Specific features of a rare form of disseminated necrobiosis lipoidica granuloma annulare type: a case report

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Abstract
Necrobiosis lipoidica (NL) is a rare dermatosis that has been shown to precede the onset of diabetes mellitus in 15% of patients. It is more common in women; the average age of the onset is 30 years. Skin lesions of classic NL begin as a small papule, typically on the bilateral pretibial area. Progressively, these papules become indurated plaques with an atrophic, yellow center and multiple telangiectatic vessels, and brown-violaceous borders. We present the case of a 57-year-old male with type II diabetes mellitus from 2004, class II obesity, hepatosteatosis and metabolic disturbances who presents a disseminated eruption from 2010, formed by indurated plaques with flattened centers and a tendency of atrophy and raised, sharply demarcated, red-violaceous borders, having a variable diameter between 1.5 and 5 cm. The laboratory findings revealed elevated glucose levels between 250 and 300 mg%, high levels of transaminases and lipids. Diagnosis of disseminated necrobiosis lipoidica granuloma annulare-type was histopathologically confirmed. For the treatment, we have used topical corticosteroids and Tacrolimus 0.1% and systemic vasodilators. We consider this rare case interesting because has some peculiarities, as: disseminated lesions extend over the characteristic areas for necrobiosis lipoidica, the characteristic features of lesions similar to granuloma annulare, gender (male) and the onset of the disease over fifth decade.

Keywords: disseminated necrobiosis lipoidica, diabetes mellitus, microangiopathy.

Introduction
The first case of necrobiosis lipoidica was reported by Oppenheim, in 1929, in a patient with diabetes mellitus and it was called “dermatitis atrophicans lipoidica diabetica” [1]. In 1932, Urbach renamed the disease necrobiosis lipoidica diabeticorum (NLD). In the following years, were reported other cases of necrobiosis lipoidica diabeticorum in non-diabetic patients, so in 1960, Rollins and Winkelmann suggested to exclude the term “diabetes”, renaming the disease as “necrobiosis lipoidica”. It is a rare disorder, more frequent in a patient with insulin dependent diabetes mellitus. The disease shows no race predilection, but is more common in women, with female/male ratio of 3:1 [2]. The average age of onset is 30 years, and rarely can occurs from infancy to the eighth decade [3, 4].

Classically, the disease affects young women suffering from diabetes mellitus, the lesions being typically located on the pretibial area, rarely on the upper limbs, face or scalp [5]. Therefore, we present a rare form of necrobiosis lipoidica with disseminated lesions localized on the upper arms, sparing the legs, occurring in a 57-year-old male previously diagnosed with type II diabetes mellitus.

Patient, Methods and Results
Patient, 57-year-old male from urban area, with a history of type II diabetes mellitus insulin-dependent from 2004, checks in with an asymptomatic, disseminated eruption from 2010 formed by round, erythematous-violaceous and infiltrative plaques with yellowish, atrophic center especially on the upper extremities. The onset of the eruption was in 2007 with well-circumscribed, erythematous papules, 3–4 mm in diameter on the bilateral pretibial area that healed with atrophic scars. After three years, the lesions appeared on upper extremities as infiltrative, reddish brown plaques with yellowish, atrophic centers.

The patient has a history of chronic bronchitis since 2006, hepatosteatosis, chronic alcoholic, class II obesity (body mass index 38.6 kg/m²) and dyslipidemia. He also had a family history of stroke, liver carcinoma and diabetes mellitus.

The local examination revealed a disseminated eruption formed by round, infiltrative plaques, isolated or conflated, with tendency to symmetry on the upper extremities (Figure 1) with comedo-like plugs in the lesion. On the left forearm, the lesions expand to form a 7/5 cm plaque.
with polycyclic borders. On the left dorsal hand, the plaques had a variable diameter between 1.5–2.5 cm slightly elevated, red-violaceous borders, yellowish, atrophic centers, telangiectatic vessels and fine, adherent scales on the surface (Figure 2).

The laboratory findings revealed elevated glucose levels between 250 and 300 mg%, high levels of transaminases (ASAT 65 IU/L, ALAT 75 IU/L), erythrocyte sedimentation rate (ESR) 35 mm, cholesterol 265 mg/dL, triglycerides 196 mg/dL, total lipids 816 mg/dL.

