulum and various sizes of phagolysosomes with the presence of phagocytosed and digested microorganisms. Phagocytized microorganisms were often found intact inside macrophages.

Uneven cell hypertrophy was often observed, and the nucleus, as a rule, acquired an intricate shape, and it possible to see accumulations of chromatin

on the periphery of the nuclei. In addition to the described changes, a constant feature was the appearance of large vacuoles, diverse in structure. Often, they were near the nucleus, were filled with crumbly or light homogeneous content.

Vacuoles could contain other often high-density structures. The described changes in the state of nuclear and perinuclear material were accompanied by compression and dehydration of the cell, which ended in its fragmentation and the formation of tightly contacting bodies of various shapes.

Diabetic foot syndrome generally presents with diverse and often unclear morphological picture, ranging from minor skin defect to vast necrotic gangrenous and inflammatory changes. Thus, finding something more specific or clinically relevant is an uneasy task. However, it is clear that in the diabetic foot, a comprehensive morphological assessment of all ulcers and defects is required to devise a proper treatment and revascularization strategy. Obviously, DFS presents with a multitude of wounds that are heterogeneous in both morphology and topography. Even one single patient may present with several wounds dispersed over more than one angiosome or manifest a large ulcer that lies on the verge of two angiosomes with different phases of wound processes and different infestation status. Furthermore, different systemic changes and peculiarities of pathogenesis may significantly variate adding to diversity in morphological picture.

Morphological studies of the DFS are usually focused on clinically relevant changes like vascular changes and deformities (e.g. Charcot's foot) formation with much less attention payed to microscopic changes in skin and soft tissues. Even lesser consideration is concentrated on the intracellular

changes as they are assumed to be sufficiently studied by biochemical and physiological methods.

Current study complements additional information to the existing database on DFS focusing on intracellular changes obtained through electronic microscopy. Such information may be useful for determining future local therapies for DFS as new types of treatment approaches are undergoing rapid development, like molecular or cellular surgery.

Conclusions

Morphological changes in diabetic foot syndrome are diverse and uneven. This study confirms that pathologic changes in diabetic foot syndrome occur not only on tissue and cellular level but involves subcellular and molecular structures, encouraging further research in this field. Microcirculatory disorders are expressed in the form of venular plethora, the phenomena of stasis in capillaries, hemolysis of erythrocytes and marginal standing of formed elements in venules and capillaries. The vascular network occupied 11.7±1.0%, but the vascular loops were unevenly spaced and markedly different in size. The appearance of foci of destruction and lysis of the newly formed epithelium are characteristic for DFS, which is not observed in the normal wound process.