J Cutan Pathol 2009: 36: 1293–1298
doi: 10.1111/j.1600-0560.2009.01256.x
John Wiley & Sons. Printed in Singapore

Journal of
Cutaneous Pathology

Adipose tissue remodeling in lipedema: adipocyte death and concurrent regeneration

Lipedema is a disease with unknown etiology presenting as bilateral and symmetric enlargement of the lower extremities due to subcutaneous deposition of the adipose tissue. Here we describe the histopathological features of the lipedema tissue and nonaffected adipose tissue obtained from a typical patient with severe lipedema. Immunohistochemical analyses indicated degenerative and regenerative changes of the lipedema tissue, characterized by crown-like structures (necrotizing adipocytes surrounded by infiltrating CD68+ macrophages; a feature commonly seen in obese adipose tissue) and proliferation of adipose-derived stem/progenitor/stromal cells (Ki67+CD34+ cells), respectively. These findings suggested increased adipogenesis in the lipedema tissue, which may further lead to hypoxia similar to that seen in obesity, resulting in adipocyte necrosis and macrophage recruitment. The confinement to the lower extremities and the difference from systemic obesity warrants further elucidation in future studies.

Suga H, Araki J, Aoi N, Kato H, Higashino T, Yoshimura K. Adipose tissue remodeling in lipedema: adipocyte death and concurrent regeneration.
J Cutan Pathol 2009; 36: 1293–1298. © 2009 John Wiley & Sons A/S.

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Accepted for publication January 5, 2009

Lipedema is a disease of unknown etiology characterized by bilateral and symmetric enlargement of the buttocks and lower extremities due to subcutaneous deposition of the adipose tissue.[1] Although it is not a rare condition, lipedema is often misdiagnosed as lymphedema presumably because most clinicians are unaware of this disease.[2,3] Several authors have reported differences in features between lipedema and lymphedema, such as absence of skin thickening, minimal improvement with elevation or compression therapy, no swelling in the foot, and an uncommon history of cellulitis in lipedema.[1–5] Lipedema also differs from simple severe obesity in some aspects. First, lipedema predominately affects women.[1,5] Although the majority of lipedema patients are overweight, a significant number of them are of normal weight and have disproportionately enlarged lower extremities. In addition, those who were overweight reported no change in lower extremity size with dieting or weight loss.[5]

Histological analysis of the adipose tissue in lipedema shows edema with moderate hyperplasia, and most commonly reveals a lack of the fibrotic elements frequently seen in lymphedema.[1] Some reports describe abnormal histological findings in lipedema, such as microangiopathy as an early histological feature[1] and a high prevalence of microlymphatic aneurysms in affected limbs.[6] Another article describes abnormal elastic fibers with an increased number of mast cells in lipedema tissue.[7] Although these reports hint that aberrant cellular activity actively occurs in lipedema, detailed analyses of adipose tissue from lipedemic individuals at the cellular level have not been performed. Furthermore, there have been no reports that describe proliferative changes accounting for the enlargement of adipose tissue.

Here we describe a case of severe lipedema showing typical clinical features. Immunohistochemical comparative analyses of the affected and normal adipose tissues from the same patient revealed histopathological findings of cellular remodeling in the affected adipose tissue.

Case report

A 74-year-old Pakistani woman presented with bilateral enlargement of the lower extremities and buttocks that had gradually developed over 30 years. Although the patient suffered from no pain and had no history of cellulitis, she had gait difficulties due to the enlarged lower extremities (Fig.1). She had no other remarkable medical conditions or family history. Physical examination revealed massive bilateral enlargement of the lower extremities involving the buttocks without palpable subcutaneous indurations or dermal thickening. No abnormality was identified in the feet, and Stemmer's sign was negative: a skin fold at the base of the second toe lifted normally when pinched. Magnetic resonance imaging (MRI) revealed massive circumferential enlargement of the subcutaneous tissue. Together with these clinical findings, the patient was diagnosed with lipedema. Biopsy samples from the medial patellar fat pad (lipedema tissue) and inguinal subcutaneous tissue (nonaffected adipose tissue) were examined histologically. No prior trauma had occurred at the biopsy sites in the recent or remote past.