After obtaining informed consent of the patient was performed local anesthesia with Lidocainum 1% and afforded a skin specimen of 0.3/0.5 cm from the lesion. This skin fragment was immediately fixed in neutral formalin 10% and included in paraffin using the current technique. Sectioning of biological material was made at Microm HM350 microtome equipped with a rotary transfer system sections in a water bath (STS, Microm). For histological study were used classical stains of Hematoxylin–Eosin (HE) and trichrome Goldner–Szekely (GS).

Histopathological exam showed atrophic epidermis, a central area of necrosis surrounded by inflammatory response in the deep dermis (Figure 3). The inflammatory infiltrate shows epithelioid cells with imprecise borders, abundant cytoplasm, lymphocytes and a few capillaries with turgescent endothelium and reduced lumen (Figures 4 and 5). The epithelioid cells are arranged in palisades around the connective tissue and are separated by fibrosis. The collagen was pale, fragmented and disorganized (Figure 6). The trichrome GS staining revealed abundant lymphocytic inflammatory infiltrate around the sebaceous gland (Figure 7) and altered collagen fibers. At the dermal–subcutaneous junction, a perivascular moderate inflammatory infiltrate formed by plasma cells – specific feature of NL –, is present (Figure 8).

For the immunohistochemical study were performed 4-μm thick sections, which were collected onto slides coated with poly-L-Lysine and after this they were kept to thermostat at 37°C for 24 hours to increase the adhesion of biological material to the slides. Then, following dewaxing and hydration of histological sections, the biological material was incubated for 30 minutes in a solution of 3% hydrogen peroxide. The sections were then washed in tap water and then have been cooked in the microwave for antigen unmasking, in a solution of sodium citrate, pH 6, for 21 minutes (seven cycles of 3 minutes). After boiling, they were allowed to cool for 15 minutes and washed in phosphate-buffered saline (PBS) for 5 minutes. The non-specific sites were blocked using 2% skim milk for 30 minutes. The sections were incubated overnight with the primary antibody in a refrigerator at 4°C. In the next day, biotinylated secondary antibody was applied (αMs/αRb) for 30 minutes followed by Streptavidin-HRP passage for 30 minutes. The signal was detected with 3,3'-Diaminobenzidine (DAB) (Dako) and was performed contrasting with Hematoxylin.

In our study, we used the following antibodies (Table 1):

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Code</th>
<th>Clone</th>
<th>Antigen retrieval</th>
<th>Specificity</th>
<th>Dilution</th>
<th>Source</th>
</tr>
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<tr>
<td>CD20</td>
<td>M0755</td>
<td>L26</td>
<td>Sodium citrate buffer, pH 6</td>
<td>B-lymphocytes</td>
<td>1:100</td>
<td>Dako</td>
</tr>
<tr>
<td>CD3</td>
<td>A0452</td>
<td>F7.2.38</td>
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<td>T-lymphocytes</td>
<td>1:100</td>
<td>Dako</td>
</tr>
<tr>
<td>CD68</td>
<td>M0814</td>
<td>KP1</td>
<td>Sodium citrate buffer, pH 6</td>
<td>Macrophages</td>
<td>1:200</td>
<td>Dako</td>
</tr>
<tr>
<td>CD34</td>
<td>M7165</td>
<td>QBEEnd 10</td>
<td>Sodium citrate buffer, pH 6</td>
<td>Vascular endothelium</td>
<td>1:50</td>
<td>Dako</td>
</tr>
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The immunohistochemical study revealed a chronic inflammatory infiltrate (Figures 9 and 10) present in the lower two-thirds of the dermis, rich in lymphocytes and histiocytes (epithelioid) cells (Figure 11), with a tendency to organize in a nodular fashion, located mainly around the altered connective tissue, sebaceous glands and blood vessels (Figure 12).

Based on the clinical, laboratory findings and histopathological examinations we established the diagnosis of disseminated necrobiosis lipoidica granuloma annulare-
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Type II diabetes mellitus insulin-dependent, class II obesity, dyslipidemia and hepatosteatosis.

