Zagazig University Medical Journal
www.zumj.journals.ekb.eg

Manuscript ID ZUMJ-1901-1032 (R1)
DOI **10.21608/zumj.2019.7305.1032**

ORIGINAL ARTICLE

THE DIAGNOSTIC ROLES OF C4D EXPRESSION IN SOME AUTOIMMUNE BULLOUS DERMATOSES IN ZAGAZIG UNIVERSITY HOSPITALS; AN IMMUNOHISTOCHEMICAL STUDY

Kamal Ahmed El Kashishy[1], Mohammed Ibrahim Metwally[2], Abeer Magdy Hassan[1] and Ebtehal El Sayed Mohammed Soliman[1]

[1]Pathology Department, Faculty of Medicine, Zagazig University. Egypt.
[2]Dermatology, Venereology and Andrology Department, Faculty of Medicine, Zagazig University. Egypt.

Corresponding author :
Ebtehal El Sayed Mohammed Soliman
Pathology Department, Faculty of Medicine, Zagazig University. Egypt.
allah.inmyheart.1990@yahoo.com

Submit Date 2019-08-26
Revise Date 2019-10-07
Accept Date 2019-10-13

ABSTRACT

Background: Autoimmune bullous skin dermatoses (AIBD) diagnosis relies on direct immunofluorescence examination performed on frozen tissue sections. However, this is not always available for DIF; therefore, alternative techniques needed for diagnosis. We tested the usefulness of C4d immunohistochemistry on formalin-fixed, paraffin-embedded tissue (FFPE) sections for the diagnosis of AIBD.

Objective: to evaluate the role of C4d expression using immunohistochemistry in diagnosis of some autoimmune bullous skin diseases.

Methods: This study included FFPE tissue blocks of 35 cases from archives of pathology department, Faculty of medicine, Zagazig University in the period from January 2017 to December 2017. These 35 cases were diagnosed histopathologically as: 30 cases autoimmune bullous dermatoses (18 pemphigus vulgaris, 6 bullous pemphigoid, 3 pemphigus foliaceus and 3 drug induced pemphigus) and 5 cases erythema multiforme. Specimens were obtained as punch biopsy from the edge of a recent bullous lesion. C4d immunostaining was performed and correlated with clinicopathology.

Results: C4d immunohistochemistry was a reliable method for detecting AIBD in 29 of 30 cases diagnosed by histopathology, with 96.7% sensitivity. Also it was efficient in ruling out all the 5 negative cases ruled out by histopathology with 100% specificity.

Conclusion: When correlated with the light microscopic and clinical findings, the C4d assay defines an important diagnostic adjunct in the evaluation of some autoimmune vesiculobullous dermatoses. It may prompt further DIF testing or, in some instances, may even define a reasonable substitute for DIF and/or add to the morphologic assessment of a biopsy specimen submitted for routine light microscopic assessment.

Key words; C4d, Immunohistochemistry, Autoimmune Bullous Dermatoses.

INTRODUCTION

Autoimmune bullous skin diseases are characterized by the presence of autoantibodies in specific adhesion antigens of the epidermis or the dermoepidermal junction zone. Binding of these antibodies to their target antigens causes loss of adhesion between epidermal keratinocytes or at the basement membrane zone, which results in blister formation**[1]**.

Diagnosis of pemphigus (P), bullous pemphigoid (BP) and other autoimmune bullous dermatoses (AIBD) can be suggested clinically but must be confirmed by a pathological study, including examination of routinely processed [formalin-fixed, paraffin-

embedded (FFPE)] sections of a skin biopsy taken (ideally) from the edge of a recent bullous lesion, and direct immunofluorescence (DIF) testing of a snap-frozen (or fixed in Michel's medium before freezing) skin biopsy taken from peribullous skin**[2]**.

DIF typically shows linear IgG and/or C3 deposits along the dermal-epidermal junction (DEJ) in BP, and IgG and C3 deposits on the surface of epidermal keratinocytes (the so-called "intercellular" pattern) in P. Although DIF is essential in the diagnosis of both BP and P, suitable skin specimens for DIF (ie, snap-frozen or fixed in Michel's medium) are not always available to the pathologist, either because an AIBD were not suspected by the submitting physician (several clinically atypical presentations of P and BP exist), or because of the difficulty to handle and forward frozen skin samples suitable for DIF examination to the laboratory**[3]**.

When antigen retrieval techniques became available, attempts were made to perform DIF on FFPE skin biopsies using these techniques (enzymatic treatment and microwave heating in citrate or urea buffer). These early studies achieved detection of immunoglobulins and/or C3 in a characteristic pattern in 50%–60% of BP and P cases**[4]**.

Commercially available mouse monoclonal and rabbit polyclonal antibodies have been produced and are able to reliably detect human C3d and C4d, stable component of classic complement activation, on FFPE biopsies using immunoenzymatic techniques**[5]**.