Methods

Harvested adipose tissues were zinc-fixed (Zinc Fixative; BD Biosciences, San Diego, CA) and paraffin-embedded. We prepared 6 – μm-thick sections and performed immunostaining using the following primary anti-bodies: mouse anti-human CD34 (clone QBEnd 10, dilution 1:500, Dako, Glostrup, Denmark), rabbit anti-human Ki67 (clone SP6, dilution 1:200, Thermo Fisher Scientific, Fremont, CA), and mouse anti-human CD68 (clone KP1, dilution 1:100, Dako). Isotypic anti-body was used to serve as a negative control for each staining. For visualization with diaminobenzidine (DAB), peroxidase-conjugated secondary anti-bodies appropriate for each primary anti-body (Nichirei Biosciences, Tokyo, Japan) were used. Nuclei were counterstained with hematoxylin. For a double fluorescence staining, the following secondary anti-bodies were used: Alexa Fluor 488-conjugated goat anti-mouse IgG and Alexa Fluor 568-conjugated goat anti-rabbit IgG (both dilution 1:200, Molecular Probes, Eugene, OR). Nuclei were stained with 4′, 6-diamidino-2-phenylindole (DAPI).

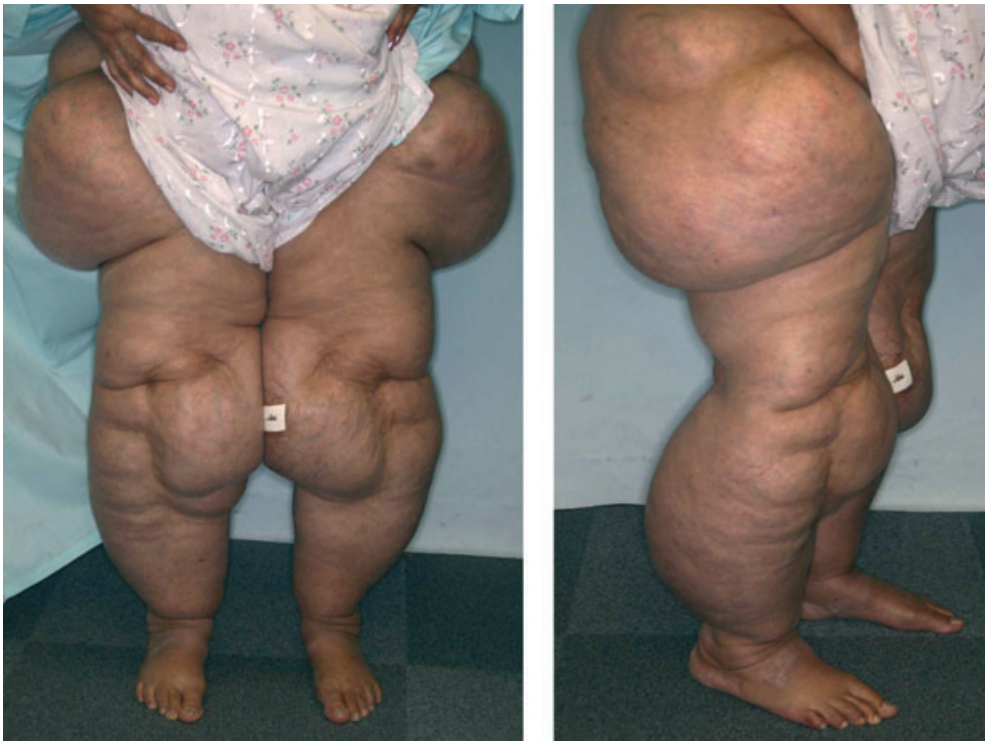

Fig. 1. Preoperative views of the patient. She presented with bilateral enlargement of the lower extremities involving the buttocks.

Observations

Hematoxylin and eosin staining of the excisional specimens showed edematous change in lipedema tissue compared with the normal adipose tissue (Fig. 2). Adipocytes in the lipedema tissue varied in size; large adipocytes with a diameter of more than 150 μm were frequently observed; in contrast, adipocytes in the normal adipose tissue were more uniform in size with an average diameter of approximately 100 μm. Though no apparent abnormality was seen in the vasculature, cellular infiltration and crown-like structures (adipocytes surrounded by macrophages; a typical finding in necrotic-like adipocyte death[8]) were observed in the lipedema tissue (Fig. 2). Immunohistochemistry for CD68 revealed that most of the adipocyte-surrounding cells in the crown-like structure were CD68+, suggesting that they were macrophages scavenging adipocyte debris that could ultimately form multinucleated giant cells[8] (Fig. 3). Furthermore, immunohistochemical staining for Ki67 showed that there were a large number of Ki67+ proliferating cells in lipedema tissue, while they were rarely seen in the nonaffected adipose tissue (Fig. 4). The Ki67+ proliferating cells were spindle-shaped (easily distinguished from macrophages by cellular shape and localization) and CD68−. Double immunostaining for Ki67 and CD34 revealed that most Ki67+ cells were also CD34+. In nonaffected adipose tissue, CD34 positivity, a marker for adipose stem/progenitor cells (ASCs), was observed between adipocytes or in the interstitial space, but the CD34+ cells were Ki67−(Fig. 5). These results suggest that degenerative changes of adipocytes are ongoing in lipedema tissue, while CD34+ ASCs are simultaneously proliferating and repairing the degenerative adipose tissue.

