Original Article

Laminin 5 and collagen IV in the diagnosis of linear IgA bullous dermatosis

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Abstract

Background: Linear IgA bullous dermatosis (LABD) is an immune-mediated heterogeneous disease, characterized by a subepidermal vesiculobullous eruption that occurs in both children and adults. It has been defined on the basis of a unique immunopathology consisting of an exclusive or predominant linear

Aim of Work: deposition of IgA along cutaneous basement membrane zone. Diagnosis depends on the use of a sophisticated immunofluorescent technique which may not be always available.

Laminin 5 and Collagen IV bind to the cell membrane through integrin or dystroglycan receptors and induce cytoskeletal rearrangements, which contribute to the basement membrane's final mat-like structure.

Subjects and Methods: We evaluated the usefulness of immunopathological detection of Laminin 5 and collagen IV in the diagnosis of LABD.

Conclusion: The use of Laminin 5 and collagen IV immunohistochemistry (especially collagen IV) on paraffin sections can be considered as an adjuvant diagnostic tool for LABD, especially in cases where direct immunofluorescence were not performed.

Key words: Laminin 5, Collagen IV, basement membrane zone, ultrastructure, linear IgA bullous dermatoses.

Introduction

Linear IgA Bullous Dermatosis (LABD) is a pruritic, vesiculobullous autoimmune disease characterized by the linear deposition of IgA autoantibodies at the cutaneous basement membrane zone (BMZ) and subepidermal blisters that resemble skin lesions of bullous pemphigoid (BP) or dermatitis herpetiformis (DH)[1-4]. There is a childhood variant of LABD termed chronic bullous dermatosis of childhood (CBDC) or linear IgA bullous dermatosis of childhood which share identical histologic and immunopathologic characteristics and may be considered the same disease[3].

For a long time, the nosology of LABD remained controversial. It has been previously reported as a mixed bullous disease, a form of DH, a type of BP or a distinct disease entity. The reason for this confusion is that it, sometimes, combines features of both DH and BP.

The clinical features of LABD may resemble those of DH, with a primarily pruritic papulovesicular eruption but it also may resemble BP lesions in the form of tense vesicles and bullae developing de novo or on an urticarial base[5-8]. The torso and limbs are most frequently involved. Involvement of the hands, feet, perineum, and face may also be seen. Mucous membrane involvement has been reported in up to 64% of CBDC and 80% of LABD patients [3, 9-11]. In CBDC, there is a typical localization on the lower abdomen and perineum with the characteristic string of pearls or cluster of jewels appearance[3].

Biopsy of a lesion reveals a subepidermal bulla with a superficial dermal infiltrate of neutrophils. There may be papillary microabscesses composed of neutrophils, as in typical DH, but usually the neutrophils tend to be scattered more evenly along the BMZ in LABD. Occasionally eosinophils may be admixed along the neutrophilic infiltrate as may be seen in BP. It is often difficult to distinguish LABD from DH or from Bullous eruption of systemic lupus erythematosus on histology alone[12-14].

The main diagnostic feature of LABD is the presence of BMZ-specific IgA antibody in a linear distribution on direct immunofluorescence (DIF) of perilesional skin in the absence of other immunoglobulins. Some cases of LABD with IgG antibodies in addition to IgA antibodies deposited in a linear fashion at the BMZ have been reported[15-20].

In LABD, specific antibody binding has been found to various antigens on Western immunoblot. The most well characterized is a_{97}-kDa antigen found in a modified epidermal extract ($LABD_{97}$)[21]. This antigen is recognized by adult and childhood LABD sera that bind to the epidermal side of BMZ-split skin[22]. This antigen is identical to a portion of the extracellular domain of the 180-kDa antigen recognized by circulating IgG antibodies in BP ($BPAg_2$)[23].

Different studies have found IgA deposition in the lamina lucida, lamina densa, sublamina densa, in the hemidesmosome, or on the basal surface of keratinocytes[24,25]. Prost et al[26] described the localization of IgA deposits as a “mirror image” pattern on each side of the lamina densa, i.e. the lamina lucida and sublamina densa. Indirect electron microscope (IEM) using LABD sera with high titers of antibodies specific for $LABD_{97}$ has demonstrated binding of these antibodies in the lamina lucida[24, 25]. Indirect IEM using LABD sera with high titres of antibodies specific for collagen VII also shows binding to the anchoring fibrils[27, 28].

