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Research Article

Evaluation of E-Cadherin Expression in Patients with Pemphigus Vulgaris via Immunohistochemistry

Saeid Ghorbanivalikchali,[1] **Azadeh Rakhshan**,[2] **Fariba Ghalamkarpour**,[1] **and Fahimeh Abdollahimajd**[3,4]

[1]*Skin Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
[2]*Department of Pathology, Shohada-e Tajrish Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
[3]*Department of Dermatology, Shohada-e Tajrish Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
[4]*Clinical Research Development Unit, Shohada-e Tajrish Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

Correspondence should be addressed to Fariba Ghalamkarpour; fghalamkarpour@yahoo.com and Fahimeh Abdollahimajd; fabdollahimajd@sbmu.ac.ir

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Pemphigus is a group of autoimmune bullous diseases that can affect the skin and mucous membranes, and it is vital to recognize the exact pathogenesis of this disease. This study aimed to investigate the role of E-cadherin in the pathogenesis of pemphigus vulgaris (PV) and compare the expression of E-cadherin in the lesions of PV patients with that in healthy individuals' skin. Thirty tissue samples from histopathologically confirmed PV patients as the case group and 30 skin samples from healthy individuals as the control group were evaluated for E-cadherin expression via the immunohistochemical method. Data analysis was performed using SPSS software version 25; chi-squared and Fisher's exact tests were used to examine the relationship between qualitative variables. Immunohistochemical staining revealed decreased E-cadherin expression in the basal and suprabasal layers of the epidermis of PV patients compared with healthy individuals ($P < 0.001$). E-cadherin expression was 1+ in 53.3% of patients, 2+ in 40% of patients, and 3+ in only one (3.3%) patient. On the other hand, the expression of E-cadherin in other layers of the epidermis was 1+ in one patient, 2+ in five patients (25%), and 3+ in 14 patients (70%). Also, the expression of E-cadherin in all layers of the epidermis was 3+ in all controls. E-cadherin expression in the basal and suprabasal layers of the epidermis appears to be lower in patients with PV compared with controls. Therefore, E-cadherin immunohistochemical staining helps diagnose PV along with other diagnostic methods. Moreover, these findings may shed light on the role of E-cadherin as a potential target for disease treatment aiming at disease stabilization. However, more studies are needed to clarify this issue.

1. Introduction

Pemphigus is a group of autoimmune bullous diseases affecting the skin and mucous membranes [1–6]. In this disease, autoantibodies target the desmosomes, leading to acantholysis and causing blisters in the epidermis [4–12]. Pemphigus vulgaris (PV) and pemphigus foliaceus (PF) are the two most common subtypes of pemphigus [1, 9, 13]. Although several studies have examined the pathogenesis of PV, the exact pathological mechanism of the disease remains elusive [1].

Cadherins are calcium-dependent intramembrane proteins that fulfill a vital role in intercellular adhesion [14, 15]. These proteins are divided into classical, proto, desmosomal, and atypical types, and E-cadherin is one of the classical cadherins expressed in the epidermis [14]. E-cadherin appears to be an immunological target for pemphigus autoantibodies as part of the adhesion proteins of desmosomes [16]. Some studies have demonstrated that the level of anti-E-cadherin antibodies in the serum of patients with pemphigus is increased [16].

Many studies have investigated the role of desmogleins in the pathogenesis of PV [4, 13–20], but only one study has examined the immunohistochemical expression of E-cadherin in PV lesions [21]. Therefore, we assessed the role of E-cadherin in PV pathogenesis and compared the expression of E-cadherin in the lesions of these patients with that in healthy individuals' skin.

2. Patients and Methods

This study was approved by the Ethics Committee of our institute. All patients provided informed consent.

2.1. Subjects. In this study, we included 30 new cases of PV who were referred to our teaching dermatology clinic in 2019, did not suffer from other systemic and cutaneous diseases, had no history of medication usage, and were diagnosed by a dermatologist and confirmed by a dermatopathologist as the case group. In the control group, we included skin samples from 30 healthy individuals who did not suffer from systemic and cutaneous diseases, had no history of medication usage, and had undergone cosmetic surgery for reasons such as abdominoplasty and mammoplasty. These individuals did not have any cutaneous or systemic diseases and were all over 16 years old. Disease severity was assessed according to the pemphigus vulgaris activity score (PVAS), which considers the type, number, and distribution of skin and mucosal lesions and the presence or absence of the Nikolsky sign. The total score ranges from 0 to 18.

