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NEÜ YAYINEVİ

An Overview of The Results of Direct Immunofluorescence in Mucocutaneous Biopsies: Single Center Experience

Mukokutanöz Biyopsilerde Direkt İmmünfloresan Değerlendirme Sonuçlarına Bir Bakış, Tek Merkez Deneyimi

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Öz

Amaç: Direkt immünfloresan ve histopatolojik inceleme yapılan cilt/mukoza biyopsilerinin immünfloresan sonuçlarını, histomorfolojik bulgularını, ön tanılarıyla uyumunu karşılaştırmak ve immünfloresan incelemenin diagnostik rolünü değerlendirmek amaçlanmıştır.
Hastalar ve Yöntem: 01 Ocak 2019 – 31 Aralık 2019 tarihleri arasında patoloji laboratuvarına gönderilen toplam 207 hastanın biyopsileri değerlendirildi. Ön tanılara göre gruplar oluşturuldu, histolojik ve direkt immünfloresan bulgular kaydedildi. SPSS programında Kappa istatistikleri ve McNemar testi kullanıldı. P<0.05 istatistiksel olarak anlamlı kabul edildi.
Bulgular: Direkt immünfloresan sonuçları 115 hastada negatif, 91 hastada pozitifti ve bir hastada değerlendirme yapılamamıştı. Büllöz pemfigoidli 44 hastanın 19'unda (%43,2), pemfiguslu 18 hastanın 10'unda (%55,6), lupus eritematozuslu 26/51 (%50,9), liken planuslu 10/15 (%66,7), Henoch Shönlein purpuralı 6/6 (%100), diğer vaskülitler için 37/62 (%59,7) hastada histopatolojik uyum izlenmiştir. Direkt immünfloresan pozitifliği büllöz pemfigoid için 19/19, pemfigus için 10/10, lupus eritematozus için 11/26, liken planus için 2/10, Henoch Shönlein purpurası için 6/6, diğer vaskülitler için 28/37 olguda saptanmıştır (κ = 0.021).
Sonuç: İmmün aracılı dermatolojik hastalıkların tanısında ve vezikülobüllöz hastalıkların ayırıcı tanısında direkt immünfloresan inceleme, klinik ve histolojik bulgulara önemli ölçüde destek olmaktadır.

Anahtar Kelimeler: Direkt immünfloresan, deri, vezikülobülloz hastalıklar, lupus eritematozus, vaskülit

Abstract

Aim: It was aimed to compare skin/mucosa biopsies performed using direct immunofluorescence and histopathological examination, compliance with the preliminary diagnosis and to evaluate the diagnostic value of immunofluorescence examination.
Patients and Methods: A total of 207 patients' biopsies sent to the pathology laboratory between January 01, 2019 and December 31, 2019 were evaluated. Groups were formed according to the preliminary diagnosis, and histological and direct immunofluorescence findings were recorded. Kappa statistics and McNemar test were used in SPSS program. P<0.05 was considered statistically significant.
Results: Direct immunofluorescence results were negative in 115 patients, positive in 91 patients and could not be evaluated in one patient. Histological agreement was found in 19 of 44 (43.2%) patients with bullous pemphigoid, 10 of 18 (55.6%) with pemphigus, 26/51 (50.9%) with lupus erythematosus, 10/15 (66.7%) with lichen planus, 6/6 (100%) with Henoch Shönlein purpuras and 37/62 (59.7%) with other vasculitides. Direct immunofluorescence positivity was found as 19/19 for bullous pemphigoid, 10/10 for pemphigus, 11/26 for lupus erythematosus, 2/10 for lichen planus, 6/6 for Henoch Shönlein purpura, and 28/37 for other vasculitides (κ = 0.021).
Conclusion: Direct immunofluorescence examination significantly supports clinical and histopathological findings in the diagnosis of immune mediated dermatologic diseases and in the differential diagnosis of vesiculobullous diseases.

Keywords: Direct immunofluorescence, skin, vesiculobullous diseases, lupus erythematosus, vasculitis

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INTRODUCTION

The skin is an external organ, so it can be observed in the first place and is easily accessible for biopsy. The diagnosis of de novo skin lesions and systemic autoimmune diseases (such as systemic lupus erythematosus (SLE) and vasculitides) can be established through biopsy. However, histopathology alone may not be sufficient in some cases and the diagnosis may need to be supported by assisting techniques (1). Direct immunofluorescence (DIF) is one of these ancillary methods (2). Historically, Coons developed the immunofluorescence (IF) method with a blue fluorescent compound, beta anthracene in the 1940s, diagnostic immunopathology in dermatopathology started with the definition of the lupus band test in 1963 (3). In 1964, Beutner and Jordon demonstrated circulating antibodies in the serum of pemphigus patients using the indirect immunofluorescence (IIF) method. Thus, the immunofluorescence technique took its place as a method used in the diagnosis and management of various vesiculobullous, autoimmune and connective tissue diseases (4).

