

CAN OBSTRUCTIVE INTRALYMPHATIC GRANULOMAS BE THE CAUSE OF CHEILITIS GRANULOMATOSA?

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ABSTRACT

Cheilitis granulomatosa (ChG), also known as Miescher's cheilitis, is an uncommon, immunologically mediated non-necrotizing granulomatous inflammatory disease characterized by recurrent, painless swelling of the lips. The aim of the study was a pathomorphological and immunohistochemical assessment of cases clinically classified as ChG to investigate potential pathological mechanisms of ChG symptoms and to verify the hypothesis of intravascular granulomas as a cause of lymphatic vessel obstruction and localized edema in ChG. We report 6 patients with ChG who clinically presented localized edema of the lips. Lip biopsy with pathomorphological and immunohistochemical examination was performed in all cases. We found discrete, non-necrotizing granulomas which were adjacent to numerous blood and lymphatic vessels. The lumen of lymphatic channels was dilated and was either empty or contained lymph and few macrophages or was completely occluded by nearby granulomas. All patients demonstrated a characteristic pattern of lymphangiectasia and perivascular lymphatic aggregates with evidence of non-necrotizing granulomas. None manifested intralymphatic granulomas. These results do not support the view that lymphatic vessel obstruction is caused by intravascular

histiocytic granulomas described as the main part in the etiology of lymphatic edema in ChG. However, perivascular granulomas and dilation of lymphatic vessels confirm presence of inflammatory lymphostasis in all studied cases of ChG.

Keywords: cheilitis granulomatosa, Melkersson-Rosenthal syndrome, intralymphatic granulomas, lymphatic obstruction, histology

Cheilitis granulomatosa (ChG), also known as Miescher's cheilitis, is an uncommon, immunologically mediated non-necrotizing granulomatous inflammatory disease characterized by recurrent, painless swelling of the lips that can mimic angioedema. The swelling is transient and initially affects the upper lip. In some cases it may affect both lips, causing discomfort and disturbing the sense of aesthetics. It is considered as a manifestation of orofacial granulomatosis (1). ChG may occur as an isolated sign or may be one of the three basic symptoms characterizing Melkersson-Rosenthal syndrome (MR): cheilitis, lower motoneuron facial nerve palsy, and fissured tongue (2,3). The pathomechanisms and causal agents of the two varieties of ChG are unknown, which results in lumping together both ChG and MR cases as one group of

granulomatous inflammations with immunological background (4). Diagnosis of the isolated ChG has to involve exclusion of such morbidities as Crohn disease, sarcoidosis, Quincke's edema, a foreign body reaction, and infection. Crohn disease, however very rarely, can present its initial manifestations in facial/labial localizations, and sarcoidosis located on the lips may be clinically identical with ChG. In rare instances, histoplasmosis and tuberculosis may also display orofacial localization. Differential diagnosis and management require a skillful interpretation of clinical and pathomorphological findings (5). Microscopic examination is essential for establishing the final diagnosis of ChG. Subsequently, presence of additional clinical manifestations allows identification of MR syndrome. In the case of ChG, pathomorphological examination shows granulomatous inflammation, macrophage, lymphocyte, and plasmocyte perivascular infiltrates as well as small non-caseating granulomas distributed near vessels. There are also reported cases of ChG with intravascular granulomas in dilated lymphatic vessels (6-9). Such localization of granulomas might be responsible for dynamic changes of edema, appearance of lymphedema and restoration of lymphatic flow proved by lymphoscintigraphy in patients undergoing clinical improvement (10).

The aim of this study was pathomorphological and immunohistochemical assessment of cases clinically classified as ChG to investigate potential pathological mechanism and to verify the hypothesis of intravascular granulomas as a cause of lymphatic vessel obstruction and localized edema in ChG.

MATERIALS AND METHODS

Patients

We report 6 patients who clinically presented localized edema of the lips. Patients were hospitalized in Department of Internal Disease and Allergology at Wroclaw Medical University where they underwent lip biopsy.

The demographic data and medical history of patients are presented in *Table 1*.