Discussion

This case presented with typical clinical features and MRI findings[2] of lipedema, allowing for a relatively straightforward diagnosis. Treatment options for lipedema are limited and controversial. Conservative therapies such as dieting, leg elevation, and compression seem to have minimal effect.[5] One report described that suction lipectomy with limited skin excision improved the contour and size of the tissue in some cases,[3] while another report does not recommend surgical treatments such as lipectomy or liposuction because of the possible risk of secondary lymphedema.[1]

Detailed histological analyses of the affected tissue revealed degenerative changes of adipose tissue characterized by accumulation of macrophages and the formation of crown-like structure. Interestingly, these changes are similar to the findings observed in obese mice and humans.[8–10] In obesity, increased adipocyte size correlates with the frequency of

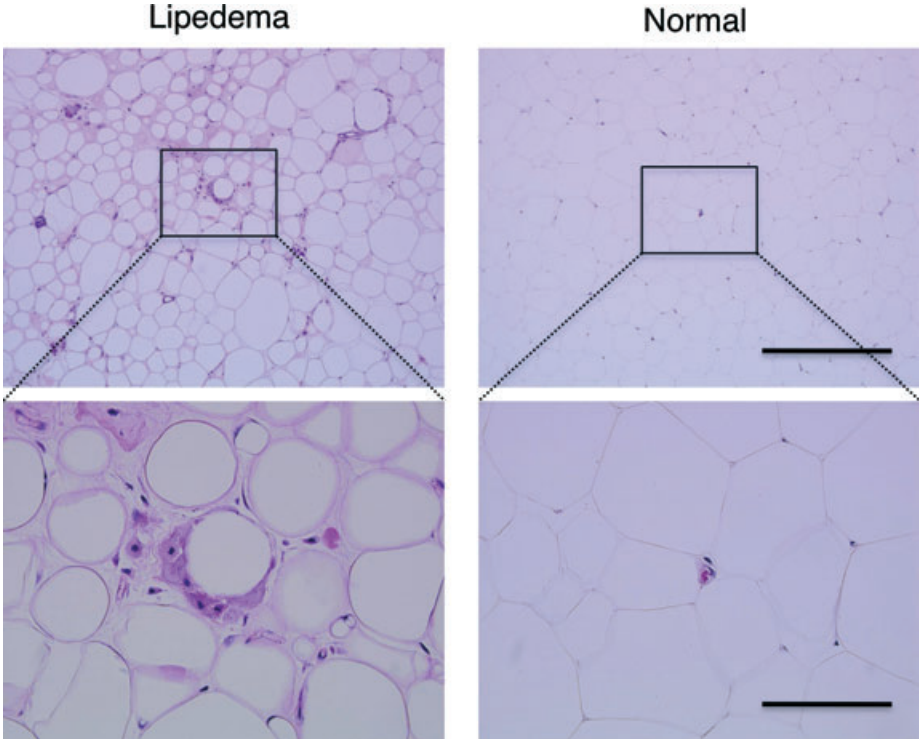

Fig. 2. Hematoxylin and eosin. Edematous adipose tissue, large adipocytes, and crown-like structures were observed in lipedema. Scalebar = 400 μm (top panels) and 100 μm (bottom panels).

