The Utility of Tissue and Epidermal Transglutaminase Immunohistochemistry in Dermatitis Herpetiformis

Aida Valencia-Guerrero, Karen Dresser, Kristine M. Cornejo
Department of Pathology, UMass Memorial Medical Center, University of Massachusetts Medical School, Worcester, MA, USA

Abstract

Dermatitis herpetiformis (DH), also known as Duhring-Brocq dermatitis, is an autoimmune, chronic subepidermal blistering disorder characterized by an intensely pruritic, papulovesicular eruption. It is linked to gluten sensitivity and considered to be a cutaneous manifestation of celiac disease. It has been demonstrated that sera from most patients with DH demonstrate autoantibodies against epidermal transglutaminase (eTG) and tissue transglutaminase (tTG). Therefore, the aim of the study was to evaluate the diagnostic utility of eTG and tTG immunohistochemistry in identifying patients with DH. A total of 15 skin biopsies from patients with an established diagnosis of DH confirmed by histopathology and direct immunofluorescence (DIF) studies were evaluated. Six cases were used as controls in which the clinical differential included DH and were found to be nondiagnostic by both histopathology and DIF evaluations. Eight (55%) of the DH cases were positive for eTG expression, while none of the controls showed any immunoreactivity ($P = 0.0456$). All 15 (100%) DH cases showed faint granular staining for tTG in the epidermal basal layers with similar results in all 6 (100%) control cases. Our study indicates eTG immunohistochemistry may aid in identifying patients with DH, with a diagnostic sensitivity and specificity of 55% and 100%, respectively. tTG does not appear to be a useful immunomarker in this setting.

Keywords: Dermatitis herpetiformis, celiac disease, gluten-sensitive enteropathy, immunohistochemistry

Introduction

Dermatitis herpetiformis (DH), also known as Duhring-Brocq dermatitis, is an autoimmune, chronic blistering disorder characterized by an intensely pruritic, papulovesicular eruption secondary to gluten hypersensitivity. Celiac disease (CD) is the gastrointestinal manifestation of a gluten-sensitive enteropathy, which results in intestinal mucosal atrophy. Although all patients with DH have an intestinal gluten sensitivity, only a portion will display symptoms of CD and/or pathologic intestinal changes.

Nine different types of proteins belong to the transglutaminase (TG) family of which two are pertinent to DH, tissue TG (tTG) which is also called TG type 2 and epidermal TG (eTG) also known as TG type 3 (TG3). Studies have shown that while tTG is the dominant autoantigen in CD, the main target of IgA in DH is eTG. The objective of our study was to evaluate the expression of both tTG and eTG immunohistochemistry in biopsy specimens submitted from patients diagnosed with DH to assess its possible diagnostic utility.

Case Selection

The study protocol was approved by the University of Massachusetts Medical School Institutional Review Board. A total of 15 cases were retrieved from the surgical pathology files of the University of Massachusetts Memorial Medical Center from 2000 to 2016. Inclusion criteria included both a clinical and pathologic diagnosis of DH, detected by histopathologic features on skin biopsy with a positive concurrent direct immunofluorescence (DIF) specimen which revealed the typical granular or fibrillar IgA staining within the superficial dermal papillae. Six negative cases were used as controls in which the clinical differential included DH and were found to be nondiagnostic by both histopathology and DIF evaluations.

Address for correspondence: Dr. Kristine M. Cornejo, Department of Pathology, Massachusetts General Hospital, 55 Fruit Street, Warren 831B, Boston, MA 02114, USA. E-mail: kcornejo@partners.org

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differential diagnosis that included DH but were diagnosed with other inflammatory conditions based on the histologic findings and negative DIF results such as a hypersensitivity reaction or allergic contact dermatitis, among others.

**Immunohistochemical staining and analysis**

Immunohistochemical studies were performed on 5-μm sections of formalin-fixed, paraffin-embedded tissue. The sections were stained with anti-TG2 (tTG) monoclonal mouse antibody (CUB7402, ab2386, 1:300, Abcam, Cambridge, MA) and with anti-TG3 (eTG) polyclonal rabbit antibody (AP 1005.1, 1:1600, Immunodiagnostics). Antigen retrieval was performed with citrate (pH 6.0) and ethylenediaminetetraacetic acid (pH 8.0), respectively. Positive staining was defined as a dark-brown cytoplasmic and granular staining in the epidermal basal layer. No staining was considered negative. Sections of tissue with known positive positivity for the target proteins were used as positive controls. For each marker, a two-tailed Fisher’s exact test was run to determine whether the results were significant.