Pathomorphological and Immunohistochemical (IHC) Examination

Samples were fixed in 4% buffered formalin, dehydrated, and embedded in paraffin. The paraffin blocks were cut into 7 μm sections and stained with hematoxylin and eosin to initiate diagnosis. For IHC reactions, 4 μm -thick paraffin sections were cut, sections dewaxed, re-hydrated, and the epitopes were exposed using Pre-Treatment Link Rinse Station in Target Retrieval Solution (pH 6 for Ki-67; pH 9 for the rest of the markers; 97°C, 20 min). Immunohistochemical reactions were performed in Autostainer Link 48, using the visualization system of EnVision™ FLEX+. Activity of endogenous peroxidase was blocked by 5 min exposure to Peroxidase-Blocking Reagent. Sections were then rinsed with Wash Buffer and incubated for 20 min at room temperature with primary antibodies against all tested markers, i.e., Ki-67, CD68, CD31, Podoplanin, and Sox-18 (described in *Table 2*). All antibodies were applied in ready-to-use dilutions, except anti-Sox-18 (1:50). Secondary goat anti-rabbit antibodies (HRP) were coupled to a dextran core and linked to peroxidase. The color reaction was obtained using 3,3'-diaminobenzidine tetrachlorohydrate (DAB+Chromogen) and all slides were counterstained with Mayer's hematoxylin. All reagents, equipment, and antibodies, except Sox-18 (Abcam, Bristol, United Kingdom), were obtained from DakoCytomation, Glostrup, Denmark.

Evaluation of IHC Reaction

For evaluation, observations were conducted under x100, x300, x400 magnification and scoring for brown-labeled nuclei or cytoplasm expressing tested markers (BX-43 light microscope equipped with Cell^D software for computer-assisted image

TABLE 1
Demographic Data and Medical History of Study Patients

Initials	Age (years)	Sex	Date of first symptoms	Symptoms of ChG	Medical history	Follow-up
R-P	52	F	2009	lip swelling and erythema, metallic taste, mouth dryness	aphtosis, lip sores, tinnitus, tetany, contact allergy to Nickel	constant lip swelling
Z-Z	25	M	2010	recurrent upper lip swelling	none	recurrent upper lip swelling
M-H	59	F	2008	recurrent lip swelling, cheek edema	cholelithiasis, gastroduodenitis, contact allergy to Nickel and fragrance mix, psoriasis	recurrent lip swelling
M-D	35	F	2000	constant, fluctuating upper lip swelling	Wolff-Parkinson-White syndrome, atopy (dog and cat dander).	constant lip swelling, stomach tumor (GIST diagnosed in 2013)
B-Z	27	F	2009	constant upper lip swelling	none	lip swelling
S-W	44	F	2010	recurrent upper lip swelling	crural varicosis	recurrent upper lip swelling

analysis; Olympus, Tokyo, Japan). The intensity of the IHC reactions in coded preparations was independently evaluated by two pathologists. In the case of a discordant evaluation, a re-evaluation with a double-headed microscope was performed until a consensus was achieved.

RESULTS

Histopathological examination showed a keratinized squamous stratified epithelium with underlying connective tissue (*Fig. 1A*; hematoxylin and eosin). In the basal layer of the epithelium active proliferation was visualized (*Fig. 1B*; Ki-67 antigen). The subjacent connective tissue showed diffuse

edema and infiltration of macrophages forming perivascular aggregates (*Fig. 1C*; CD68). Those discrete, non-necrotizing granulomas were adjacent to numerous blood and lymphatic vessels (*Figs. 1D,E,F*; CD31, Podoplanin, Sox-18, respectively). The lumen of lymphatic channels was dilated and was either empty, or contained lymph and few macrophages, or was completely occluded by nearby lying granulomas. All specimens demonstrated a characteristic pattern of lymphangiectasia and perivascular lymphatic aggregates with evidence of non-necrotizing granulomas. None of them manifested intralymphatic granulomas.

