Ezrin may confine neutrophil orientation in a chemotactic gradient towards the vessels in a case of Sweet syndrome

Ana Maria Abreu-Velez¹, Bruce R Smoller², Michael S. Howard¹

¹Georgia Dermatopathology Associates, Atlanta, Georgia, USA, ²Department of Pathology and Laboratory Medicine, Professor, Department of Dermatology, University of Rochester School of Medicine and Dentistry

Corresponding author: Ana Maria Abreu-Velez, M.D., Ph.D., E-mail: abreuvelez@yahoo.com

ABSTRACT

Sweet syndrome is a neutrophil dermatosis. Here we present a case of a 63 male who presented with tender, reddish purple macules and papules on his trunk. Skin biopsies were taken for hematoxylin and eosin stain (H&E), immunohistochemical staining (IHC) and direct immunofluorescence examination (DIF). H&E stains demonstrated the histologic features previously described in SS, along with other findings. Strong expression of CD31, CD34, Von Willebrand Factor, podoplanin and vimentin (vessels markers) as well as Ham 56, CD68 and myeloperoxidase were seen. The DIF showed expression of ezrin in the vessels and in the areas overwhelmed by neutrophils. Our data suggest that ezrin may confine neutrophil orientation in a chemotactic gradient towards the vessels and may play an import role in Sweet syndrome. We also showed that vascular involvement, skin appendageal inflammation and cluster damaged dermal extracellular matrix maybe also part of the Sweet syndrome.

Key words: Sweet syndrome; Neutrophilic dermatosis; Ezrin; CD31; Von Willebrand factor; Podoplanin; HAM 56; Neutrophil transmigration

INTRODUCTION

Acute febrile neutrophilic dermatosis (Sweet syndrome) (SS), was first described in 1964 by Robert Douglas Sweet and later studied by multiple authors. This disease usually occurs in middle-aged women and can occur after non-specific infection of the respiratory or gastrointestinal tract. SS is considered by some authors to have three clinical settings: a), classical (or idiopathic), b), malignancy-associated and 3), drug-induced, especially including bortezomib medication. SS has a histiocytoid variant. Some people associate SS with immature neutrophils, and some others with rare extracutaneous manifestations including cardiovascular involvement, coronary artery occlusion, involvement of the eyes, joints, and oral mucosa as well as systemic manifestations involving the lungs, liver, kidneys, and central nervous system.

Clinically, SS syndrome usually presents clinically with raised erythematous plaques with pseudo-blistering and occasionally pustules may occur on the face, neck, chest, and extremities, accompanied by fever and general malaise [1-4]. Ezrin (also known as cytovillin or villin-2), is a cytoplasmic peripheral membrane protein and is a member of the ezrin, radixin and moesin (ERM) protein family. Ezrin is a membrane of the cytoskeleton linker protein that plays a key role in cell surface structure adhesion, migration, and organization. Ezrin has been shown to play a role in neutrophil migration [5,6]. Therefore we decided to test for the presence of ezrin in a patient skin biopsy.

CASE REPORT

A 63 male presented with a diffuse eruption on the trunk that began as red –purple maculo-papules
that later became confluent, forming tender plaques (Fig. 1). The skin lesions were accompanied by mild fever. The clinical diagnosis was Sweet Syndrome (SS), and dapsone was used for treatment. Skin biopsies were taken for hematoxylin and eosin stain (H&E), direct immunofluorescence examination (DIF) and immunohistochemical (IHC) staining, and these tests were performed as previously described [7,8]. In brief, for the DIF, we used, in addition to the routine antibodies to IgA, A, M, E, D, C1Q, C3C, albumin and fibrinogen, an antibody to ezrin (3C12), Cat # MA5-13862 from Invitrogen, at 1:75 dilution. We used for its secondary antibody a Texas red goat anti-mouse IgG secondary antibody both from (Carlsbad, California, USA). For the IHC we used antibodies to Von Willebrand Factor, clone F8/86, to podoplanin clone D2-40, to CD68, clone EBM11, CD31, clone JC70A, CD34 Class II clone QBEnd 10, and rabbit anti-human myeloperoxidase all from Dako, Carpinteria, CA, USA). We also used macrophage (HAM-56) mouse monoclonal antibody 279M-1 HAM-56 from Cell marque (from Sigma-Aldrich, Rocklin, CA, USA). The tests were performed in a Leica Bond Max machine.

**Figure 1:** (a) Shows multiple erythematous plaques and vesicles in the legs of the patients (black arrows). (b) H&E stain shown in the papillary dermis, a strong infiltrate of numerous neutrophils with an admixed with few cells including lymphocytes, histiocytes and occasional eosinophils in the dermis, (black arrows, 200X). (c) IHC stains showing positive stain with HAM 56 in the vessels where the neutrophils and other inflammatory cells were seen, (dark stain, red arrows). (d) Double IHC stain showing positive stain with in fuchsia with myeloperoxidase (+++) (black arrow) cells also stain with CD68 near the vessels in brown (red arrow) (400X). (e) H&E stain shows neutrophils infiltrate in a liner pattern along the piloerector muscle (black arrow) (400X). (f) IHC stain positive in the corneal cluster with myeloperoxidase (+++) (dark stain, red arrow) (400X). (g) IHC stains with CD31 in an elongated and dilated vessel (dark stain, red arrow) surrounded by neutrophils. (h) DIF shows overexpression of ezrin along the dermal vessels and those neoformed in the dermis (red stain, yellow arrows), (100X). The nuclei of the cells were stain in blue with 4’,6-diamidino-2-phenylindole (DAPI). (i) IHC showing a dilated and deformed upper dermal lymphatic positive for D2-40 (+++),(fuchsia stain), (black arrows).
The H&E stain demonstrated focal subcorneal collections of neutrophils with focal papillary dermal edema and the presence of early subepidermal vesiculation due to dermal edema (Fig. 1). Within the papillary dermis, a dense infiltrate of neutrophils was observed, admixed with lymphocytes, histiocytes and occasional eosinophils. Abundant cellular debris was also observed. Newly formed vessels were detected in the dermis. Of interest, a dense inflammatory infiltrate was also seen around the pilosebaceous glands and the arrector pili muscles, mainly consisting of neutrophils, as well as scattered lymphocytes, histiocytes and eosinophils (Fig. 2). Multiple skin appendages were damaged and they appeared to be decreased in number and size. The dermal extracellular matrix was also damaged in multiple spots, especially near the mesenchymal-endothelial cells junctions (Fig. 2). Focal leukocytoclastic debris was also seen, but frank vasculitis was not identified. The DIF was negative for IgG, IgA, IgM, IgE, complement/C1q, complement/C3, albumin, and fibrinogen. Ezrin was very positive (++++) in the area where the majority of neutrophils were seen in the dermis, as well as where the newly formed vessels were observed with the routine H&E staining (Fig. 1).