Using the following formula and considering $p1 = 0.4$, $\beta = 0.8$, $\alpha = 0.05$, $p2 = 0.25$, and $B = 0.5$ in the study by Bakry et al. [22], the sample size was calculated to be 30 in each group:

$$N = \frac{\left[z_{a/2} * \sqrt{V(0)} + z_{1-\beta^*}\sqrt{V(\beta^*)}\right]^2 (1 + 2 * P_1 * \delta)}{P_1 * \beta^{*2}}. \quad (1)$$

2.2. Histopathological Examination. Serial 4 μm cut sections were obtained from formalin-fixed paraffin-embedded blocks. Hematoxylin and eosin (H&E) staining was performed on the sections, and an experienced dermatopathologist performed the histopathologic evaluation.

2.3. Immunohistochemical Staining. Immunohistochemical staining of E-cadherin was performed using a mouse anti-human E-cadherin monoclonal antibody (Clone HECD-1; Master Diagnostica, Spain). From paraffin blocks, 4-μm pieces were placed on a charged slide at 60°C overnight, then the samples were deparaffinized, rehydrated, and under heat conditions (95°C, pH: 8, boiled in EDTA buffer for 20 min) were exposed to the retrieval epitope. The specimens were washed with distilled water and cooled to room temperature for 20 minutes. Endogenous peroxidase was blocked by utilizing a peroxidase solution for 10 minutes.

The primary antibodies were incubated for 20 minutes and washed with phosphate-buffered saline and immunohistochemistry (IHC) wash buffer. Secondary antibodies were added and left for 15 minutes, followed by horseradish peroxidase for 30 minutes. Antibodies were detected with DAB (3-3-diaminobenzidine) for two minutes and then washed with distilled water. Slide mounting was performed after hematoxylin staining.

Cells of the surface epithelium of the skin, mucosa, and skin appendages were examined for E-cadherin expression. The intensity and pattern of E-cadherin expression in these areas were calculated. E-cadherin expression intensity was defined as 0 (loss of expression), 1+ (weak, incomplete membranous expression), 2+ (moderate to strong, incomplete membranous expression), or 3+ (moderate to strong, complete membranous expression). Also, the distribution of nonexpressed areas of E-cadherin was defined as focal, patchy, or diffuse (Figures 1(a)–1(e)).

2.4. Statistical Analysis. We used the statistical package for the social sciences (SPSS) software (version 25.0, Armonk, NY: IBM Corp., USA) for data analysis. Quantitative variables were described using means and standard deviations, while qualitative variables were described using frequencies and percentages. Fisher's exact test was used to compare qualitative data. $P < 0.05$ was regarded as statistically significant.

3. Results

In this study, 30 patients with PV were studied as the case group and 30 healthy individuals as the control group. The mean age was 46.27 ± 12.14 years in the PV group and 41.47 ± 11.95 years in the control group. In the PV group, 8 (26.7%) participants were males and 22 (73.3%) were females; in the control group, 1 (3.3%) was male and 29 (96.7%) were females. The female predominance in the latter group was expected as the controls were selected from individuals who had undergone mammoplasty or abdominoplasty. Pemphigus vulgaris was only cutaneous in 6.7%, only mucosal in 10%, and mucocutaneous in 83.3%. The mean disease severity in this group based on the PVAS was 7.40 ± 3.76 (min = 1.5 and max = 16).

E-cadherin expression in the basal and suprabasal layers of the epidermis was +1 in 53.3%, +2 in 40% of patients, and +3 in only one patient (3.3%). On the other hand, E-cadherin expression in other layers of the epidermis was +1 in one patient, +2 in five patients (25%), and +3 in 14 patients (70%). The expression of E-cadherin in all layers of the epidermis in all controls was +3 (Figure 1). The nonstained distribution in basal and suprabasal layers and other epidermal layers was mostly diffuse (69% and 25%, respectively) (Table 1). The results of immunohistochemical staining showed a decrease in the expression of E-cadherin in the basal and suprabasal layers of the epidermis of PV patients compared with healthy individuals ($P < 0.001$).