Choosing the right method of treatment was difficult due to the patient’s comorbidities. The patient received systemic treatment with vasodilators (Pentoxifyllinum 400 mg, twice daily), topical corticosteroids in occlusive dressings and topical Tacrolimus 0.1%. Under treatment, the disease evolution slightly improved with partial disappearance of the skin lesions.

Figure 3 – Necrobiosis lipoidica: atrophic epidermis and necrosis area surrounded by inflammatory infiltrate. HE staining, ×100.

Figure 4 – Necrobiosis lipoidica: inflammatory infiltrate with epitheloid cells, lymphocytes and capillaries. HE staining, ×200.

Figure 5 – Necrobiosis lipoidica: details. HE staining, ×200.

Figure 6 – Necrobiosis lipoidica: epithelioid cells arranged in palisades around the altered connective tissue. HE staining, ×40.

Figure 7 – Necrobiosis lipoidica: image of sebaceous gland with abundant lymphocytic inflammatory infiltrate around the gland. Trichrome GS staining, ×100.

Figure 8 – Necrobiosis lipoidica: inflammatory infiltrate with plasma cells at the dermal epidermal junction. HE staining, ×200.
Discussion

The etiopathogenesis of the disease is elusive, possible theories being given. Because of the strong relationship between diabetes and NL, many studies have focused on the implication of diabetic microangiopathy in the etiology of both diseases. A deposit of glycoprotein in blood vessel walls may be the cause of diabetic microangiopathy [6]. The vasculitic process based on the deposition of IgM immunoglobulin, the third component of complement and fibrinogen in the blood vessel walls and in the dermal epidermal junction suggested that the antibody-mediated vasculitis may initiate the blood vessel changes and subsequent lesions of necrobiosis lipoidica [6, 7]. An additional etiologic theory focuses on the abnormal collagen in necrobiosis lipoidica lesions. The basement membrane thickening in NL could be explained by higher lysyl-oxidase levels in some diabetic persons, which are responsible for increased collagen cross-linking [7]. In present, trauma is considered a possible etiologic agent, patients who developed typical lesions of NL in scars and also at insulin injection sites were reported [8]. Another possible etiology is represented by inflammatory and metabolic changes, the granuloma formation in NL, being probably determined by an increased number of macrophages due to impaired neutrophil migration [5].

In our case, we considered that the NL appeared because of the diabetes mellitus and dyslipidemia, but we could not correlate the extent of the cutaneous eruption beyond the classic affected areas to trauma, such as repeated insulin injections.

Clinically, skin lesions of classic necrobiosis lipoidica begin as erythematous, 1 to 3 mm, well-circumscribed papules that expand centrifugally with active borders, to form reddish brown, sharply demarcated, patches or plaques with yellow, atrophic, shiny (porcelain-like appearance) centers with telangiectatic vessels on surface. The necrobiosis lesions usually occur on the bilateral pretibial area, and rarely could be disseminated on the face, scalp, trunk, upper extremities, and penis. In evolution, the lesions can remit spontaneously (20% of the cases), but scars and residual atrophy are present [4]. Painful ulceration can occur in approximately 35% of cases, most of it being triggered by trauma. Rarely, the lesions can degenerate into squamous cell carcinoma [9, 10].

In our case, the cutaneous eruption arose typically...
on the pretibial area, but in evolution, the lesions affected forearms and the dorsum of the hands.

There are, however, some atypical forms that can be hard to recognize:

Granuloma annulare form, which present annular lesions that resemble granuloma annulare [4, 5].

Xanthomas form has yellow annular lesions of necrobiosis lipoidica with a fatty infiltration that can resemble xanthomas, especially when the lesions are not localized on the lower limbs [5].

Sarcoidosis form can appear clinically and histologically like necrobiosis lipoidica, and the lesions occur on the upper half of the body, on the face and on the scalp [11].

Miescher’s granulomatosis or granulomatosis disciformis chronica et progressiva with bilateral and symmetrically lesions localized on the lower limbs. Are not associated with diabetes mellitus.

Laboratory findings are not helpful in the diagnosis of necrobiosis lipoidica. Still, is necessary checking for glucose intolerance to evaluate for the presence or absence of diabetes mellitus.

Because our patient was a chronic alcohol consumer has always had high blood sugar levels associated with elevated transaminases and dyslipidemia.

