

Case Report

Survivin, p53, MAC, Complement/C3, fibrinogen and HLA-ABC within hair follicles in central and centrifugal cicatricial alopecia

Ana Maria Abreu-Velez¹, M.D., Ph.D., A. Deo Klein², III, M.D., Michael S Howard¹, M.D.

Georgia Dermatopathology Associates¹, Atlanta, Georgia, USA.
Statesboro Dermatology², Statesboro, Georgia, USA.

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Abstract

Context: Central centrifugal cicatricial alopecia (CCCA; originally entitled follicular degeneration syndrome, or hot comb alopecia) was first described in African American women utilizing hot combs and/or strong chemical hair care products.

Case Report: A 67 year old African American female was evaluated for the presence of alopecic areas occurring on the scalp vertex, and spreading centrifugally. The alopecic lesions appeared as diffuse patches, including atrophic small areas surrounding individual hair follicles. **Patients and Methods:** Skin biopsies for hematoxylin and eosin examination, as well as for direct immunofluorescence and immunohistochemistry analyses were performed. **Results:** hematoxylin and eosin staining demonstrated histopathologic findings of premature desquamation of the inner root sheath and eccentric thinning of the follicular epithelium, supporting the diagnosis of CCCA. Direct immunofluorescence revealed strong depositions of Complement/C3, fibrinogen and kappa light chains around the hair follicles. Immunohistochemistry demonstrated increased expressions of HLA-ABC (as in African American patients with insulin independent diabetes mellitus). We also detected positive p53, bcl-2 and MAC staining in the hair follicle areas. **Conclusions:** Follicular degeneration syndrome may have an important immunological component previously not described, and multicolor immunofluorescence may be useful in establishing an early diagnosis.

Keywords: Central centrifugal cicatricial alopecia, follicular degeneration syndrome, hot comb alopecia, survivin, p53, bcl-2, MAC, HLA-ABC, complement/C3.

Correspondence to: Ana Maria Abreu Velez, M.D., Ph.D., Georgia Dermatopathology Associates, 1534 North Decatur Road, NE; Suite 206, Atlanta, Georgia 30307, USA. Email: abreuvelez@yahoo.com

Introduction

Central centrifugal cicatricial alopecia (CCCA) was formerly titled follicular degeneration syndrome (FSD), central progressive alopecia or "hot comb alopecia". FSD was first identified in African-American women (female to male ratio of 3:1), and was considered to be associated with excessive use of hot combs, as well as oil pomades and other hair care chemicals [1-5]. It was thought that the oils applied to the hair were heated by the hot comb and thus liquified. The liquid oil was then thought to travel down the hair shaft into the hair follicular unit opening, and to irritate the skin causing inflammation around the upper follicles [1-6]. However, it is now recognized that, while hot

combing might elicit the condition in some individuals, it can also present in the absence of any cosmetic procedure [1-5]. With this additional discovery, the condition has been retitled follicular degeneration syndrome and also entitled central centrifugal alopecia.

Case Report

A 67 year old African American female presented complaining of alopecic areas occurring on the scalp vertex and gradually spreading centrifugally. These areas progressed to diffuse areas of alopecia, including atrophic areas around the hair follicles. The patient's hair stylist used had utilized multiple chemical relaxers, as well as

selected traumatic hair stylish practices over a period of years.

Due to the fact that the southeast region of the United States includes many African American patients suffering from lupus [5], the referring dermatologist obtained a skin biopsy for hematoxylin and eosin (H & E) stain, as well as a skin biopsy in Michel's medium for direct immunofluorescence (DIF) analysis. Our laboratory has standardized comparable methods to evaluate deposits of immunoglobulins and complement in both 1) paraffin fixed specimens for immunohistochemistry (IHC) studies and 2) DIF studies.

Thus, positive findings by DIF are routinely compared with pertinent H&E and IHC results. We utilized an archival biopsy from a healthy female African-American patient of similar age as a control for the IHC studies.

Direct immunofluorescence (DIF)

In brief, skin cryosections were prepared, and incubated with multiple fluorochromes as previously reported [6-9]. We utilized a normal skin as negative control from patients going under aesthetic plastic surgery.