The IHC stains for vascular markers dermis (Von Willebrand factor, CD31, CD34, vimentin, and D2-40) were strongly expressed throughout the entire. Most of the dermal vessels demonstrated altered shapes. The neutrophils were found in the same areas of the cells positive for HAM 56 and CD68 (Fig. 1). Neutrophils were also present around most of skin appendages. Neutrophilic dust and cellular debris were found in the dermis (Figs. 1 and 2). HAM-56 was positive in cells around the vessels, around the sweat glands and as extremely positive around the mesenchymal-endothelial cells junction’s dermal vessels (colocalizing with extracellular dermal damage). Myeloperoxidase was also positive in the epidermis (++++) and inside the vessels (++++) (Fig. 1). DIF of the skin shows overexpression of ezrin (++++) in the dermal vessel and in the areas where the neutrophils were seen (Fig. 1).

STATEMENT OF ETHICS

Although Institutional Review Board (IRB) approval for a case report is not needed, the US Health Insurance, Portability and Accountability Act of 1996 (HIPAA). Privacy Rule restricts how protected health information (individually identifiable health information) is disclosed and nothing about this report violates those rules.

DISCUSSION

In this report we describe a case of Sweet syndrome with histopathologic features previously not described such as alteration of vessels with neovascularization, a peri-appendageal inflammatory infiltrate of neutrophils and damage of several skin appendages. We also describe the expression of Ezrin in the areas populated by neutrophils including the vessels in the dermis. In this case we observed histologic features of Sweet Syndrome displaying perfect colocalization of epidermal blisters with a myeloperoxidase marker. The presence of myeloperoxidase in the blisters suggests that this enzyme may contribute to the blister formation (when present). We also detected an important finding occurring with the neovascularization in the dermis.

Additionally we observed the damage of multiple skin appendages. These two findings have not been described before in Sweet syndrome. Additionally the expression of vascular markers (e.g. Von Willebrand factor, CD31, CD34, vimentin, and D2-40) and the colocalization of HAM-56 within close proximity to the vessels may indicate some possible antigen presentation involving endothelial and other vessels components.

Of interest, this is the first description of the presence of Ezrin being overexpressed in most of the dermal vessels within the areas where neutrophils were seen. Ezrin links the cortical cytoskeleton to the plasma membrane and plays a role in regulating changes in cell shape. Recently, a study reported that NSC668394 (a pharmacological inhibitor) inhibits a key step for ezrin activity, i.e. phosphorylation at threonine 567. The authors also pointed out that in neutrophils, another key regulatory step is the Ca2+-mediating cleavage of ezrin by calpain. The authors furthermore showed that neutrophils with NSC668394-inhibited ezrin phosphorylation remained both phagocytic and chemotactically competent. However, phagocytosis was slightly impaired and chemotaxis could not be maintained over longer periods with aberrant morphology. The authors presented evidence that although phosphorylation of ezrin plays a minor role in limiting the rapid changes in cell shape in neutrophils, inhibition of ezrin phosphorylation by NSC668394
prevented multiple and prolonged shape changes during extended chemotaxis [6].

It is known that during inflammation, the selectin induced sluggish rolling of neutrophils on venules cooperates with chemokine signaling to mediate neutrophil recruitment into tissues. It has been also suggested that the pathophysiological roles of ezrin/radixin/moesin proteins can alter the leukocyte rolling as shown in in mice deficient in moesin, a member of the ezrin-radixin-moesin family [9,10].

In this report we also showed that HAM-56 and CD68 positive cells were present at the mesenchymal-
endothelial cells junctions near altered dermal extracellular matrix clusters. Under inflammatory conditions or during interactions with other cell subsets, neutrophils inherently express or can de novo produce the receptors needed for antigen presentation. For several authors these observations support that neutrophils have the capacity to function as antigen presenting cells [11]. Some authors also have demonstrated that tumor necrosis factor-α and IL-17A activates and induces pericyte-mediated basement membrane remodeling in human neutrophilic dermatoses [12]. Indeed, in this case we observe that the neutrophils and their products had strong affinity to the vessels. It is conceivable that they released inflammatory arsenals causing the shape alterations to vessels as well as those that gave rise to the skin damaged appendages.

We conclude that in this case of Sweet syndrome, the vessels markers are strongly expressed, altered in their shape and that ezrin is co-localized. This protein may play a significant role in the neutrophil migration. Further studies are needed.

**Consent**

The examination of the patient was conducted according to the Declaration of Helsinki principles.

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

**REFERENCES**