DIF is technically a one-step procedure that shows antibodies that bind to antigens on the skin or mucosa in vivo (4). It is used in punch biopsy samples that are taken from the lesional and perilesional areas according to the preliminary clinical diagnosis (5,6). While evaluating the DIF findings, the main analyzed parameters are; the primary site of deposition, type, density, and pattern of immune deposits (2). These parameters gain diagnostic importance especially in diseases with autoimmune separation (4). Studies focusing on the compatibility of DIF results with clinical and histological findings in immune-mediated diseases are generally observed in the literature. However, there are few studies in which DIF examination is evaluated together with clinical and histological findings for immune-mediated diseases and diseases/conditions in the differential diagnosis. We planned to consider the final diagnostic results together in biopsies with DIF inconsistency with clinical findings.

In this study, it was aimed to compare evaluated results of DIF and histopathological examination of mucocutaneous biopsies in about 1 year, and to determine the contribution of DIF to diagnosis. It is hoped that this study will contribute to the literature as the experience of single center.

PATIENTS AND METHODS

After receiving ethics committee approval; skin and mucosa biopsies and immunofluorescent samples sent to the pathology laboratory for routine examination between January 01, 2019 and December 31, 2019 were retrospectively analyzed. For this purpose, clinical preliminary diagnoses were grouped (such as pemphigus, bullous pemphigoid (BP), Duhring's disease, vasculitis, discoid lupus erythematosus (DLE), and lichen planus (LP)), and the main histological findings (such as intraepithelial/subepithelial separation, vasculopathy, clasia, dermal mucin deposition) and DIF results (Presence of immunoglobulin G, M, A, and C3 deposits and positivity patterns, if any) were recorded. Patients' age, gender and lesion localization was also recorded.

Statistical analysis of clinical preliminary diagnoses, histological findings and DIF results were obtained using SPSS software with Kappa statistics and McNemar test. $p<0.05$ values were considered statistically significant. For the Kappa values: 0.00 – 0.20 indicated mild agreement, 0.21 – 0.40 low agreement, 0.41 – 0.60 moderate agreement, 0.61 – 0.80 significant agreement and 0.81 – 1.00 almost excellent agreement.

RESULTS

A total of 207 patients were included in the study. The youngest patient was 3 yo, and the oldest patient 97 yo. Of all the patients, 88 were male and 119 were female. Biopsy localizations were recorded as feet (7), legs (66), legs and trunk (1), trunk (44), arms (19), arms and legs (1), hands (5), hands and feet (1), neck (3), hairy skin (16), face (23), oral mucosa (19) and lips (2). DIF results were negative in 115 patients and positive in 91 patients, and could not be evaluated in one patient, statistical analysis of DIF results was made except for this patient. Results of the patients characterized by subepithelial separation (Table1):

Pathologic diagnosis was made as BP in 18 patients with the clinical preliminary diagnosis of BP and all of these patients showed DIF positivity. Subepidermal separation was found in 15 patients, IgG and C3 (7/18), and C3 (7/18) positivity mostly found in the epidermis basal membrane with DIF (Figure 1). Chronic bullous dermatosis of childhood (CBDC) was diagnosed with DIF and histological findings in one patient. BP was ruled out with histological and DIF findings in 13 patients. Results of the patients characterized by intraepithelial separation (Table 2):

There were 8 patients with the clinical primary

Table 1. Clinical preliminary diagnosis, pathological diagnosis, DIF results, immune deposits and histological findings of the patients characterized by pemphigoid group and subepidermal subepithelial separation.