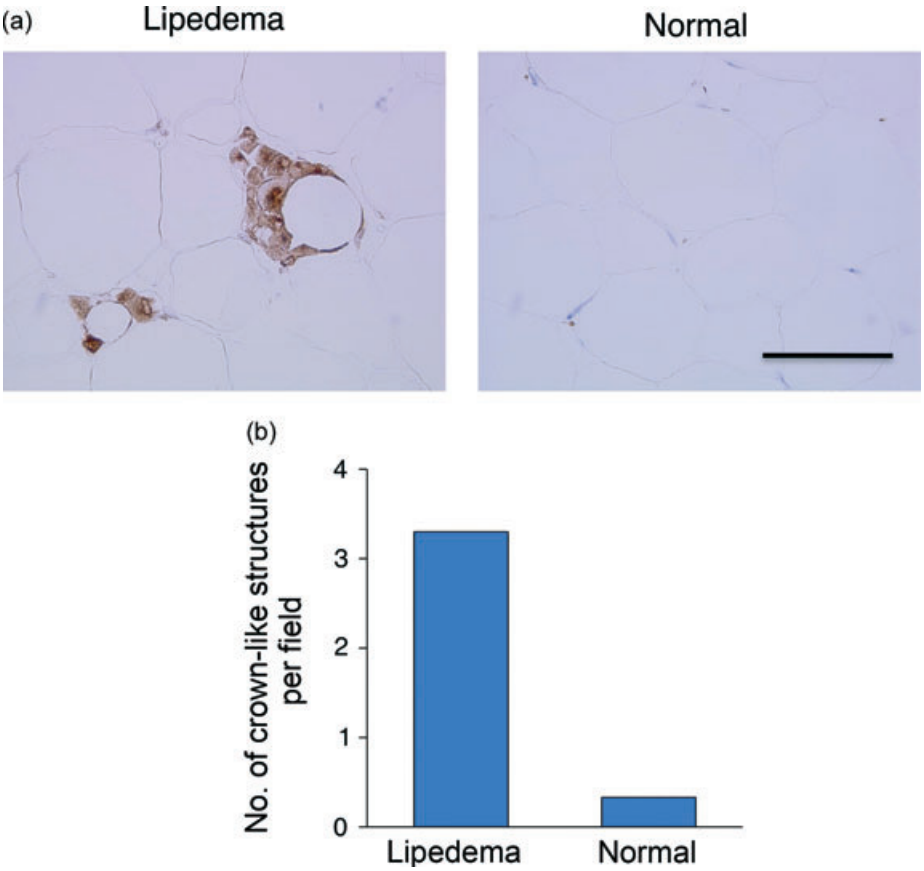

Fig. 3. a) Immunohistochemistry for CD68. CD68+ cells surrounded a necrotic adipocyte in the crown-like structures frequently seen in lipedema tissue. Scale bar = 100 μm. b) Number of crown-like structures per field. Crown-like structures were counted using three randomly selected fields (×100).

adipocyte necrosis which results in the recruitment of macrophages;[8] these infiltrative macrophages play a crucial role in the development of obesity-related insulin resistance.[10] In lipedema tissue, it was suggested that some adipocytes undergo necrosis and are subsequently scavenged by recruited macrophages, similar to what is seen in obesity. Although the cause of adipocyte death in both obesity and lipedema remains unclear, recent studies have identified adipose hypoxia and dysregulation of adipose function in obesity.[11,12] Hypoxia is most likely induced by excessive hypertrophy and may be a key factor responsible for degenerative changes such as adipocyte death and subsequent macrophage accumulation in lipedema as well. Some previously reported histological findings such as microangiopathy,[1] microlymphatic aneurysms,[6] and abnormal elastic fibers with an increased number of mast cells[7] may all be associated with these degenerative changes secondary to hypoxia.

Another interesting finding is an increase of cells positive for both Ki67 and CD34; such proliferative change in lipedema has not been previously reported in the literature. ASCs have been reported as CD34+ cells in the adipose tissue of mice and humans.[13–17] ASCs not only function as tissue-specific progenitors[16–18] but are also multipotent[13,14] and secrete angiogenic growth factors such as hepatocyte growth factor (HGF) under certain circumstances.[15] It was recently revealed that adipose tissue has a very slow rate of turnover[18] and its perivascular progenitor cells (namely ASCs) differentiate into replacement adipocytes.[14,17] Thus, ASCs play important roles in physiological turnover,[18] hyperplasia (obesity), and atrophy of adipose tissue, as well as in incidental remodeling, such as post-injury repair.[15] Increased proliferation of ASCs in lipedema suggests increased adipogenesis in the affected adipose tissue, which accounts for the massive enlargement of adipose tissue in lipedema. The rapid increase in adipogenesis could lead to hypoxia as seen in obesity, resulting in adipocyte necrosis and macrophage recruitment as described above. The mechanisms underlying confinement to the lower extremities and the difference of lipedema from systemic obesity should be elucidated in future studies.