**RESULTS**

Fifteen DH cases were analyzed and are summarized in Table 1. The patients comprised of 12 males and 3 females and were from 16 to 64 years of age (mean: 43.5 years). A total of 8 (55%) DH cases were focally positive for eTG, while none (0%) of the controls showed any immunoreactivity [P = 0.0456, Figure 1a-c]. Evaluation of circulating anti-tTG IgA was performed in a total of 6 of 15 patients and 3 (50%) were noted to contain antibodies. Of these 3 patients, 2 (66%) revealed focal reactivity for eTG by immunohistochemistry. Both cases, who were negative for circulating tTG IgA antibodies, were also negative for eTG immunoreactivity. Testing for antiendomysial antibodies or anti-eTG IgA was not performed.

All 15 (100%) DH cases showed granular cytoplasmic staining for tTG in the epidermal basal layers with similar results in all 6 (100%) control cases [Figure 1d-f].

**DISCUSSION**

DH is histologically characterized by a subepidermal blistering disease containing a neutrophilic infiltrate with papillary microabssceses and the presence of granular or fibrillary IgA deposits along the dermoepidermal junction with accentuation in the dermal papillae on DIF evaluation, which targets the TG family of proteins.[1,10] Two of the nine TG proteins are pertinent to DH, which are eTG and tTG.[1,5] The dominant autoantigen in DH is eTG, while the major autoantigen in CD is tTG.[1,5-7]

There are two types of anti-eTG antibodies described, one that binds to eTG exclusively and a second that also cross-reacts with tTG, which is in part due to the 64% structural homology between the tTG and eTG molecules within its enzymatically active domains.[1,2] Therefore, sera from patients with DH often demonstrate autoantibodies to both eTG and tTG.[2]

The pathogenic mechanism underlying DH is complex, multifactorial, and not completely understood. Anti-TG antibodies (anti-TG) seem to play an important role in the histopathogenesis of both DH and CD, and the presence of circulating anti-TG is often used to aid in the diagnosis and follow-up of these patients.[3] Levels of circulating tTG antibodies have been found to correlate with disease activity in CD, with reduction after receiving treatment and/or following a gluten-free diet.[2,3] Similarly, there is a reduction in eTG antibodies with activity although it has been reported to persist much longer than anti-tTG antibodies.[2,3,4,9] In addition, the IgA deposits have been found to persist within the skin, despite therapy and reduction in circulating eTG antibodies.[3,7] The hypothesis for this persistence is based on active cross-linking of the insoluble aggregates of IgA and eTG with tight binding to the papillary dermal extracellular matrix.[3,7] Only eTG and not tTG aggregates with IgA in the skin in patients with DH.[1,2]

Physiologically, tTG is widely present in many tissues including the skin and is found in the basal epidermal keratinocyte layers.[3,10] eTG is primarily expressed in the superficial epidermal keratinocytes and not in the superficial papillary dermis and is involved in the maintenance of the integrity of the cornified envelope, contributing to the intact function of the skin barrier through its involvement with cross-linking.[1,3,7,10] There are two theories which explain the occurrence of IgA deposition at the dermoepidermal junction, rather than within the superficial epidermis. The first and most probable is that eTG is released hematogeneously and form immune complexes with IgA, which then deposit at the dermoepidermal junction and papillary dermis.[1,5,7] This hypothesis is further supported by the presence of an IgA nephropathy in some patients with DH and the identification of eTG in cutaneous vessels.[3,11-13] The second theory is that eTG is released as a result of trauma, depositing along the

