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# Direct immunofluorescence of plucked hair for evaluation of immunologic remission in pemphigus vulgaris

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**Background:** Negative direct immunofluorescence (DIF) is a predictor of immunologic remission in pemphigus vulgaris. Recently, it has been shown that plucked hair can be used as substrate for DIF in the diagnosis of pemphigus.

**Objective:** We sought to compare hair DIF in patients with pemphigus vulgaris in clinical remission with conventional DIF for the assessment of immunologic remission.

**Methods:** A total of 55 patients with pemphigus vulgaris fulfilling the following inclusion criteria were enrolled: absence of any lesion and daily prednisolone dosage equal or less than 10 mg without adjuvant drug in the preceding 6 months. Biopsy specimen and plucked hair were processed for DIF. Interstitial deposition of IgG and/or C3 was considered positive.

**Results:** Conventional DIF and hair DIF were positive in 28 (50.9%) and 36 (65.5%) patients, respectively. Hair DIF had a sensitivity of 0.79 (95% confidence interval [CI] 0.59-0.92), a specificity of 0.48 (95% CI 0.29-0.68), a positive predictive value of 0.61 (95% CI 0.44-0.77), and a negative predictive value of 0.68 (95% CI 0.43-0.87).

**Limitations:** Small sample size is the main limitation of this study.

**Conclusions:** The sensitivity of hair DIF was not high enough to allow us to suggest it as a substitute for conventional DIF. On the other hand, one cannot disregard positive cases of hair DIF in the setting of negative biopsy DIF. As hair plucking is less invasive than biopsy, the following approach could be suggested: hair DIF may be repeated in patients in clinical remission until negative; then conventional DIF should be performed, too. The physician can decide to stop treatment only when DIF assays on both substrates are negative. (J Am Acad Dermatol 2011;65:e173-7.)

**Key words:** direct immunofluorescence; outer root sheath; pemphigus; plucked hair; remission.

**“W**hen should I discontinue the drug?” This question is not a minor concern of dermatologists caring for patients with pemphigus. Many authorities rely on clinical remission; others would rather assess immunologic remission in the appropriate clinical setting.<sup>1-4</sup>

#### Abbreviations used:

CI: confidence interval  
DIF: direct immunofluorescence  
ORS: outer root sheath  
PV: pemphigus vulgaris

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Negative direct immunofluorescence (DIF) has been repeatedly shown to be a better predictor of immunologic remission compared with indirect immunofluorescence.<sup>1-4</sup> However, obtaining a sample for DIF imposes an oral or skin biopsy—a relatively invasive and unpleasant procedure to the patient. The need for repeating the test until negative further complicates the problem; many patients are reluctant to accept it. Hence finding a less-invasive way for collecting a suitable substrate would be of much help. Recently, Schaerer and Trüeb<sup>5</sup> showed that outer root sheath (ORS) of plucked anagen hair can be used as substrate for DIF in the diagnosis of pemphigus. The value of this method was proven in further studies on patients with untreated or relapsed pemphigus.<sup>6,7</sup> The current study was undertaken to compare ORS DIF in patients with pemphigus vulgaris (PV) in clinical remission with conventional DIF, to assess the immunologic remission.

## METHODS

The study was approved by the Institutional Review Board of Tehran University of Medical Sciences. A total of 55 consecutive patients with PV in clinical remission fulfilling the following inclusion criteria were voluntarily enrolled: intercellular deposition of IgG and/or C3 in the biopsy specimen before treatment; the absence of any new, old, or nonhealing lesion in the preceding 6 months; daily prednisolone dosage equal or less than 10 mg; and no adjuvant in the preceding 6 months. An oral biopsy specimen for DIF was obtained from patients with the history of mucosal or mucocutaneous phenotype. In patients with the cutaneous phenotype, the upper aspect of the trunk was biopsied for DIF. In addition, approximately 30 hairs were plucked from the scalp in the same way as for the trichogram.<sup>8</sup> Hair plucking was performed in the dermatopathology laboratory with a forceps. The specimen disc was covered with a thick layer of gel and put in the cryostat for 1 to 2 minutes. The hairs were plucked and the distal ends of the shafts were cut to about 2 cm. The shortened hairs with their root sheaths were immediately put horizontally over the gel on the disc and the forceps blades were opened. The disc was covered by another layer of gel and left in the cryostat  $-20^{\circ}\text{C}$ . The specimen was ready for

sectioning after a few minutes. The slides were stained with fluorescein isothiocyanate–labeled rabbit anti-IgG and C3c antibodies (Dako, Glostrup, Denmark). The hair specimens were examined independently by two of the authors. They were unaware of the results of the conventional DIF. The detection of intercellular deposits (latticelike) of IgG and/or C3

was considered positive for the diagnosis of pemphigus (Fig 1). Desmoglein 1 and 3 enzyme-linked immunosorbent assay were performed in 9 patients. Indirect immunofluorescence assay was not done.

Age, gender, phenotype of PV (mucosal predominant, mucocutaneous, cutaneous predominant), history of scalp involvement, duration of the disease, duration of fulfilling the inclusion criteria of the study, and duration of clinical remission (absence of new, old, or nonhealing lesion) were recorded in a questionnaire. The data were analyzed by software (SPSS, Version 16, SPSS Inc, Chicago, IL).

Conventional DIF is known as the best method for assessing immunologic remission in PV and was used as the gold standard in statistical analysis with which hair DIF was compared. Sensitivity of hair DIF (ie, the percentage of patients with positive conventional DIF whose hair DIF was positive), specificity of hair DIF (ie, the percentage of patients with negative conventional DIF whose hair DIF was negative), positive predictive value of hair DIF (ie, the percentage of patients with positive hair DIF whose conventional DIF was also positive), and negative predictive value of hair DIF (ie, the percentage of patients with negative hair DIF whose conventional DIF was also negative) were calculated (Table I).

Positive predictive value will enable us to tell how likely it is that the patient is not in immunologic remission if his/her hair DIF is positive. Negative predictive value will enable us to tell how likely it is that the patient is in immunologic remission if his/her hair DIF is negative.

Kappa was calculated to evaluate the concordance of hair DIF and conventional DIF. *P* value less than .05 was considered significant.

After informing the patients about the two results of DIF and explaining the implications of continuing or stopping the steroid, they were left free to decide about their future treatment and followed up.

## CAPSULE SUMMARY

- Direct immunofluorescence (DIF) of plucked hair has been used for the diagnosis of pemphigus.
- We compared hair DIF with conventional DIF in patients with pemphigus in clinical remission to evaluate for immunologic remission. We found a sensitivity of 79% for hair DIF; however, one third of patients with negative conventional DIF had positive hair DIF.
- As hair plucking is less invasive than biopsy, we suggest a combined approach, ie, repeating hair DIF until negative results are obtained, and then performing conventional DIF.

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