Clinical diagnosis (n)	Pathological diagnosis (n)	DIF (n)	Immune deposition (n)	Histological finding (n)
BP (18)	BP (18)	Positive (18)	At BMZ IgG and C3 linear (6) C3 linear (5) IgM, IgA and C3 linear (2) IgM and C3 linear (1) IgG linear (1) At BMZ C3 linear (1) C3 granular (1) IgG, C3 linear and granular (1)	Subepidermal separation yes (15) Subepidermal separation no (3)
CBDC (1)	CBDC (1)	Positive (1)	At BMZ IgM and IgA linear (1)	Subepidermal separation yes (1)
BP, PV (1)	Differential diagnosis could not be made (1)	Negative (1)		Subepidermal or intraepidermal separation no (1)
BP, LP, LIGAD, Urticaria, Kaposi (7)	Differential diagnosis could not be made (7)	Positive (4) Negative (2) Could not be evaluated (1)	At BMZ C3 (1) PV C3 (2), PV C3 and IgM (1)	Subepidermal seperation yes (7): BP, LP distinction could not be made (1/7) Vasculopathy yes (3/7) Ulceration yes (1/7) Could not be differentiated because of DIF negativity (1/7) and failure to evaluate (1/7)
BP, Atopic dermatitis, Prurigo, Duhring's disease, SCLE, Dermatomyositis, Drug eruption, SJS (13)	Prurigo (6) Spongiotic dermatitis (1) Atopic dermatitis (2) Dermatomyositis (1) Drug eruption (1) SJS (1) Subcorneal pustular dermatosis (1)	Positive (2), Negative (4) Positive (1) Negative (2) Negative (1) Negative (1) Negative (1) Negative (1)	PV C3 (1), At BMZ granular C3 (1) PV C3 (1)	Subepidermal separation no (13) Dermal mucin positive (1/13)
CBDC, LIGAD, Strophulus, Nummular dermatitis (4)	Nummular dermatitis (1) Strophulus (3)	Positive (1) Negative (3)	At BMZ partly granular C3 (1)	Spongiotic dermatitis (1) Severe spongiosis (3)
Duhring's disease, Prurigo, Viral eruption, Atopic dermatitis, Psoriasis (8)	Prurigo (2) Spongiotic dermatitis (3) Atopic dermatitis (2) Perivascular dermatitis (1)	Positive (1), Negative (1) Negative (3) Negative (2) Negative (1)	PV sparse fine granular C3 (1)	

DIF: direct immunofluorescence, BP: bullous pemphigoid, CBDC: chronic bullous dermatosis of childhood, PV (clinical diagnosis): pemphigus vulgaris, LIGAD: linear immunoglobulin A dermatosis, SCLE: subacute cutaneous lupus erythematosus, SJS: Stevens–Johnson syndrome, BMZ: basement membrane zone, PV (immune deposition): perivascular.

and pathological diagnosis of PV. While there was suprabasal separation in 6 patients, DIF was positive in all of these patients and intercellular IgG and C3 deposits were found in the epidermis in 4 patients, C3 deposit in 3 patients and intercellular IgA and C3 deposits in the hair follicle epithelium in 1 patient (Figure 2). Subcorneal separation was present and DIF was positive in 2 patients with pemphigus foliaceus (PF). Results of the patients characterized by LE and LP (Table 3):

The pathological diagnosis was DLE in 22 of 51 patients and histologically DLE compatible findings and dermal mucin deposition were found with Alcian-Blue. Of the 22 patients, DIF was negative in 12 and among the remaining 10 patients, epidermis basal membrane deposits were found with mostly of IgM (Figure 3). Of 51 patients, 3 were diagnosed with subacute cutaneous lupus and 1 with cutaneous

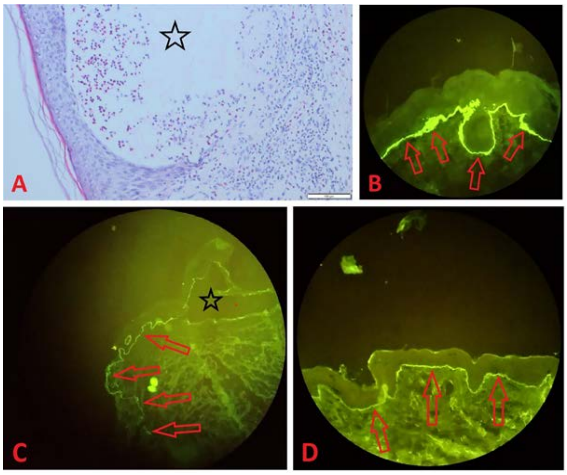

Figure 1. A: Subepidermal separation and bulla formation (star) are seen in the histological section of the skin tissue in the case of bullous pemphigoid (Hematoxylin/Eosin, 100x). B, C, D: Immune deposits are observed in the epidermis basal membrane zone in DIF images of different bullous pemphigoid patients (arrows). B: strong C3, C: moderate C3, D: moderate IgA positivity (There is also subepidermal separation at C (star)).