Collagen IV and Laminin isotypes bind to the cell membrane through integrin or dystroglycan receptors and induce cytoskeletal rearrangements, which contribute to the basement membrane’s final mat-like structure. Immunopathology utilizing optical microscopy can contribute to the differentiation between

several autoimmune bullous dermatoses. This technique uses antibodies recognizing certain specific macromolecular components of the dermoepidermal junction (DEJ), such as Laminin 4, 5, 6, BP antigen, plectin, collagen IV, collagen VII, epiligrin and fibronectin[29].

The aim of this study is to evaluate the usefulness of immunopathological detection of Laminin 5 and collagen IV to identify the site of blister in LABD in routine paraffin sections.

Patients and Methods

Twenty patients of linear IgA bullous dermatosis (children) who fulfilled the clinical picture, and showed predominantly positive linear IgA deposits at the BMZ by direct immunofluorescence, were the subject of this study.

Skin Biopsies:

Two biopsies were taken from every patient.

- The first biopsy was fixed in 10% formalin, then embedded in paraffin, and sectioned into 5μm sections to be used for routine hematoxylin-eosin (H and E), PAS and Wilder's Silver reticulum staining as well as immunohistochemical staining of collagen IV and Laminin 5 (20 cases).

- The second biopsy was fixed in glutaraldehyde for electron microscopic examination (10 cases).

Immunohistochemical Staining:

Immunoperoxidase technique was used for demonstration of type IV collagen and Laminin 5 epitopes, to identify the site of the split.

Type IV Collagen:

Prior to staining, antigen retrieval was carried out by heating tissue sections with pepsin (Cat. no. S 2002, Dako®, Carpinteria, IX, CA, USA,) diluted in 0.2 N HCl, for 10 minutes at 37ºC. The primary antibody used were mouse anti-human type IV collagen (Cat. No.10760, ICN biomedicals INC., Castmesa, CA 92626, USA). These were used in a dilution of 1:500 and were incubated with the tissue sections overnight at 4ºC in a humidified chamber.

Laminin 5:

Prior to staining, antigen retrieval was carried out by heating tissue sections with protease XXIV (Cat.no.BK-1405-15, Inno Genex®, San Ramon, CA94583, USA,) for 1 minute at room temperature. The primary antibodies used were mouse antihuman Laminin 5 (Cat. no. L1225-35, US Biological®, Swampscott, MA, 01907, USA) that recognize gamma 2 chain unique for Laminin 5. These were used in a dilution of 1:200 and were incubated with the tissue sections overnight at 4ºC in a humidified chamber.

Electron Microscopy:

Biopsies were fixed in 3% buffered glutaraldehyde for 1.5 to 2 hours at 4ºC and then washed with cacodylate buffer solution (pH 7.3) for 15 minutes at 4ºC. This was followed by post fixation in 1% buffered osmium tetroxide, dehydration in ascending grades of ethyl alcohol, and embedding in Epon 812. Sections were prepared using Nova ultra microtome and stained with uranyl acetate and lead citrate. The sections were examined under JEOL 100 C-X (JEOL Ltd., Tokyo, Japan) transmission electron microscope.

Statistical Analysis:

Data were coded, entered and analyzed using SPSS software package for statistical science (SPSS for Windows, version 13.0.1, SPSS Inc., USA). Statistical analysis included descriptive analysis as mean value and standard deviation

(SD), frequencies, and correlation coefficient (r) that was expressed in terms of P-value. The level of significance of P value was 0.05.

Results

The study was conducted on 20 patients, their age ranged between 4 and 15 years with a mean±SD of 8.25±3.02 years. Age at diagnosis ranged between 3-13 years with a mean±SD of 7±2.77 years. The age at onset of the disease ranged between 2-12 years with a mean±SD of 5.65±2.83 years. The duration of the disease ranged between 0.17-3 years with a mean±SD of 1.25±1.0 years. Sex distribution shows that 35% were females (7 patients), and 65% were males (13 patients).

