REVIEW

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Histopathological changes in major amputations due to diabetic foot – a review

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Abstract

Diabetes mellitus is the leading cause of non-traumatic amputations worldwide. Ulcer of the diabetic foot is one of the most prevalent lesions of diabetic patients and it occurs in the natural evolution of the disease as a tardive complication. Neuropathy is the main determinant of foot ulcer. A key role is played by the loss of sensitive nerves, which prove to be a protective barrier against high pressure applied otherwise on the foot. The morphopathological characteristics of neuropathic lesions in patients with diabetes show important improvement associated with the pressure relieving treatment strategies. Therefore, pressure seems to impose a continuous mechanical stress on the wounded foot and it also sustains a chronic inflammatory condition, which slows down the healing process. Atherosclerosis is an imminent process to every person, nonetheless patients with diabetes mellitus have this process highly accelerated and more diffuse. One of the main characteristics of macrovascular lesions in diabetes is Mönckeberg's medial calcific sclerosis, calcification of the muscular layer, which clinically translates into an ankle-brachial index of 1 or above. Diabetes affects not only the large vessels, but it also produces microvascular lesions, which in time leads to diseases like retinopathy or nephropathy. Osteomyelitis is very common in the diabetic foot infections and the medical treatments are not satisfying. It is also believed to be a consequence of peripheral neuropathy that diabetes comes with. Osteomyelitis plays an important role in the prevalence of amputations in patients with diabetes. Obtaining clean, infection free margins is the most important goal, because residual osteomyelitis is a strong predictor of clinical failure and comes with many postoperative complications, even the necessity to operate again or have a major amputation later in evolution.

Keywords: amputation, diabetic foot, osteomyelitis, atherosclerosis.

Introduction

Diabetes mellitus is the leading cause of non-traumatic amputations worldwide. Patients suffering from diabetes mellitus have a risk for lower limb amputations 15 times higher than the general population. Regarding the incidence and prevalence of amputations, though apparently they could be easily evaluated, it was found to be important differences in the way they are calculated. In some statistics, reporting incidence is 1:1 000 000 of the general population and in others at 1:10 000 from people at risk (with diabetes). When the prevalence of diabetes in a community is not well documented, reporting the incidence of amputations at the general population is recommended. Instead, when certain communities are adopting systematic programs to diagnose diabetes is it expected that the incidence of amputations attributable to diabetes will increase [1]. In some assessments, only considered the first amputation episode at one patient and in others repetitive episodes. A full evaluation should also include the presence of surgical resection of bone fragments (in 15–27% of the episodes of ulcers the affected bone is also excised) and even the cases in which self-amputation occurs. A high incidence of major amputations could be attributed to an increased prevalence of diabetes, reduced accessibility and the limited resources of medical services, but also a more "aggressive" attitude of the care team [2]. On the other hand, a lower incidence of amputations may reflect a lower prevalence of correct management of diabetes and leg ulcers, but also an overly conservative attitude of the care team, that is frequently to the detriment of the patient's quality of life. Chronic arterial obstructive atherosclerotic limb is the most common form of peripheral vascular interest in patients with diabetes and consists in the progressive reduction of blood flow in the arteries of the lower limbs due to the progressive narrowing of the lumen produced by atherosclerotic plaques [3]. Morphopathologically, there are many similarities between diabetic and non-diabetic patients, the common substrate of large vessel damage being atherosclerosis. A particularly atherogenic morbid association is represented by type 2 diabetes, obesity, dyslipidemia, hypertension, hyperuricemia, all of which are elements of the metabolic syndrome [4]. Diabetic macroangiopathy is considered to be the result of accelerated progression of atherosclerosis, stimulated by increased adhesion and platelet aggregation, increased levels of lipoprotein oxidation products, increased levels of plasminogen activator inhibitor (PAI), growth factors and increased vascular permeability, all being potentiated by hyperglycemia. Increased vascular permeability for molecules, such as albumin, fibrinogen, atherogenic lipoprotein may represent primary alteration in diabetic macroangiopathy and an early sign of progression of vascular wall sclerosis [5]. These changes also seem to be related to the loss of glycosaminoglycans in the vascular wall. Therefore, the use of exogenous glycosaminoglycans can be justified therapeutically. Diabetic nephropathy is also a marker for generalized vascular

disease. The most important aspect of addressing this complication of diabetes in recent years is precisely the recognition that besides the so-called "classical causes", such as arteriopathy, neuropathy and infection, an equally important role in causing lesions and their rapid evolution towards the need. Major amputations also have apparently minor factors, such as foot trauma, skin and nail fungal trauma, limiting joint mobility, patient activity, and even malfunctions in the healthcare system [6].