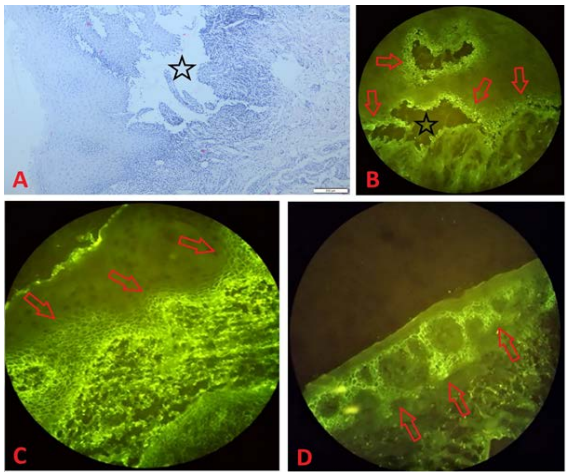

Figure 2. A: In the oral mucosal biopsy of a patient of pemphigus vulgaris, suprabasal separation (star) is observed in the surface epithelium (Hematoxylin/Eosin, 40x). B, C, D: In DIF images of different pemphigus vulgaris patients, intracellular immune deposits are observed in the epithelial lower rows (arrows). B, D: C3 positivity C: IgG positivity. B and C are oral mucosa samples, D is skin biopsy sample (There is also suprabasal separation at B (star)).

Table 2. Clinical preliminary diagnosis, pathological diagnosis, DIF results, immune deposits and histological findings of the patients characterized by pemphigus group and suprabasal intraepithelial separation.

Clinical diagnosis (n)	Pathological diagnosis (n)	DIF (n)	Immune deposition (n)	Histological finding (n)
PV (8)	PV (8)	Positive (8)	Intraepithelial IgG and C3 (3) C3 (3) In hair follicle intraepithelial IgA and C3 (1) Intraepithelial IgG and C3 (1)	Suprabasal separation yes (6) Suprabasal separation no (1) Ulceration yes (1)
PV, Drug reaction, Mucositis, Contact dermatitis, Leukoplakia (3)	PV excluded (3)	Negative (3)		Nondiagnostic (1) Spongiotic dermatitis (1) Ulceration (1)
PF, BP, Duhring's disease, Pemphigus erythematosus (2)	PF (2)	Positive (2)	In spinous layer intercellular IgG and C3 (1) IgG (1)	Subcorneal separation yes (2)
PF, Psoriasis, Seborrheic dermatitis, DLE, PV, AGEP, Subcorneal pustular dermatosis (5)	Psoriasiform spongiotic dermatitis (1) Psoriasis (2) Chronic dermatitis (1) Subcorneal pustular dermatosis (1)	Positive (1) Negative (2) Negative (1) Negative (1)	At BMZ IgM linear (1)	Suprabasal or intraepithelial separation no (5)
Darier's disease, Hailey-Hailey disease, Acanthosis nigricans (3)	Darier's disease (1) Hailey-Hailey disease (2)	Negative (1) Negative (2)		Suprabasal acantholytic separation (1) Suprabasal acantholytic separation (2)

DIF: direct immunofluorescence, PV: pemphigus vulgaris, BP: bullous pemphigoid, PF: pemphigus foliaceus, DLE: discoid lupus erythematosus, AGEP: acute generalized exanthematous pustulosis, BMZ: basement membrane zone.

Table 3. Clinical preliminary diagnosis, pathological diagnosis, DIF results, immune deposits and histological findings of the patients characterized by lupus erythematosus and lichen planus.

Clinical diagnosis (n)	Pathological diagnosis (n)	DIF (n)	Immune deposition (n)	Histological finding (n)
DLE, SCLE, LP, Actinic LP, Cutaneous Lupus, LPP, PLE, Rosacea, Atopic dermatitis, Pityriasis rosea, Dermatitis artefacta (51)	DLE (22)	Positive (10), Negative (12)	At BMZ IgM granular (1), linear (2) C3 granular (2) IgG granular (1) IgM and C3 granular (1) IgM and C3 linear (1) IgM, IgA and C3 linear (1) IgG, IgA and C3 linear (1)	Dermal mucin positive (22)
	SCLE (3)	Negative (3)		Dermal mucin positive (3)
	Cutaneous lupus (1)	Positive (1)	PV C3 (1)	Dermal mucin and vasculopathy positive (1)
	Psoriasis (3)	Positive (1), Negative (2)	At BMZ sparse C3 granular (1)	
	Actinic LP (3), LPP (3), PLE (3), Rosacea (3), Granulomatous inflammation (3), Spongiotic dermatitis (2), Pityriasis rosea (1), Dermatitis artefacta (1), LE, LPP and PLE could not be differentiated (3)	Negative (22)		
LP, Contact dermatitis, SCLE, BP, PV, GVHD, Vasculitis (15)	LP (9)	Positive (2), Negative (7)	At BMZ C3 fine granular (1), PV sparse C3 granular (1)	Lichenoid reaction yes (9)
	LP excluded (3)	Negative (3)		Lichenoid reaction no (3)
	Ulcer (3)	Negative (3)		Ulcer, favor of LP (1) Ulcer, favor of lichenoid GVHD (1) Ulcer, no comments for LP (1)