DISCUSSION

TABLE 2
Description of Markers Utilized for Immunohistochemical Examination

Marker	Labels	Description
Ki-67	proliferation	The antigen is expressed during all active phases of the cell cycle (G1, S, G2 and M-phases), but absent in resting cells (G0-phase). In diagnostic histopathology its valuable for allowing direct monitoring of the growth fraction of tested cells
CD 68	mononuclear phagocyte lineage cells	The antibody against CD68 is of value for demonstration of monocytes and macrophages in normal and pathological specimens
CD 31	blood vessels	The antibody against CD31 strongly labels endothelial cells and is valuable for the demonstration of capillaries and small vessels
Podoplanin	lymphatic vessels and lymphangiogenesis	The expression has been demonstrated in lymphatic endothelium whereas it is absent with vascular endothelium what is useful in identifying lymphatic and blood vessels
Sox-18	angiogenesis and lymphangiogenesis	The protein has been shown to play a role in blood and lymphatic vessel development valuable for allowing precise vessels demonstration

Cases of ChG, pose a serious diagnostic problem and require histopathological confirmation. Perilymphatic granuloma and granulomatous lymphangitis in histopathology are considered characteristic features of MR (11). These features typical for MR were found in all studied patients with cheilitis. We observed extravascular infiltrates and non-necrotizing extravascular granulomas compressing lymphatic capillaries and extensive perivascular inflammation which might inhibit lymphatic contractility, increase lymphatic capillary leakiness, and decrease lymphatic transport, contributing to development of lymphedema. Therefore, perilymphatic inflammation with compression of lymphatics appears to be the major cause of lymphedema in ChG patients.

However, there are studies reporting intralymphatic cellular aggregates that might be responsible for impaired lymphatic drainage and facial edema (6-9). Gonzales-Garcia et al. and Franz et al. described patients with ChG whose biopsy histology

showed intralymphatic granulomas (7,8). These intravascular cell aggregates were composed of histiocytes (positive expression of CD68 and negative expression of S100 and CD1a). Moreover, endothelial cells of blood (positive CD31 and factor VIII) and/or lymphatic (positive Podoplanin) vessels were found in these aggregates. Intralymphatic histiocytosis might cause stenosis, and even occlusion of lymphatic vessels resulting in lymphatic edema, which resembles the clinical picture of ChG. Such is the case with erysipelas carcinomatosum where in the course of lymphangitis carcinomatosa lymphatic drainage is blocked, vessels are dilated and clinical picture imitates erysipelas. If the description were to be true for ChG, it might be supported by changeable and dynamic character of lip enlargement in this syndrome. Intensification of lip edema might be connected to build-up of stasis and growth of granuloma while remission of the symptom might be ascribed to the granuloma's decrease.