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**Table 1: Summary of cases (n=15)**

| Case | Age | Sex | Serum IgA | Anti-tTG IgA | Gliadin panel | eTG IHC |
|------|-----|-----|-----------|--------------|---------------|---------|
| 1    | 44  | Male | NP        | NP           | NP            | +       |
| 2    | 48  | Male | NP        | NP           | NP            | +       |
| 3    | 16  | Male | NP        | NP           | NP            | +       |
| 4    | 64  | Male | NP        | NP           | NP            | −       |
| 5    | 58  | Male | NP        | NP           | NP            | −       |
| 6    | 58  | Male | NP        | NP           | NP            | −       |
| 7    | 34  | Male | NP        | NP           | NP            | −       |
| 8    | 59  | Female | NP | N           | NP            | −       |
| 9    | 49  | Male | N         | N            | −             | −       |
| 10   | 47  | Male | N         | −            | NP            | −       |
| 11   | 41  | Male | N         | −            | NP            | −       |
| 12   | 31  | Male | NP        | NP           | NP            | +       |
| 13   | 17  | Female | N | +           | NP            | +       |
| 14   | 33  | Female | N | +           | +            | −       |
| 15   | 53  | Male | N         | +            | +            | +       |

−: Negative, +: Positive, IHC: Immunohistochemistry, N: Normal, NP: Not performed, eTG: Epidermal transglutaminase, tTG: Tissue transglutaminase, IgA: Immunoglobulin A
basement membrane at the dermoepidermal junction and then forming immune complexes with circulating IgA.\textsuperscript{[1,14,15]} In this study, we attempted to explore the diagnostic utility of eTG and tTG immunohistochemistry in patients with DH. eTG expression revealed a sensitivity of 55% and specificity of 100% in identifying patients with DH. The variable expression of eTG is unclear. Only 2 of 3 cases in which the patient harbored anti-tTG IgA antibodies were positive by immunohistochemistry for eTG. These varying results in relationship to the presence of antibody levels and disease activity may be due to the fluctuating persistence of IgA and eTG deposits in the skin. Unfortunately, serum antiendomysial antibodies and anti-eTG IgA levels were not evaluated and would be a better correlate in patients with DH and are a pitfall of this study.

Although the utility of tTG and eTG immunohistochemistry in patients with DH has not been previously studied, tTG immunohistochemistry has been evaluated in the small bowel of patients with CD with variable results.\textsuperscript{[16-21]} Biagi et al. concluded that staining of the surface enterocytes with anti-tTG is specific for CD, highlighting 80% of untreated CD patients while absent in all normal control biopsies.\textsuperscript{[16]} Almarzoogi and colleagues also noted similar findings in which overexpression was identified in 83% of patients with CD and only 45% of normal control patients ($P = 0.0012$).\textsuperscript{[19]} However, there was no significant statistical difference when comparing expression of tTG in CD patients (83%) with patients with inflammatory bowel disease (71%).\textsuperscript{[19]} Additional studies have also identified that CD patients overexpress tTG within the epithelium and lamina propria of the small bowel but that the findings are not specific and present in other diseases and may represent a common mucosal reaction to inflammation or atrophy.\textsuperscript{[17,18]}

On the other hand, Sakly et al. found less expression of tTG within enterocytes in CD patients when compared to the control group but higher expression within the lamina propria.\textsuperscript{[20]} Tuncer and colleagues failed to demonstrate an increase in epithelial distribution of tTG by immunohistochemistry and showed similar expression among the patients with CD and the control group with equal distribution within the muscularis mucosa and pericryptal fibroblasts.\textsuperscript{[20]} Similarly, all of the DH and control cases in our study showed reactivity for tTG, mainly localized to the basal keratinocytes. This correlates with previous studies that have demonstrated expression of tTG in other inflammatory conditions such as lichen planus and psoriasis as well as in normal skin.\textsuperscript{[22,23]} These findings overall suggest that tTG is nonspecific and do not appear to be useful in this setting.

**Conclusion**

In summary, based up our findings, eTG appears to be a specific marker in identifying patients with DH, albeit with a low sensitivity. tTG seems to be expressed in normal skin and in reactive and inflammatory conditions and does not appear to be a helpful immunohistochemical marker in this setting. Due to the small cohort, additional studies analyzing eTG immunohistochemistry in conjunction with serum antiendomysial and/or anti-IgA eTG levels are warranted.

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Nil.

**Conflicts of interest**

There are no conflicts of interest.

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