DIF: direct immunofluorescence, DLE: discoid lupus erythematosus, SCLE: subacute cutaneous lupus erythematosus, LP: lichen planus, LPP: lichen planopilaris, PLE: polymorphous light eruption, BP: bullous pemphigoid, PV (clinical diagnosis): pemphigus vulgaris, GVHD: graft versus host disease, BMZ: basement membrane zone, PV (immune deposition): perivascular.

lupus, 1 of which was DIF positive.

Results of the patients characterized by vasculitis were as follows (Table 4, Figure 4):

Six patients with clinical preliminary diagnosis and the pathological diagnosis of HSP were identified. DIF was positive in all patients with histologically detected LCV; perivascular IgA and C3 were found in four patients, IgM, IgA and C3 deposits in 2. The pathological diagnosis was LCV in 23 patients; while histological leukocytoclasis and DIF positivity were found in 17. C3 was the most common with DIF followed by IgA and C3. There were 13 patients diagnosed with vasculopathy, and DIF was negative in 10. Whereas, the diagnosis of vasculitis was ruled out with histological findings and DIF negativity in 12 cases.

When all patients were evaluated in terms of the subgroups, clinical and histopathological/DIF agreement rates ranged from 0% to 100%. A rate of 0% was found in 8 patients with the primary diagnosis of Duhring's patient, while a 100% rate was observed in 3 patients with the primary diagnosis of Darier disease, Hailey-Hailey disease and 6 patients with the primary diagnosis of HSP. Kappa agreement value between histopathological and DIF results in terms of total cases was mild ($\kappa = 0.021$). $p<0.001$ value was

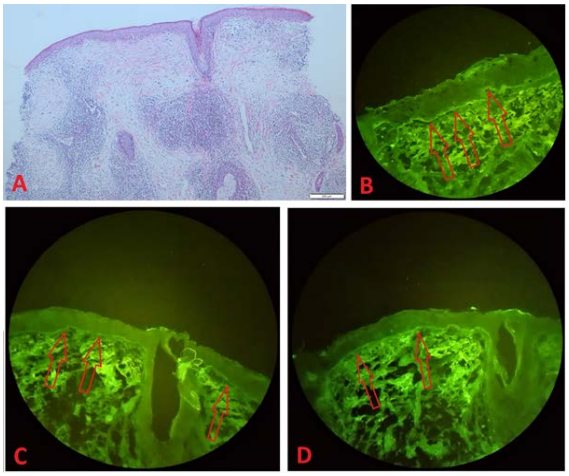

Figure 3. A: Skin biopsy specimen of a discoid lupus erythematosus patient shows flattening of the epidermis, basal vacuolar changes, periadnexal and perivascular lymphocytic infiltration and interstitial mucin deposition in the dermis (Hematoxylin/Eosin, 40x). B, C, D: IgG positivity at B, IgA positivity at C and C3 positivity at D in the epidermis basal membrane zone in the DIF study of the same patient (arrows).

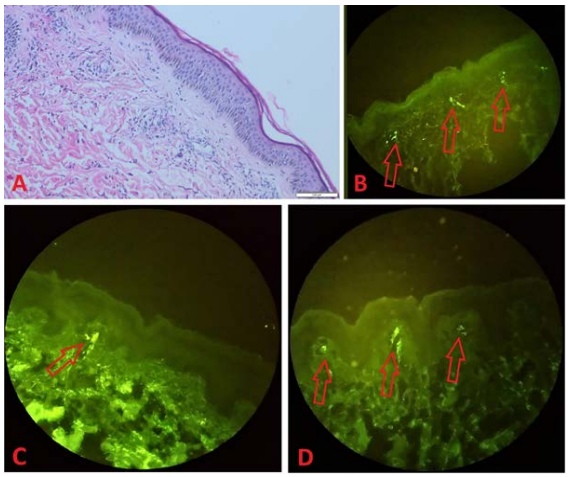

Figure 4. A: In a patient of leukocytoclastic vasculitis, leukocytoclasis and signs of vascular damage with nuclear particles in the dermis are observed (Hematoxylin/Eosin, 100x). B, C, D: Perivascular immune deposits are observed in the upper dermis in different leukocytoclastic vasculitis patients (arrows). C3 positivity at B, IgA positivity at C and D.