The histopathological exam remains the main investigation that confirms the necrobiosis lipoidica diagnosis but direct immunofluorescence and electron microscopy can also be useful. The necrobiosis lipoidica lesions have a very characteristic appearance: the interstitial and palisaded granulomas are arranged in a tier-like (layered) fashion and are admixed with areas of collagen degeneration. The necrobiosis areas are irregular aspect and are surrounded by histiocytes and multinucleated Langhans’ cells or foreign body giant cells. The granulomas are composed of histiocytes (some of them multinucleated), lymphocytes, occasional plasma cells, and eosinophils. The vascular changes are more prominent in diabetic patients and are the following: superficial vessels increased in number, and in the deep vessels intimal proliferation, thickening of their walls with PAS-positive foci, epithelioid granulomas may be present in or adjacent to blood wall. A very important observation is the finding of atypical plasma cells or very dense plasma cell infiltration. In such cases, the lesion is associated with plasma cell dyscrasia and circulating monoclonal antibodies [1, 5, 6].

In the case of our patient, histopathological exam showed a central area of collagen necrosis surrounded by inflammatory response in the deep dermis. The inflammatory infiltrate shows epithelioid cells, lymphocytes and a few capillaries with turgid endothelium and reduced lumen. The epithelioid cells are arranged in palisades around the connective tissue and are separated by fibrosis. The trichrome GS staining revealed abundant lymphocytic inflammatory infiltrate around the sebaceous gland and altered collagen fibers. At the dermal–subcutaneous junction, a perivascular moderate inflammatory infiltrate formed by plasma cells, a specific feature of NL, is present.

Direct immunofluorescence microscopy of necrobiosis lipoidica shows vascular thickening due to immunoglobulin M (IgM), IgA, C3, and fibrinogen in the blood vessels [7]. Electron microscopy reveals loss of cross-striation of collagen fibrils and a marked variation in the diameter of individual collagen fibrils [7].

In our case of disseminated NL granuloma annulare-type, demonstrated immunohistochemical staining for granuloma-forming cells, especially focusing on IL-17-producing cells and CD163+ proinflammatory macrophages. The mean CD34+ microvessel density per mm² in the granuloma annulare group was 79.04, which was significantly higher than in the NL group (64.84; p=0.009) and in the controls (52.03; p<0.001). The obtained results confirm the similarity of the histological features of NL and granuloma annulare. However, in granuloma annulare, the biopsy changes in angiogenesis were more marked in the granuloma annulare than in the NL group.

In conclusion, imbalance in the process of angiogenesis is one of the factors involved in development of both NL and granuloma annulare [12].

Differential diagnosis can be made with the following: granuloma annulare, sarcoidosis, xanthomas, necrobiotic xantogranuloma, rheumatoid nodules, localized scleroderma.

Granuloma annulare is clinically characterized by small, firm, reddish brown, asymptomatic papules or nodules grouped in annular rings with centrifugal extension. The disease is particularly common in those individuals with a strong family history of diabetes mellitus and the lesions are usually localized to the dorsal hands, elbows, knees, ankles and fingers [4]. Histologically, degenerative collagen within the middle dermis, but also in lower dermis and hypodermis in subcutaneous forms is characteristic [5].

Sarcoidosis is characterized by slightly elevated, round to oval, red-brownish, firm or pasty plaques with smooth, telangiectatic surface. The eruption usually affects the scalp, the face, the trunk, the shoulders, the extremities and the buttocks. When they are located on the lower extremities, they can resemble necrobiosis lipoidica. Histopathologically, dermal and hypodermal, well circumscribed nodules formed by aggregates of epithelioid cells, giant cells and lymphocytes that can arranged like a mantle, are present [11].

Xanthomas can clinically resemble necrobiosis lipoidica but telangiectatic vessels and atrophy are missing [2, 5].

Necrobiotic xantogranuloma (NXG) is a rare disorder that can mimic necrobiosis lipoidica clinically and histologically. Clinically, is characterized by yellow or red-orange, indurated plaques that most commonly involves the periorbital area and the trunk. Histopathologically, areas of hyaline necrobiosis surrounded by granulomatous structures, a palisade of histiocytes and giant cells (Touton cells) are present in the dermis along with lymphoid follicles and perivascular lympho-plasmacytic infiltrate [1, 2].