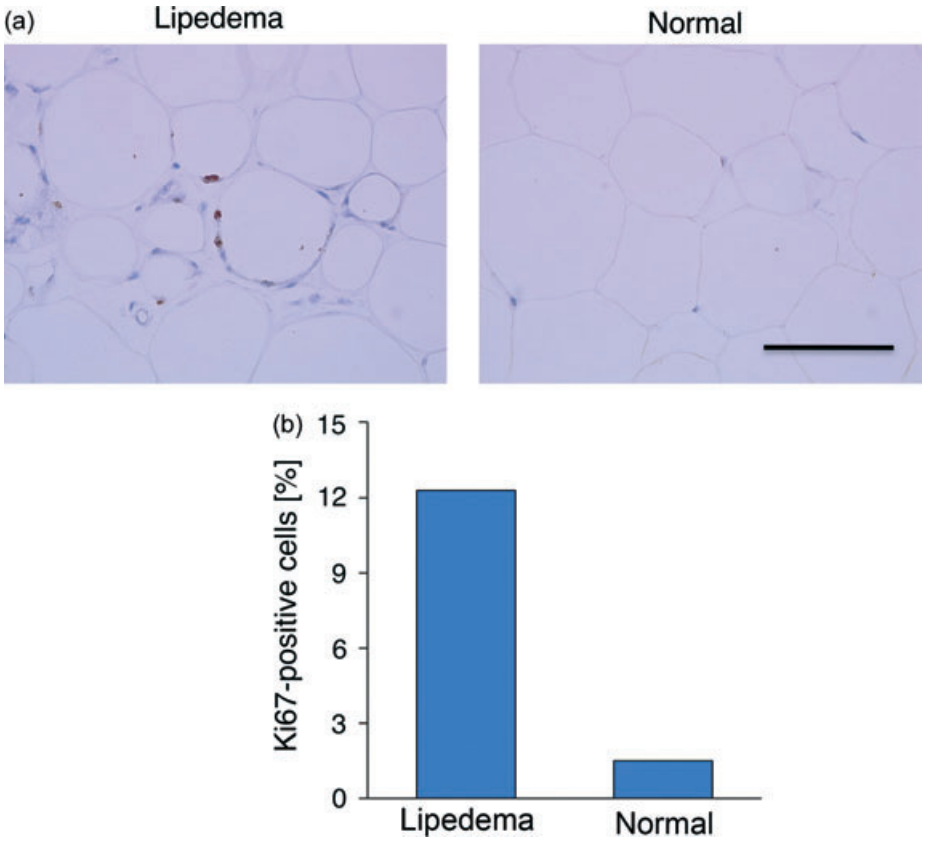

Fig. 4. a): Immunohistochemistry for Ki67. There were a higher number of Ki67+ cells in lipedema. Scale bar = 100 μm. b) Percentage of Ki67+ cells. Ki67+ cell number and total cell number were counted using three randomly selected fields (×100).

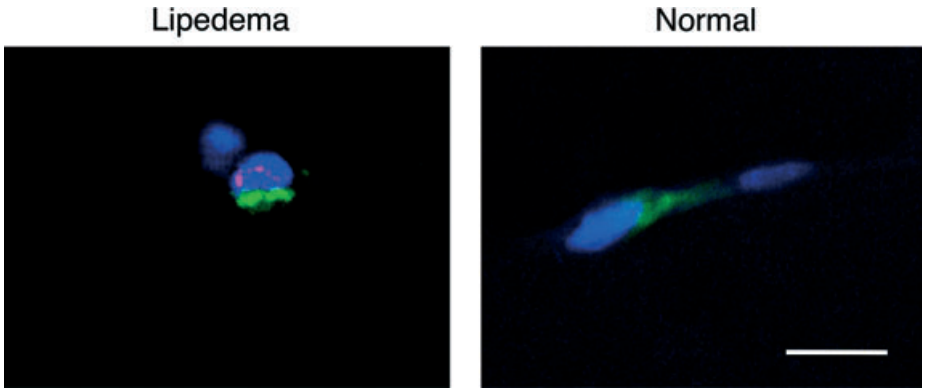

Fig. 5. Double staining for Ki67 (red) and CD34 (green). Ki67+ cells in lipedema tissue were also positive for CD34. Nuclei were stained with 4′, 6-diamidino-2-phenylindole (DAPI) (blue). Scale bar = 10 μm.

Conclusions

Comparative immunohistochemical analyses of lipedema and intact adipose tissue indicated concurrent degenerative and regenerative changes of the lipedema tissue, characterized by necrotizing adipocytes and proliferation of adipose-derived stem/progenitor/stromal cells, respectively. These findings suggested increased adipogenesis in lipedema tissue, which may further lead to hypoxia similar to that seen in obesity, resulting in adipocyte necrosis and macrophage recruitment.

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