Complement fragment 4d (C4d), an index of complement activation by the classical pathway, is well established as a marker for antibody deposition in renal allograft biopsies. It is reliably detected by immunohistochemistry on formalin-fixed paraffin embedded kidney biopsies and correlates with serologic studies that confirm the presence of donor-specific renal allograft antibodies**[6]**.

C4d immunohistochemical stain is sensitive method to confirm immunoreactant deposition in formalin fixed paraffin embedded tissue in bullous pemphigoid. The usefulness of immunohistochemical detection (IHC) of C4d in the diagnosis of inflammatory dermatoses, including AIBD, has been addressed in some studies; these included a limited number of biopsies or studied a variety of inflammatory dermatoses without targeting specifically AIBD, and their results were sometimes contradictory**[7]**.

MATERIALS AND METHODS

Materials:

This study included formalin fixed, paraffin embedded tissue blocks from 35 cases diagnosed histopathologically as:

- 30 cases autoimmune bullous dermatoses (18 cases of pemphigus vulgaris, 6 cases of bullous pemphigoid, 3 cases of pemphigus foliaceus and 3 cases of drug induced pemphigus).
- 5 cases erythema multiforme.

Blocks were also selected from archives of pathology department, Faculty of medicine, Zagazig University in the period from January 2017 to December 2017. Specimens of these paraffin blocks were obtained as punch biopsy from the edge of a recent bullous lesion. Written informed consents were obtained from all participants and the study was approved by the research ethical committee of Faculty of Medicine, Zagazig University. The work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Methods:

1. **Clinical study:**

Clinical data concerning age, sex, family history, drug history and clinical presentation were obtained from the patients' files.

2. **Histopathological study:**

Four µm thick sections were cut from formalin fixed, paraffin embedded tissue specimens and stained with hematoxylin & eosin for histopathological examination.

3. **Immunohistochemical study:**

I. **Immunohistochemical stain:** Immunohistochemical reactions were carried out using streptavidin-biotin immunoperoxidase staining technique. Immunohistochemical staining was carried out using C4d (rabbit monoclonal anti-C4d antibody [dil 1:100, clone A24-T, Biocare medical, CA, USA]).

The universal kit:

Super sensitive link-label IHC detection system, Biotin-Streptavidin amplified (B-SA)

detection system, (Code No.QD000-5L Multilink. Detection kit, HRP, Bio Genex, CA, USA) was used in this study. The following materials were included in this kit:

- Peroxidase block: one vial (6ml) of 3%hydrogen peroxide in water.
- Secondary antibody (link): one vial (6 ml) of prediluted biotinylated, anti-immunoglobulins for mouse, rabbit in phosphate buffered saline (PBS) with carrier protein and less than 0.09% sodium azide.
- Lable: One vial (6 ml) of ready-to-use horse radish (HRP) peroxidase-conjugated streptavidin in PBS with carrier protein and less than 0.1% procilin 300.
- Chromogen: One vial (2ml) of 3, 3 diaminobenzidine (DAB) chromogen solution.
- Substrate: Six vials of ready to use substrate buffer.

II. **Immunohistochemical procedure was perfor-med as the following:**

- Two consecutive sections 5 μm thick were cut, mounted on positively charged slides coated with poly L-lysine.
- Sections were dewaxed and then inserted in xylene for 30 minutes.
- Slides were rehydrated in descending grades of alcohol, followed distilled water for 5 min and then in phosphate buffer saline (PBS) for 5 min.
- This was followed by incubation in 3% hydrogen peroxide in water to block endogenous peroxidase.
- Antigen retrieval was done using Dako target retrieval solution (PH 6.0) in a microwave for 20 min, and then washed in phosphate buffer saline (PBS) (pH 7.3).
- Excess liquid was tap off using lint less tissue (as gauze).

Application of primary antibody:

- A rabbit monoclonal anti-C4d antibody was placed on each slide to cover the specimen for 30 min.
- Slides were rinsed with buffer solution with avoiding flow directly on the tissue and excess buffers were tap off and slides were wiped
- Application of secondary antibody.
- Sections were incubated with polyclonal antirabbit immunoglobulin for 15 minutes at room temperature followed by washing in buffer.
- Sections were incubated with streptavidin-HRP for 15 minutes followed by washing.

Detection system:

- Sections were incubated with diaminobenzidine (DAB) substrate as a chromagen then rinsed gently with distilled water.
- They were washed and counterstained with Mayer's hematoxylin for 30 seconds, then rinsed gently in ascending grades of alcohol.
- Slides were cleared in xylene and mounted with a cover slip.