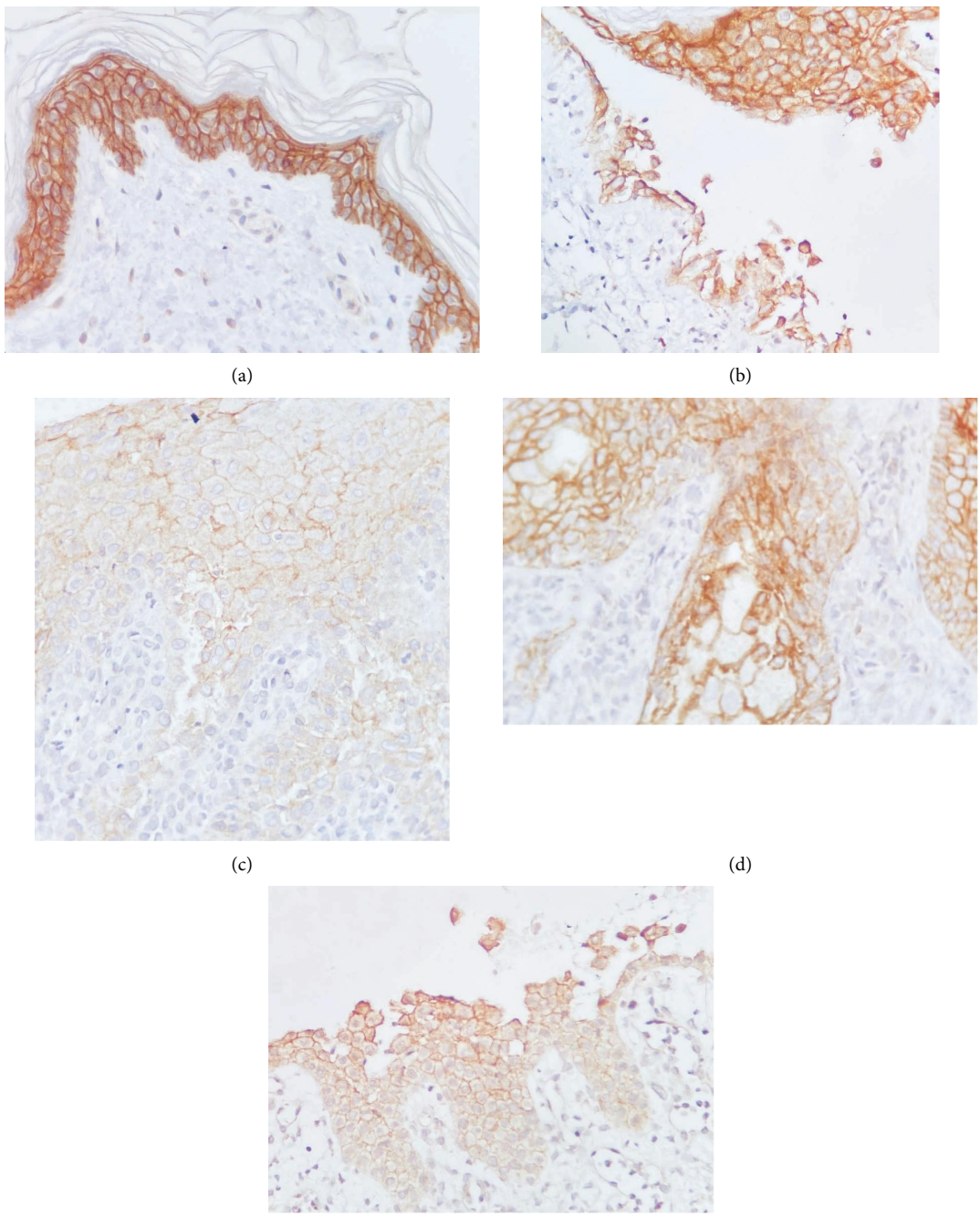

Figure 1: (a) Normal skin showing strong (3+) membraneous staining of E-cadherin (×400). (b) Suprabasal blister with acantholysis. The blister floor shows weak (2+) staining and the blister roof has a normal 3+ staining pattern (×400). (c) Suprabasal blister of pemphigus vulgaris with loss of E-cadherin expression in the floor of the blister in some parts and 1+ staining in the blister roof (×400). (d) Follicular epithelium in a case of pemphigus vulgaris shows suprabasal weak (1+) E-cadherin expression and 2+ membranous staining in upper layers (same as surface epithelium in this case), ×400. (e) Oral mucosa biopsy in a case of pemphigus shows 1+ basal and suprabasal staining of E-cadherin and 2+ staining in the upper layers of the epithelium (×400).