However, our present study does not

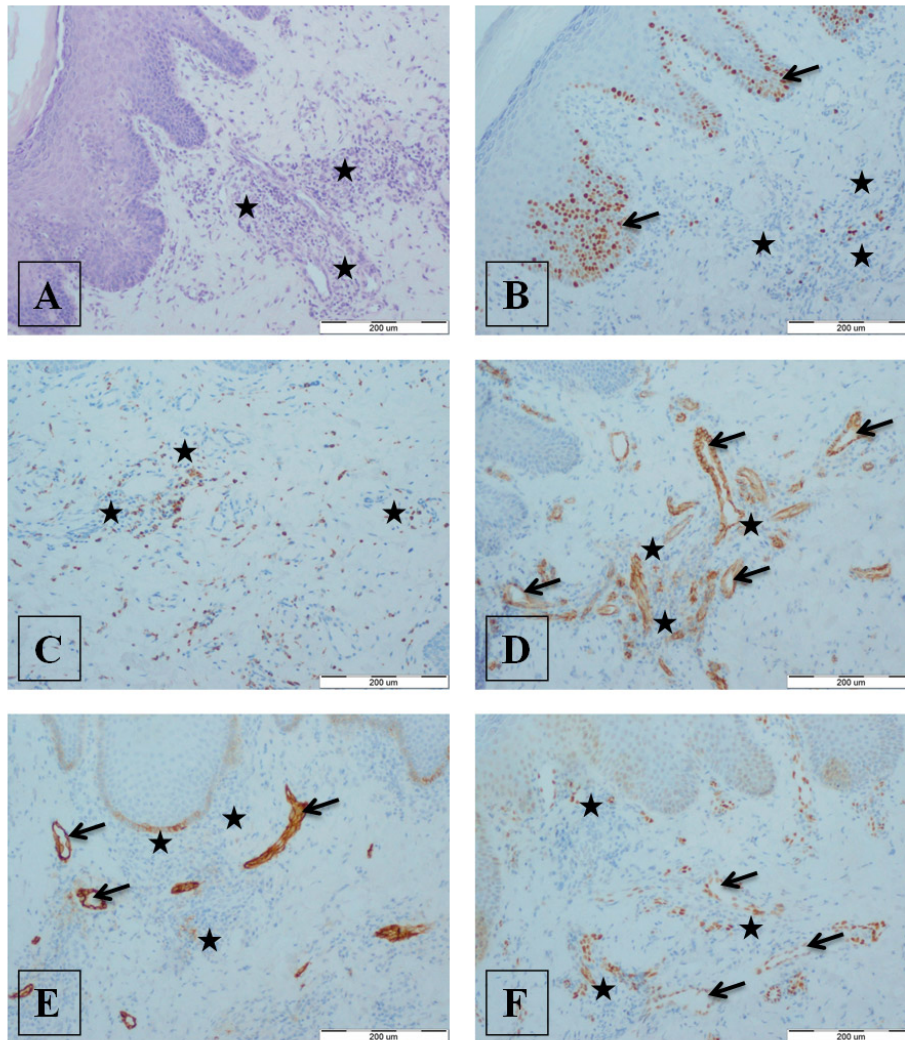


Fig. 1. Microscopic view of a histological specimens from biopsied lip skin tissue. On all images, asterisks highlight perivascular aggregates forming non-necrotizing granulomas and diffuse edema. Magnification x100. (A). Hematoxylin-eosin staining. Characteristic image of keratinized squamous stratified epithelium with underlying connective tissue. In the connective tissue, visible diffuse edema and inflammatory infiltration forming perivascular aggregates is seen; (B). Immunohistochemical expression of Ki-67 in the basal layer of the epithelium demonstrates active proliferation by a strong reaction (brown-labeled nuclei, highlighted by arrows); (C). Expression of CD68 in the connective tissue shows cells of mononuclear phagocyte lineage in diffuse edema and infiltration regions visible; (D). Expression of CD31 displays a strong reaction in many capillaries and small blood vessels (arrows); (E). Expression of D2-40 highlights the lymphatic vessels (arrows); (F). Expression of Sox-18 displays the development of blood and lymphatic vessels in edema and infiltration regions.

support the view that lymphatic vessel obstruction is caused by intravascular histiocytic granulomas described as the main part in the etiology of lymphatic edema in ChG, and the pathomorphological images

were convincing in establishing a diagnosis. However, it is likely in some cases of ChG and MR that microscopic appearance may vary depending on the character and intensity of the host immune responses. Such a

conclusion might be supported by the study of Kaminagakura et al. who showed non-necrotizing granulomatous aggregation in the complete form of MR while the mono-symptomatic form featured lack of granulomatous formation of inflammatory cells (12). Nevertheless, other possibilities should be taken into consideration including whether the intravascular localization of granulomas might represent a particular stage of the disease or, alternatively, ChG and MR might possibly be distinct variants of a granulomatous disease. There are also other granulomatous diseases, for example granulomatosis with polyangiitis that may show histologic features similar to ChG and affect both venous and arterial vessels down to the capillary level, which must be considered in a differential diagnosis.

In summary, histopathological findings in many cases of ChG are not always obvious or specific but they are invariably characterized by the common presence of the perivascular inflammatory response and extravascular non-caseating granulomas.

ETHICAL APPROVAL

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed, written consent was obtained from the individual participants included in the study.

CONFLICT OF INTEREST AND DISCLOSURE

All authors declare that no competing financial interests exist.

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