The course of the disease showed remissions and exacerbations in 80% of patients. Some of these exacerbations were attributed to a preceding upper respiratory tract infection, drug intake and sea food ingestion.

Pruritus was a variable feature; it was severe (20%), moderate (40%), mild (35%), or even entirely absent (5%). The lesions were essentially formed of vesicles (100%) and bullae (70%). The contents of the bullae were clear serous or haemorrhagic fluid.

The vesicles and bullae, arised on apparently normal or erythematous base. Iris like lesions were present in 20% of cases. The vesicles and bullae were noticed to form the characteristic "String of Pearls" (45%) (Fig.1) and "Cluster of Jewels" sign (10%) in some cases. However, annular or polycyclic lesions (70%) were more apparent. The sites of predilection were abdomen (60%), inner thighs (50%), and genitalia (40%), although the upper and lower limbs (55%) were among the most commonly affected sites. However, mucous membrane involvement was not encountered in any case of our patients.

Histopathologically, a subepidermal separation was observed in all cases (100%). However, a classic histopathological DH-like picture with neutrophilic predominance and microabscess formation was seen in 30% of cases (Fig 2A), while 25% had shown the characteristic picture of BP with eosinophilic predominance and no microabscess. The remaining 45% revealed a non diagnostic histopathological picture intermediate between DH and BP. Mononuclear cell infiltrate was evidently increased (60%). Meanwhile marked epidermal changes (15%), basal cell vacuolization (40%) and rete tip infiltration by inflammatory cells (30%) were seen, although not regularly observed.

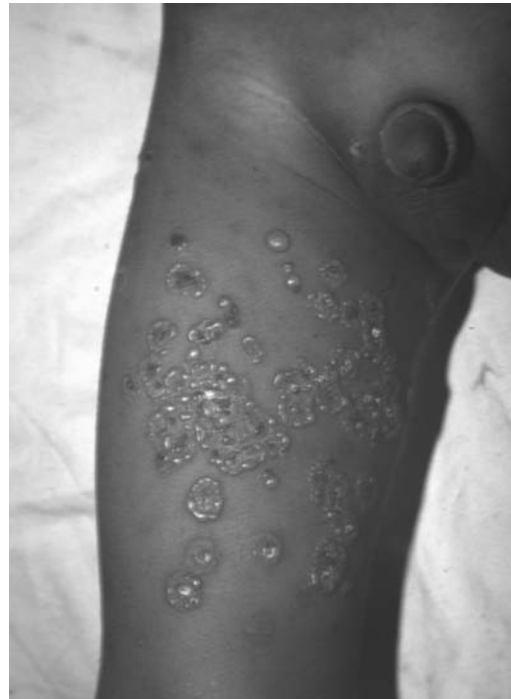

FIG .1. Characteristic String of Pearls Sign of LABD.

In the group of patients with a mixed DH-BP like picture, the prominent feature was a rich cellular infiltrate in the papillary layer of the dermis and within the bulla cavity so that microabscesses were very uncommon or entirely absent. The inflammatory cells were scattered throughout the papillae or arranged in

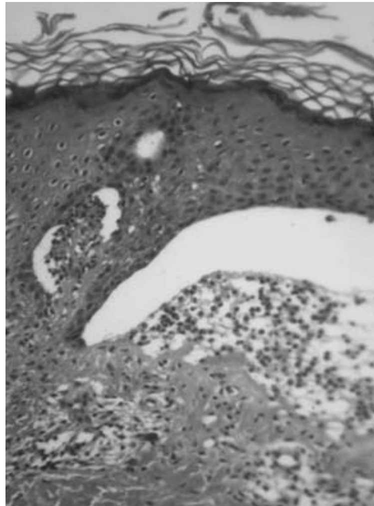

FIG .2a. H&E Stain Showing Bullae (B) formation with neutrophilic infiltrate (N).