Morphopathology of diabetic ulcer

Ulcer of the diabetic foot is one of the most prevalent lesions of diabetic patients and it occurs in the natural evolution of the disease as a tardive complication [7].

Comparing diabetic ulcers and venous ulcers to the acute ones, it is observed that the chronic ulcers, older than eight months present more inflammatory cells and a large amount of extracellular matrix than the acute ulcers, with duration of less than half a month. Studies observed vessel wall thickening and inflammatory cellular clusters around the capillaries, associated by elastic lamina disruption in samples taken from foot ulcers in diabetic patients [8].

It may suggest that the ulcers are unable to purchase from the acute to the reparative stages. The diabetic ulcers have a low proliferation rate and morphological abnormalities of fibroblasts compared to other types of ulcers, with little to no connection with glycemic levels. This findings lead to the idea of the pressure burden on the diabetic neuropathic foot with all the alterations it stimulates [9].

Neuropathy is one of the main determinants of foot ulcer. A key role is played by the loss of sensitive nerves, which prove to be a protective barrier against high pressure applied on otherwise on the foot. Moreover, the architectural changes of the foot due to the motor neuropathy expose the surfaces on which the tensions apply, imposing an even higher burden on the already affected foot [10].

Therefore, local hyperkeratosis forms followed shortly, if not observed in time, by superficial lesions that tend to become deeper, even get infected and, in the end, producing a real ulcer. The length and depth of the ulcer are usually proportioned with the postural stress [11].

Few are known in literature about of histopathology of such open lesions and studies do not reach a common conclusion, except the fact that the healing process of the wounds is impaired at different stages.

Patients who suffered a surgical necrectomy on diabetic ulcers were characterized by a chronic, intense and diffuse inflammatory reaction. Leukocytes, lymphocytes, macrophages and all the inflammatory cells are dispersed on the whole surface of the wound, forming nodular clusters, mainly around the arterioles, frequently infiltrating the muscular layer and perivascular areas, the histology image being highly similar with panarteritis [12].

Vessels, tendons and sweat glands were profusely modified or even fragmented. Cell debris, fragments of extracellular matrix and amorphous necrotic tissue were present on the microscope field, showing progressive dehydration and a degenerative process [13].

Neoangiogenesis and granular tissue formation are barely visible, compensated by hyperkeratosis. The hyperkeratosis is seen on the margins of the ulcer, infiltrating the epidermal tissue beneath. On the other hand, the acanthosis implying the basal epidermal layer is seen on the surface of the ulcer. The dermal layer is hypertrophic, with a high rate of fibrosis, interfering with the normal structure of the extracellular matrix [14].

On the other hand, patients with ulcer treated with hydrocolloid dressing and pressure free dressing showed a less inflammatory reaction.

Biopsies from these ulcers showed granulation, a tissue full of fibroblasts, neovascularization which starts from the non-affected borders of the ulcer, also the base of the ulcer or cutaneous annexes. The new arterioles formed present a single layer of endothelial cell. Fibroblasts use these vessels to rejuvenate the necrotic tissue into granulation and fibrosis. There are spotted even cells undergoing mitosis. Regarding the epidermal layer, on the edges of the ulcer, keratinocytes upgrade their replicative activity and their number at the same time. It is observed fibroblasts' migration towards the core of the ulcer, much more active than in the surgically treated ones. All the regenerative markers, cutaneous annexes, granulation tissue and capillaries are present [15].

The morphopathological characteristics of neuropathic lesions of the patients with diabetes show important improvement associated with the pressure relieving treatment strategies [16].

Therefore, pressure seems to impose o continuous mechanical stress on the wounded foot and it, also, sustains a chronic inflammatory condition, which slows down the healing process [17].

On the contrary, leaving the affected area to rest and depressurize it stimulates the active reparatory process, composed of tissue granulation and neoangiogenesis, a global regenerative reaction of cells. After mere 20 days of pressure relief treatment, there are signs of important inflammatory infiltrate reduction, granulating tissue emerges, cell migration from the borders and the base to the core of the ulcer, resulting in a 50% reduction in size [18].