Immunohistochemistry (IHC)

IHC was performed as previously described. We utilized antibodies against human HLA-ABC, p53, bcl-2, membrane attack complex (MAC; complement/C5b-9), anti-kappa light chains, and immunoglobulins A, G, M, D and E; all antibodies were obtained from Dako [6-9].

Results

Microscopic description

H&E staining demonstrated premature desquamation of the inner root sheath, and eccentric thinning of the follicular epithelium. A mild to moderate perivascular and perifundibular infiltrate was also noted, with attendant loss of hair follicular units. No significant interface alterations were appreciated (Figure 1). The infundibular epithelium was also noticeably atrophic. Focal dermal follicular stelae scars were identified. A Verhoeff elastin special stain confirmed focal scarring within the dermis (Figure 1). Focally isolated arrector pili muscles were also observed.

Discussion

Healthy appearances and beauty are valued in diverse cultures. Some diseases occur secondary to the patient's pursuit of such physical qualities. The presented case seems to be a case of CCCA, previously titled FSD or hot comb alopecia [10]. A CCCA diagnosis is based on clinical, epidemiological, family history, histopathologic and laboratory data [1-5]. In our patient, the clinical history reflected multiple hair treatments, the patient also presented alopecic areas occurring on the scalp vertex and gradually spreading centrifugally. Histologically, we observed premature desquamation of the hair follicle inner root sheath and eccentric thinning of the follicular epithelium,

supporting the diagnosis of CCCA [11]. Early in the course of CCCA, a brisk dermal lymphocytic infiltrate is often observed histologically; skin biopsies consistently demonstrate inflammation of affected hair follicles, and the previously noted premature degeneration of the hair follicle inner root sheath.