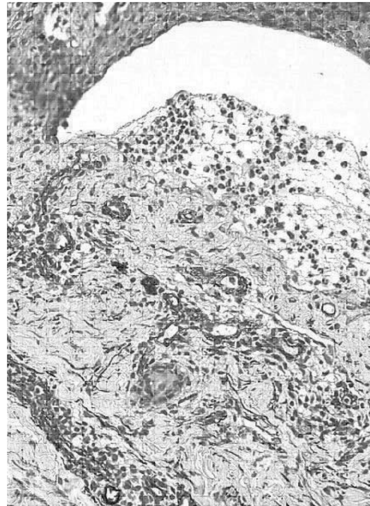

FIG .2b. Collagen Staining (Arrow show brownish staining of Collagen IV at the base of Bullae and around blood vessels).

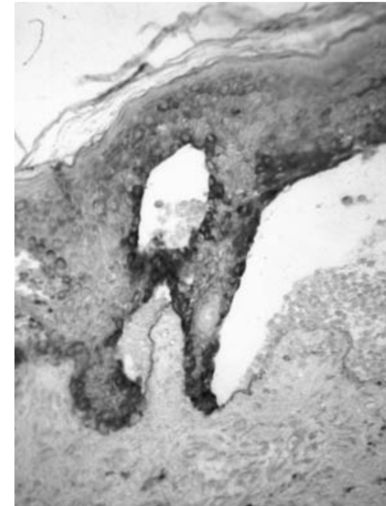

FIG .2c. Laminin 5 Staining Arrow show brownish staining at the base of the bullae.

a some what linear, closely aggregated manner immediately beneath the epidermis or at the base of the bulla. These cells were mostly neutrophils with eosinophils and mononuclear cells.

Pattern of DIF was homogenous linear (HL) in all patients (100%). IgA was present in all patients (100%), while IgG was present in 5 patients (25%), and IgM was present in 5 patients (25%). Fibrin was present in 12 patients (60%) and C_3 was present in 10 patients (50%).

Histochemical examination using Wilder's Silver Reticulum Stain and PAS revealed that the BM, when not destroyed, was located in the floor of the bullae (35%) or at the site of dermoepidermal separation.

Collagen IV staining (Fig 2b) showed characteristic brownish pigmentation at the floor of the bullae in 19 patients (95%). Meanwhile, Laminin 5 staining (Fig 2C) showed pigmentation at the floor of the bullae in 14 patients (70%). Moreover, Collagen IV characteristic staining around the dermal blood vessels was evident (Fig. 2B).

Laminin 5 staining correlated significantly with histochemical staining with Silver & PAS stains ($r= 0.48$, $p= 0.03$), While, Collagen IV showed insignificant correlation ($r= 0.17$, $p= 0.48$).

Electron microscopic findings (done in 10 patients only) showed that epidermal changes were present in all patients (100%). In perilesional or early lesions (Fig .3) the lamina densa was preserved in most of the regions with only localized interruptions, whereas in fully developed lesions (Fig 4) it was destroyed nearly throughout its course. The early site of cleavage seemed to be present between lamina densa, when not destroyed, and the basal cell plasma membrane that is to say in the lamina lucida. This

FIG .3. EM examination of the Bullae (Early Lesion).
Ma: Macrophage K: Keratinocyte
Arrow is aiming to the lamina densa which is partially preserved.

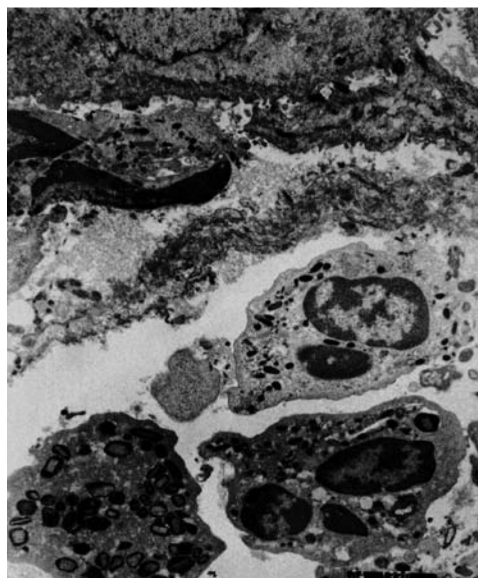

FIG .4. EM examination of the Bullae (Fully developed lesion).
B: Bullae K: Keratinocyte
N: Neutrophil E: Eosinophil
Arrow is aiming to the nearly destroyed lamina densa.

finding showed a highly significant correlation with histochemical examination using Wilder's Silver Reticulum Stain and PAS of lesional skin ($r = 1$, $p < 0.0001$).