Not only an important, but also a surprising aspect of this research is that the glycemia control proved no influence what so ever [19].

Macro- and microvascular lesions in diabetes

Atherosclerosis is an imminent process to every person, nonetheless patients with diabetes mellitus have this process highly accelerated and more diffuse. Macrovascular atherosclerosis in patients with diabetes usually affects the distal portion of the lower limb, the infragenicular arteries, in comparison to peripheral arterial occlusive disease, which affects more often the aorto-iliac and femoro-popliteal regions. Aorto-iliac disease incidence is much lower in diabetes compared to peripheral artery disease, but it produces a bigger impairment of the below-knee part. It simultaneously affects the femoral, popliteal and tibial sections, compared to the non-diabetic patients, which present more localized atherosclerotic plaques. Nevertheless, the morphology is similar [20].

One of the main characteristics of macrovascular lesions in diabetes is Mönckeberg's medial calcific sclerosis, calcification of the muscular layer, which clinically translates into an ankle-brachial index of 1 or above. Studies show that Mönckeberg medial calcification is more prevalent on the tibio-fibular trunk and the anterior tibial artery. Examinations of the amputated legs prove that the calcification of the media is usually present in diabetic patients, more frequently than the arterial obstruction [21].

Diabetes affects not only the large vessels, but it also produces microvascular lesions, which in time leads to diseases like retinopathy or nephropathy. Diabetic micro-angiopathy from the histological point of view is seen to present hyaline material deposits, Periodic Acid–Schiff (PAS)-positive, surrounding the arterioles and capillaries of the lower limbs. Due to the PAS-positive deposits in skin and muscles of the legs examined in amputated limbs, it has emerged the so-called lesions of small vessels or peripheral diabetic microangiopathy, similar to the hyaline deposits in the retina and kidney [22].

In order to fully understand the burden of diabetes on the arterial health, studies demonstrate that diabetes bypasses the protective cardiovascular effects of estrogens. The risk of cardiovascular diseases is two times higher in male patients with diabetes than in the general population and three times higher in female patients. This explains the high number of women with diabetes who need to undergo lower limbs amputations during the natural evolution of the disease [23].

Osteomyelitis of the diabetic foot

Osteomyelitis is very common in the diabetic foot infections and the medical treatments are not satisfying. It is also believed to be a consequence of peripheral neuropathy that diabetes comes with. The pathogen, usually *Staphylococcus aureus* from the skin crosses the epidermal layer barrier through the diabetic lesions, such as open wounds or ulcers and spreads from the soft tissue to the bone. Due to diabetes, the tissues have poor immunity response and local defense [24].

As a treatment, it is preferred the surgical treatment, followed by histopathological and microbiological examination. Surgical debridement and resection of the infected bone is the best option, when possible [25]. Afterwards antimicrobial therapy targeted against pathogens isolated from the surgically removed bone pieces should be continued for six weeks after amputation [26].

An exact diagnosis of foot osteomyelitis is a challenge. Even bone probes testing in diabetes has a positive predictive value varying from 53% in outpatients to 97% in hospitalized patients. Its accuracy as a diagnostic instrument can be debated, according to the referred histological characteristics taken into consideration. Due to the problems of reaching a positive diagnosis of osteomyelitis, treatment approaches have an important delay [27].

Osteomyelitis plays an important role in the prevalence of amputations in patients with diabetes. Obtaining clean, infection free margins is the most important goal, because residual osteomyelitis is a strong predictor of clinical failure and comes with many postoperative complications, even the necessity to operate again or have a major amputation later in evolution [28, 29]. Although with the right treatment conducted carefully, osteomyelitis can be satisfyingly managed.

Conclusions

Chronic inflammation associated with diabetes and pressure lesions promotes the development of hyperkeratosis, cell death and accumulation of cell debris. The large number of patients affected and the impressive consumption of resources required for the treatment of infections of the diabetic foot have a major socio-economical impact. Amputation remains a therapeutic approach when the infection cannot be controlled and becomes life threatening for the patient. The amputation rate can be reduced by over 50% if the preventive sanitary and hygienic education measures are applied regularly and correctly, if peripheral vascular disease is early diagnosed and is a multidisciplinary approach is adopted in case of foot injury.

Conflict of interests

The authors declare no conflict of interests.

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