Table 4. Clinical preliminary diagnosis, pathological diagnosis, DIF results, immune deposits and histological findings of the patients characterized by vasculitis group.

Clinical diagnosis (n)	Pathological diagnosis (n)	DIF (n)	Immune deposition (n)	Histological finding (n)
HSP (6)	HSP (6)	Positive (6)	PV IgA and C3 (4) IgM, IgA and C3 (1) IgA, C3 and IgM at BMZ and PV (1)	LCV yes (6)
LCV, Drug eruption, PPD, Urticaria, Urticarial vasculitis, PLEVA, Lymphomatoid papulosis, Stasis dermatitis, Liveoid vasculitis, Psoriasis (62)	LCV (23)	Positive (17), Negative (6)	PV C3 (8), IgA (1) IgA and C3 (3) IgM, IgA and C3 (2) IgM and C3 (1) PV and at BMZ C3 (1) IgA and C3 (1)	Leukocytoclasis (23)
	Small vessel vasculitis (6)	Positive (6)	PV C3 (3) IgA and C3 (2), IgM and C3 (1)	
	Lymphocytic vasculitis (4)	Positive (2), Negative (2)	PV C3 (2)	
	Livedoid vasculitis (2)	Positive (2)	PV C3 (1), IgM and C3 (1)	
	Urticarial vasculitis (2)	Positive (1), Negative (1)	PV C3 (1)	
	Vasculopathy (13)	Positive (3), Negative (10)	PV C3 (2), IgM and C3 (1)	
	Vasculitis no (12)	Negative (12)		

DIF: direct immunofluorescence, HSP: Henoch Schönlein purpura, LCV: leukocytoclastic vasculitis, PPD: pigmented purpuric dermatosis, PLEVA: pityriasis lichenoides et varioliformis acuta, BMZ: basement membrane zone, PV (immune deposition): perivascular.

found between the histopathology and DIF evaluation with the McNemar test.

DISCUSSION

The DIF test is useful in the diagnosis of autoimmune bullous diseases and in differentiating from histologically similar conditions. Additionally, it can be helpful in addition to clinical and histological findings, in conditions such as connective tissue diseases, vasculitides, LP and occasionally psoriasis (7). Localization of immune complexes, their pattern, presence of inflammatory infiltrate, and types of antibodies are evaluated in the diagnosis of dermatological diseases (8).

In this study, pathologic agreement was observed in 19 (43.2%) of 44 patients with the primary diagnosis of BP, differential diagnosis could not be established in 8 (18.2%) patients and exclusion could be provided in 17 (38.6%) patients. There was no agreement between histological and DIF findings with the primary diagnosis of Duhring's disease. Pathological agreement was obtained in 10 (55.6%) of 18 patients with the primary clinical diagnosis of pemphigus and exclusion was made in 8 (44.4%) patients. Clinical agreement was obtained between histological and DIF findings in 26 (50.9%) patients with the primary diagnosis of LE, pathological diagnosis could not be made in 3 (5.9%) patients and exclusion was made in 22 (43.1%) patients. In the LP group, pathology was consistent in 10 (66.7%) of 15 patients, one patient's (6.7%) biopsy could not be evaluated due to ulceration and LP was excluded in 4 (26.7%) patients. In the vasculitis group, pathological agreement was observed in all 6 patients (100%) for HSP, and 37 (59.7%) of 62 patients with livedoid vasculitis, urticarial vasculitis and their differential diagnoses, especially LCV, in the clinical primary diagnosis were diagnosed as vasculitis, 13 (20.9%) as vasculopathy, while vasculitis was ruled out in 12 (19.4%) patients. Although DIF negativity was observed in 42 patients, the diagnosis was established with histopathological findings (12 DLE, 3 SCLE, 1 dermatomyositis, 7 LP, 6 LCV, 3 vasculitis, 10 vasculopathy). While no histopathological finding was found in 5 patients, the diagnosis was obtained using DIF positivity in these patients (3 BP, 2 PV).

Several studies comparing DIF, clinical and histopathological findings have reported that DIF results were consistent with clinical and histological findings. In a study analyzing 215 biopsies, DIF positivity was found in 103 patients, clinical and histological agreement was good, and positivity was found by 98.1% in the pemfigus group, 96% in the BP group and 100% in the HSP group. DIF negativity contributed to rule out immune mediated diseases in 110 patients (7). In another study evaluating 204 skin biopsies with 51 being in patients aged between 0 and 14 years, 151 DIF positivities were found, with 132 being consistent with the clinical diagnosis (2). In a study by Lebe et al. (9), histological findings and DIF images were evaluated in 197 cases diagnosed with vesiculobullous dermatitis, and the rates of agreement among clinical and histopathological/DIF diagnoses were close to our results with 58.8% in PV, 53.8% in PF, 37.9% in BP and 5.2% in dermatitis herpetiformis (Duhring's disease). In another study, the concordance of clinical, histological and DIF findings in 92 patients diagnosed with autoimmune bullous dermatosis was evaluated, statistically high agreement was found between clinical, histological and DIF diagnoses for the intraepidermal separation subgroup, and low agreement for the subepidermal separation subgroup (10).