4. Discussion

Our study represents only the second investigation on the immunohistochemical expression of E-cadherin in tissue samples of patients with PV [21]. We found decreased E-cadherin expression in the basal and suprabasal layers of the epidermis in all but one patient. On the other hand, in most patients (70%), no decrease in E-cadherin expression was observed in other layers of the epidermis. The distribution of lack of staining in the basal and suprabasal layers and other epidermal layers was mostly diffuse. Also, no decrease in E-cadherin expression in the epidermal layers

Table 1: Frequency and percentage of qualitative variables in the pemphigus vulgaris (PV) and control groups.

Variable	Groups	Number	Percent
Type of PV	Cutaneous	2	6.7
	Mucosal	3	10.0
	Mucocutaneous	25	83.3
E-cadherin expression in basal and suprabasal layers of epidermis in the PV group	0	1	3.33
	1+	16	53.33
	2+	12	40.0
	3+	1	3.33
E-cadherin expression in other layers of epidermis in the PV group	1+	1	5.0
	2+	5	25.0
	3+	14	70.0
Distribution of loss of staining in basal and suprabasal layers of epidermis in the PV group	Focal	1	3.4
	Patchy	8	27.6
	Diffuse	20	69.0
Distribution of loss of staining in other layers of epidermis in the PV group	None	10	62.5
	Patchy	2	12.5
	Diffuse	4	25.0
E-cadherin expression in basal and suprabasal layers of epidermis in the control group	1+	0	0
	2+	0	0
	3+	30	100
E-cadherin expression in other layers of epidermis in the control group	1+	0	0
	2+	0	0
	3+	30	100
Distribution of loss of staining in all layers of epidermis in the control group	None	30	100
	Patchy	0	0
	Diffuse	0	0

was seen in all controls. These findings indicate a significant decrease in E-cadherin expression in the basal and suprabasal layers of patients with PV compared with controls. This study also showed no significant relationship between E-cadherin expression in the basal/suprabasal layers of the epidermis and the PV severity and type.

Mignogna et al. used immunohistochemistry to examine the expression of catenins as part of the cadherin/catenin protein complex (such as E-cadherin and B-catenin) in 7 patients with different stages of oral PV and 18 healthy individuals. In the controls, the intensity of staining gradually decreased from the spinosum layers to the keratinized layers of the epithelium, while in patients with PV, loss of expression of these molecules was observed, especially in areas with severe acantholysis. This decrease in expression was not associated with the severity of mucosal involvement or symptoms [21]. Thus, this decrease in expression in patients and its lack of relationship with the disease's severity were consistent with our study's results, but the gradual decrease in expression in the upper epithelial mucosa of healthy individuals was not consistent with the results of the current study. Since the samples of our control group were taken from the epidermis, this could be the probable source of this difference.

Tsang et al. used confocal microscopy to show a lack or decrease in E-cadherin expression in cells adjacent to blisters [23] which is consistent with the findings of this study.

According to the abovementioned studies and the results of this study, it seems that, compared with healthy individuals, patients with PV have deceased tissue E-cadherin expression in the basal and suprabasal layers, independent of disease severity and type. However, more studies with more samples are needed to confirm the aforementioned findings.

The lack of mucosal samples in the control group and the small number of eligible patients were the key limitations of this study, which should be addressed in future works.

5. Conclusion

In patients with PV, E-cadherin expression in the basal and suprabasal layers of the epidermis was significantly lower compared with the control group. Hence, E-cadherin may fulfill a role in the pathogenesis of PV. Further studies are needed to confirm this issue and evaluate the usefulness of E-cadherin immunohistochemical staining in diagnosing the disease and the role of E-cadherin as a potential target for disease treatment.

Abbreviations

H&E: Hematoxylin and eosin
IHC: Immunohistochemistry
PV: Pemphigus vulgaris
PF: Pemphigus foliaceus
PVAS: Pemphigus vulgaris activity score.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Disclosure

This article is the result of a Dermatology Specialty Thesis of Saeid Ghorbanivalikchali, MD, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

F. Ghalamkarpour contributed to the conception of the work. F. Ghalamkarpour, F. Abdollahimajd, A. Rakhshan, and S. Ghorbanivalikchali contributed to the acquisition, analysis, and interpretation of data for the work. F. Abdollahimajd and S. Ghorbanivalikchali drafted the manuscript. F. Ghalamkarpour, F. Abdollahimajd, and A. Rakhshan critically revised the manuscript. All authors gave final approval and agreed to be accountable for all aspects of the work ensuring integrity and accuracy.

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