Rheumatoid nodules appear most frequently as subcutaneous nodules rather than as atrophic plaques, occurring over extensor surfaces of the hands, elbows, knees, ankles and fingers. Histopathologically, they appear like non-specific granulomas with a central necrotic areas with fibrinoid degeneration of collagen fibers and histiocytes, lymphocytes and plasma cells arranged “in palisade” at the periphery [1].
Localized scleroderma is clinically characterized by 1 to 20 cm in diameter, well-circumscribed, pearly white, indurated plaques. The histological exam reveals induration and thickening of the collagen filaments [2, 5].

Rarely, necrobiosis lipoidica can resemble with the following: nodular vasculitis, stasis dermatitis, erythema nodosum, erythema induratum, lichen scleroatrophic, tertiary syphilis, radiodermatitis, subacute nodular migratory panniculitis, Hansen disease [5].

Until now, there is no completely effective treatment for necrobiosis lipoidica, partially because the exact etiology remains unknown. When a treatment is required, several therapeutic methods can be used with controversial results.

Corticosteroid therapy, usually in occlusive dressings and intraleisional steroids in short courses decrease inflammation and limited extent of the lesions [13]. Drugs to improve the blood flow, hemorheologic agents (Pentoxifylline), reduce blood viscosity by increasing fibrinolysis and red blood cell deformity [6, 13]. Antiplatelet agents, like Aspirin, Dipyridamole, Ticlopidine, heal ulcerated lesions of NL but not NL lesions [13].

Immunomodulators, anti-TNF (tumor necrosis factor)-alpha agents, such as Etanercept, Adalimumab, Infliximab, Thalidomide improves chronic, granulomatous, cutaneous changes [14–16]. Topical immunomodulators, Cyclosporin inhibits IL-2 production by T-helper cells, preventing T-cells clonal proliferation and decrease the immune response of NL. Mycophenolate Mofetil has a high cytostatic effect on lymphocytes and accelerates healing of ulcerated NL [17, 18].

In this case, we used a medium dosage of Pentoxifylline (800 mg/day) associated with occlusive corticosteroids for a week and Tacrolimus 0.1% ointment twice daily for a month with favorable results. We used topical corticosteroids in short use due to the well-known side effects of this treatment in the long term, high blood sugar levels and local atrophy being the most frequent. In 2004, Harth and Linse reported favorable outcome after applying Tacrolimus 0.1% ointment twice daily for a month [19].

Kreuter et al. reported some results with Fumaric Acid esters in a prospective non-controlled study [20].

There are some studies on photochemotherapy (UV-A, UV-B or PUVA) that proved good results on lesions of NL, while in others there are no improvements [21–26].

Other studies reported some benefits with bovine collagen gel applied topically or with wound healing growth factors: granulocyte–macrophage colony stimulating factors, Becaplermin gel (Regranex), recombinant human platelet-derived growth factors which promote proliferation of cells [5, 13].

Surgical treatment represented by excision and grafting of the necrobiosis lipoidica lesions, is recommended only in cases with ulceration resistant to treatment because of the high risk of relapsing and the poor healing of the graft site [1, 13].

Laser treatment determined overall cosmetic improvement with respect to erythema and telangiectasia and also realizes the stabilization of the lesions [27].

Other therapies: topical retinoids (Tretinoin), anti-hyperlipidemic drugs (Nicotinamide), antileprosy agents (Clofazimine) have antigranulomatous properties by inhibiting the mitogen-induced stimulation of the mononuclear cells from peripheral blood and stimulation of phagocytosis due to macrophages and neutrophils interaction, Benzoyl Peroxide, antimalarials (Chloroquine, Hydroxycloquine), which has immunosuppressive and anti-inflammatory properties, possibly via inhibition of macrophage migration, inhibition of prostaglandin synthesis or inhibition of platelet aggregation [13].

Conclusions

Disseminated necrobiosis lipoidica granuloma annulare type is an atypical, rare form of necrobiosis, frequently incorrect diagnosed. It is difficult to treat a disseminated clinical form of necrobiosis lipoidica in a patient with hyperglycemia, class II obesity, liver failure and metabolic disturbances.

Author contribution

All authors have contributed equally to the present work.

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