Autoimmune vesiculobullous diseases, like other inflammatory dermatoses, are a morphologically heterogeneous group, and the distinction between various bullous diseases is important for treatment and prognosis. The DIF evaluation is still accepted as the gold standard in the differentiation of these diseases (9). However, for the diagnosis of these diseases, studies have been carried out on the detection of IgG, IgA and complement by immunohistochemical method from formalin-fixed paraffin-embedded blocks in recent years (11).

BP is the most common autoimmune subepidermal bullous disease, and is characterized by antibodies developing against hemidesmosome components in the basal cells of the epidermis (9). These components are mainly BP180 (180kDa) or BPAG2 and BP230 (230kDa) or BPAG1, and are responsible for the adhesion between the epidermis and dermis (12). Clinically characteristic is large serous or hemorrhagic tense blisters on normal or erythematous skin. Histology shows subepidermal separation, mild, moderate, or intense lymphocyte, eosinophil, and/or neutrophil infiltration within the separation and in the dermis (6). In a study conducted to investigate the accuracy of the belief that false negative DIF results may be encountered if the biopsy site is the lower extremity, no difference was found in terms of false negativity in biopsies taken from the trunk, upper extremity and lower extremity (13). With DIF, a linear,

homogeneous deposit of IgG and/or C3 is almost always encountered along the basement membrane zone of the skin around the lesion, oral lesions are present in some cases with IgA deposits (14). In a study by Meijer et al. (15), 277 (91.4%) of 343 patients diagnosed with pemphigoid had IgG, 223 (73.6%) C3c, 83 (27.4%) IgA deposits, while IgG alone was seen in 60 (19.8%), with C3c in 135 (44.6%) with IgA in 20 (6.6%) and combined with C3c and IgA and 62 (20.5%) specimens. In the study of Damman et al. (16), it is reported that in cases where IgG and C3c are negative, detection of C4d with DIF may help in the diagnosis, but it is not a 100% specific BP marker. Consistently with the literature, in our study of 19 patients diagnosed with BP and CBDC, IgG and/or C3 deposits were seen in 15 (78.8%) patients, IgM and IgA deposits in 1 (5.3%) patient, IgM, IgA ve C3 in 2 (10.6%) and IgM and C3 deposits in 1 (5.3%) patient. Immunodeposits were linear in 17 patients, granular in 1 case, linear and granular in 1 patient.

Pemphigus is a rare group of autoimmune bullous diseases affecting the skin and mucous membranes. Autoantibodies (mainly IgG) found on the surface of keratinocytes and formed against desmosome components (especially desmoglein1, Dsg1, or desmoglein3, Dsg3, or both) that are involved in intraepidermal adhesion play a pathogenic role. Acantholysis, vesicles, blisters and erosions develop on the skin and/or mucous membranes because of the loss of intercellular adhesion (17,18). The main pemphigus forms according to clinical and histopathological features include PV, PF, paraneoplastic pemphigus, pemphigus herpetiformis, and IgA pemphigus (17). The diagnosis of PV, PF and IgA pemphigus is based on clinical findings, DIF examination of perilesional biopsy, serology, and histological examination of lesional biopsy (19). Intercellular deposits of IgG, less frequently C3, IgM, and IgA are seen in the epidermis with DIF (14). In a study by Arbache et al. (20), intercellular IgG and C3 deposits were most frequently observed with DIF in 277 patients in the intraepidermal separation disease group. Positivity rates were found as 91.5% and 79.5% for PV, 94%, and 73% for PF, 66%, and 33% for paraneoplastic pemphigus and intercellular IgA deposition 100% for IgA pemphigus. In this study, the DIF positivity was observed in 10 patients with the primary diagnoses of PV and PF. IgG and C3 deposits were found in 5 (50%) patients, C3 deposits in 3 (30%) patients. In one of the DIF-positive patients, immune deposits were in the hair follicle epithelium.