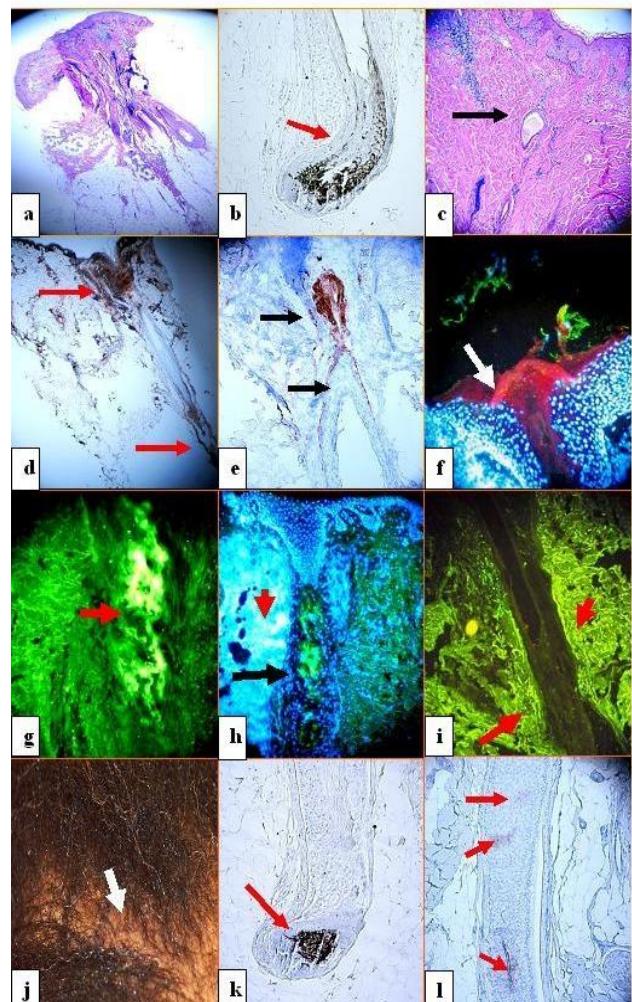


Fig. 1 **a** and **c**. H&E staining at lower and higher magnification demonstrates a mild to moderate perivascular and perifundibular infiltrate, with loss of sebaceous glands (black arrow). **b**. Positive IHC staining with anti-human p53 against the germinal area of the hair follicle (dark staining; red arrow). **d**. Overexpressed HLA-ABC by IHC in hair follicle areas (dark brown staining; red arrow). The HLA-ABC marker was also detected at a normal level in the epidermis and dermal blood vessels. **e**. Overexpressed MAC (Complement/C5b-9) by IHC inside the hair follicles and also around them (dark brown staining; red and black arrows). **f**. Positive DIF staining with rhodamine conjugated anti-human IgM inside the isthmus of the hair follicle (red staining; white arrow). The nuclei of the cells were counterstained with DAPI (blue-white staining). **g**. Strongly positive DIF staining for Complement/C3 inside the hair follicle (yellow/green staining; red arrow). **h**. Positive DIF staining for FITC conjugated Complement/C3 inside the hair follicle (green staining; black arrow). The nuclei of the keratinocytes were counterstained with 4', 6-diamidino-2-phenylindole (DAPI; blue-white staining). Please note on the left side of the hair follicle a large group of damaged cells, highlighted by the DAPI staining (red arrow). **i**. Strongly

positive DIF staining with fibrinogen around the hair follicle (green staining; red arrows). **j.** A clinically alopecic area in the patient's hair (white arrow). **k.** Positive IHC staining with anti-human survivin (dark brown staining; red arrow). **l.** Positive IHC staining in some parts of the hair follicle with bcl-2 (dark brown staining; red arrows).

Classically, treatment focuses on halting the hair follicle inflammation via topical corticosteroids and/or intradermal corticosteroid injections. No consistently reliable treatment is known, except to recommend avoiding strong chemical treatments and/or hot comb use [1-6, 10]. The clinical differential diagnoses includes folliculitis decalvans, neutrophilic cicatricial alopecia, cutaneous folliculitis, acne necrotica, lichen planopilaris, discoid lupus erythematosus, and dissecting cellulitis [12]. In FDS, DIF evaluation is classically negative, but with multicolor DIF we were able to visualize the strong deposits of Complement/C3, membrane attack complex (MAC), fibrinogen and anti-kappa light chain antibodies inside and around the hair follicles, as well as punctate areas of IgM deposition in the hair follicle matrix and hair isthmus. The combined positivity of these immunoreactants indicates both a specific and non-specific immune response. Of note, we did not find other similar studies in the medical literature for data comparison. Future studies may help to confirm our findings in CCCA.

In our case, we observed a strong presence of MAC. The MAC complex is formed as a result of activation of the complement system, and forms transmembranous channels [13]. It has been demonstrated that these channels disrupt the phospholipid bilayer of target cells, leading to cell lysis and death [13]. Thus, we suggest that the presence of MAC within the biopsy could contribute to pilosebaceous unit cell damage.

In regard to the observed positive staining for HLA-ABC, we investigated this molecule based on the fact that many African-Americans with insulin dependent diabetes mellitus display a strong HLA-ABC association. Given the fact that CCCA has been diagnosed in the relatives of many insulin dependent diabetes patients, we suggest two theories to account for the association. First, the association could be sporadic, due solely to hair care practices in the relatives; or second, the association could also be due to the combination of a genetic predisposition for CCCA, triggered by the hair care practices of the relatives [14]. We suggest a comparison of genetic linkage studies with the HLA-ABC in CCCA with linkage studies of the insulin locus in insulin-dependent diabetes mellitus; such a comparison may provide a mechanistic clues regarding disease susceptibility [14]. These loci could then potentially be utilized as susceptibility markers, and contribute to family genomic studies. The results of such studies could in turn affect family hair care recommendations and public health strategy in families of African-American patients suffering from diabetes mellitus and/or CCCA.

In our case, HLA-DPDQDR IHC staining was negative, but HLA-ABC staining was strongly positive in focal areas of significant inflammation and cell damage. A study in alopecia areata (AA) has shown that 30 out of 32 biopsies from untreated AA showed expression of HLA-ABC antigens on hair matrix epithelium, and the subinfundibular epithelium was HLA-ABC-positive in 15 out of 32 cases [15]. In the same study group HLA-DR expression was confined to dendritic cells in the epidermis and the follicular infundibulum [15]. We thus performed our HLA-DPDQDR staining, obtaining similar negative results.

Our studies also suggest that cell damage may occur in CCCA may occur through apoptotic mechanisms (e.g., positive p53 and bcl-2 stains); and further, that the body attempts to compensate for such cell damage by overexpression of survivin in the germinal centers of the hair follicles. Further studies with multiple patients are needed to confirm this pathophysiologic possibility in CCCA.

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