Disordered ultrastructure of the epidermis was evident, with prominent intercellular epidermal oedema and stretching of intercellular bridges. A variety of inflammatory cells formed of neutrophils (100%), eosinophils (70%), mononuclear cells (60%) and macrophages was observed in the bulla cavity as well as in the dermal papillae of these patients.

Discussion

Linear IgA bullous dermatosis and CBDC are rare immune-mediated blistering skin diseases that are defined by the presence of homogeneous linear deposits of IgA at the cutaneous basement membrane and are considered different presentation of the same disease process[30,31]. Patients present with lesions suggestive of epidermolysis bullosa acquisita (EBA), DH, BP, lichen planus, erythema multiforme or cicatricial pemphigoid[32-35]. These different clinical presentations appear to result

from the IgA binding to different epidermal antigens[36].

The diagnosis of LABD was confirmed by the presence of a homogenous linear IgA pattern of DIF in all patients.

In our study, EM was done in 10 cases and showed that lamina densa was destroyed in 60% and partially destroyed or preserved in 40%. The site of cleavage was in the lamina lucida in 40%, which goes on with earlier studies[37, 38]. Fibrin was detected in 40% only. The infiltrate included neutrophils in 100%, eosinophils in 70%, and mononuclear cells in 60% of the tested cases. This goes on with earlier studies[29].

Epidermal BM contains a scaffolding of two network polymers; Laminin isoforms and type IV collagen, in which diverse matrix glycoproteins such as; nidogen, perlecan, fibulins and integrins act as stabilizing bridges. Interaction of various molecules of BMZ [basal keratinocyte cell membrane, integrines, Laminin 5/ Laminin 1, Laminin 6, entactin, nidogen, type IV collagen, type VII collagen, fibronectin, type I collagen] give the major BMZ function; the adherence of epidermis to dermis[39].

Laminin 5 (Previously named: Epiligrin, nicein, BM-600) is considered one of the major lamina lucida (LL) components. It is one of the key components of the Lamina lucida/Lamina densa interface which provides stable attachment of the epidermis to the dermis (DEJ). Collagen IV is a major structural component of all BM, including cutaneous BMZ and is also found around blood vessels. It is a major component of the lamina densa. Type IV collagen has a honeycomb or reticular pattern in contrast to fibrillar pattern of other collagen types. It has numerous interruptions in its collagenous repeating segment (Gly-X-Y), that confer flexibility and allowing it to form a super structure for incorporation of other BM components[39].

The use of collagen IV and Laminin immunohistochemistry can be a useful diagnostic tool in BP cases when IF is not performed[40]. Type IV collagen mapping of BMZ helps to distinguish BP from EBA in children, since in BP it is in the base of the blister (cleavage in LL) whereas in EBA it is in the roof[40, 41]. This inspired us to study the presence of Laminin 5 and collagen IV in LABD.

Histochemical staining of BMZ (using PAS and Silver stains) was positive in only 35% of the study group, on the other hand, Laminin 5 was positive in 70%, and collagen IV in 95%. The staining was positive in the floor of the blister. This pilot study shows the significance of testing for both Laminin 5 and collagen IV in LABD patients which could be added to the investigative tests for these diseases. We did not test for circulating antibodies as previous study showed that patient's serum of LABD did not react to Laminin 5 or to collagen IV[42].

Conclusion

The use of Laminin 5 and collagen IV immunohistochemistery on routine paraffin sections (especially collagen IV) can be considered as an adjuvant diagnostic tool for LABD, especially in cases where DIF is not performed. It could also help to differentiate LABD cases from EBA since collagen IV is present in the roof of the blister in EBA and in the floor in LABD. However, it could not differentiate, by no means, between other subepidermal bullous diseases, like BP or DH.

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