LE is a complex disease and the clinical picture of LE can vary from the form in which only cutaneous lesions are seen, to the form in which skin rashes are accompanied by progressive systemic involvement (21). LE can be clinically classified mainly into systemic (acute), subacute cutaneous and chronic forms (22). Discoid lupus erythematosus (DLE) accounts for 85% of cutaneous LE cases (23). The main histological findings are epidermal atrophy, follicular dilatation, plugging, liquefactive degeneration and basement membrane thickening of the epidermal basal layer, dermal edema, telangiectasia, perivascular and periappendageal lymphocytes, histiocytes, mucin deposition (21). Vasculitic reactions can be seen in 11% of SLE cases (24). Immune deposits of various patterns (homogeneous, granular and reticular) are observed along the dermoepidermal junction with DIF and IgM are most commonly identified (21).

In a study evaluating the histopathological and DIF findings of 75 skin biopsies with DLE, DLE variants, LP-like lesions in the clinical preliminary diagnosis, histopathological characteristic DLE features were found in 60% of the patients and DIF positivity in 68% of the patients. The most common site of accumulation of immunocomplexes with DIF was the dermoepidermal junction (80%), the most commonly found (77%) was IgG deposit, followed by IgM deposit. These deposits were observed alone or along with other Ig's or C3 (23). Elbendary et al. (25) encountered most frequently IgM deposition especially in granular pattern in LE cases at the dermoepidermal junction and stromal-epithelial junction of hair follicles and sweat glands. In this study, 22 (84.6%) of 26 patients with the pathological diagnosis of LE were the patients with DLE. Interface dermatitis and dermal mucin depositions were observed in 26 patients, and vasculopathic changes in 1 patient. DIF positivity was observed in 11 (42.3%) of the 26 patients. Immune deposits were in the epidermis basement membrane zone in 10 (90.9%) patients, and in the dermal perivascular area in 1 (9.1%) patient diagnosed with vasculopathy and cutaneous lupus. The most common immunoglobulin deposit was IgM in 6 (54.5%) patients, alone, with C3, or with IgA and C3.

The term vasculitis refers to a heterogeneous group of disorders characterized by inflammation and damage of blood vessel walls. It may be limited to the skin or some other organs or may be a multisystem disease (14). The clinical signs of vascular damage are seen as edema, livedo reticularis and various hemorrhage findings (such as petechiae, purpura,

ecchymosis) and in the case of severe damage, vascular occlusion may cause ischemia and associated necrosis, gangrene and/or ulceration. The main histological findings are inflammatory cell infiltration and vascular damage (such as fibrinoid necrosis in the vessel wall). Conditions in which inflammation is not observed but vascular damage is seen are considered as vasculopathy (6). In a study evaluating the histopathological and DIF findings of 121 cases diagnosed with cutaneous small vessel vasculitis, it was observed that at least one of IgM, IgG, IgA and C3 were deposited in the perivascular or epidermal basal membrane zone. The most common immune deposit was C3 (53.7%), and the most deposited immunoglobulin was IgA (11.6%) in the perivascular area. IgA positivity was observed in all (100%) patients with HSP (26). In this study, leukocytoclasis was found in 29 (67.4%) and DIF positivity was found in 34 (79.1%) of 43 patients with pathological diagnosis of vasculitis, including HSP. C3 was the most common deposit and was observed in 33 (97.1%) patients either alone or along with immunoglobulin. Immune deposits were observed in the perivascular area in all patients, and additionally in the epidermis basal membrane zone in three patients. The most frequently deposited immunoglobulin was IgA in 15 (44.1%), in all HSP patients (100%) and it was positive in 32.1% of patients diagnosed with vasculitides other than HSP. IgM deposits were observed in 7 (20.6%) patients with 2 having HSP. Immune deposit with IgG was not observed in any patient.

The main limitations of this study are the use of data obtained from archival sources and its retrospective nature. The biopsy results examined in a certain period were analyzed, the follow-up of the cases, the course of the diseases, whether there were any biopsy results before or after them were not examined.

CONCLUSION

The DIF method makes a significant contribution to clinical and histological findings in the diagnosis or exclusion of immunopathological events affecting the skin. In our study, although the kappa fit value was mild between the histopathology and DIF findings, the contribution of DIF stands out, especially in typing of patients with vesiculobullous diseases. It also supports the clinical and histological findings of LE and vasculitis cases. The correlation between clinical and pathological examinations is crucial to obtain efficient results. It should be kept in mind that there may be false negative or false positive results in the DIF test. A definitive diagnosis can be made by evaluating the DIF and histological findings together. Biopsy localization, lesion age, type of sampling, laboratory stages, and criteria to be considered in the microscopic evaluation process are important points that enable reaching an accurate diagnosis.

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