III. Interpretation and evaluation of immuno-staining:

When correlated with the light microscopic and clinical findings, the C4d assay has significant application in the assessment of some autoimmune vesiculobullous disorders**.** Whenever the biopsies contained bullous lesions, C4d immunolabeling was as a rule found both at the roof and the floor of the blister. In **pemphigus vulgaris**, C4d immunostaining showed brown deposits on the surface of epidermal keratinocytes, predominating on the lower epidermal layers ("intercellular" pattern) and also found on the surface of acantholytic keratinocytes present in the blisters. In **bullous pemphigoid**, the C4d deposits were visualized as a brown linear labeling along the DEJ (found at the level of the blister and in noncleaved skin) and as labeling of the membrane of basal keratinocytes. In **pemphigus foliaceus**, C4d immunostaining showed intercellular deposition, predominantly within the superficial epidermal layers. **Drug induced pemphigus** showed diffuse C4d immunostaining**.**

IV. Statistical analysis:

The expression of C4d was tested using Chi-square test. This was done using SPSS version 20 (2006). The sample mean ($\overline{X}$), standard deviation (SD) and the range were obtained for numerical variables. The frequency, distribution and percentage were calculated for categorized variables. The probability value (P value) is then obtained from the (X^2) distribution tables according to a certain degree of freedom (D.F) = (number of columns -1) (number of rows -1). Level significance: for all above mentioned statistical tests done, the probability value (P-value) considered significant at 5% level ($P > 0.05$ non significant, $P < 0.05$ significant &$P < 0.001$ highly significant).

RESULTS

The age of the studied cases of AIBD ranged from 25 to 73 years old with a mean of 51.76±13.9. This study revealed that AIBD were more common in females (19 out of 30; 63.3%) than in males (11 out of 30; 36.7%); yet the result is statistically insignificant (p value = 0.144) **(Table 1).**

The age of the studied cases of erythema multiformis ranged from 20 to 44 years old with a mean of 29.6±8.96. There were 3 out of 5 cases (60.0%) being males in this study.

Our study showed that 24 out of 30 cases (80%) of the studied AIBD group had –ve family history with statistically significant difference (p<0.001) in comparison with those with 6 cases with +ve family history (20%).

Also, 27 out of 30 cases (90%) of the studied group had –ve drug history with statistically significant difference (p<0.001) in comparison with 3 cases with +ve drug history (10%).

Regarding clinical picture, 15 out of 30 (50.0%) cases had bullae on normal base, while bullae on erythematous base were evident in 15 cases (50%). Vesicles were present in 24 out of 30 cases (80%) and oral lesion were present in 16 out of 30 cases (53.3%). 24 out of 30 cases (80%) of cases had oral mucosa affection. Bullae on erythematous base and vesicles were present in all of the 30 cases (100%) **(Table 2)**.

There was no significant difference between +ve and –ve family history and drug history of the studied group.

Histopathologically, the most prevalent type of AIBD in studied group was Pemphigus Vulgaris, being 18 out of 30 cases (60%) **(fig. 1)**, followed by bullous pemphigoid, being 6 cases (20%) **(fig. 2)**. Both pemphigus foliaceus **(fig. 3)** and drug induced pemphigus **(fig. 4 A)** were detected in 3 cases out of 30 for each (10%) **(Table 3).**

Also, the most prevalent type of AIBD by immunohistochemistry in the studied group was Pemphigus Vulgaris being 18 out of 29 cases (62.1%) **(fig. 5)**, followed by bullous pemphigoid, being 5 cases (17.3%) **(fig. 6)**. Both pemphigus foliaceus **(fig. 7)** and drug induced pemphigus **(fig. 4B)** were detected in 3 cases out of 30 for each (10.3%) **(Table 4).**

There was no significant difference between immunohistochemical & histopathological findings in detecting AIBD **(Table 5).**

When comparing diagnostic performance of Immunohistochemical versus Histopathological findings in detecting the autoimmune bullous dermatoses **(Table 6)**, Immunohistochemistry had a sensitivity of 96.7 %, specificity of 100% and accuracy of 97.1% in diagnosis of AIBD. There was an excellent degree of agreement between Immunohistochemical & Histopathological findings **(Table 7)**.

Table (1): Demographic distribution of the autoimmune bullous dermatoses (AIBD) studied cases:

Demographic distribution	All patients (N=30)			
	No.	%	χ^{2}*	P value
Sex				
Male	11	36.7	2.133	0.144
Female	19	63.3		
Age (years)				
Mean ± SD	51.76±13.9			
(Range)	(25-73)			

χ2* =single sample chi square p value is significant if <0.05

Table (2): Clinical picture of the autoimmune bullous dermatoses studied group:

Symptoms	All patients (N=30)	
	No	%
Bullae on normal base		
+ve	15	50.0
-ve	15	50.0
Bullae on erythematous base		
+ve	15	50.0
-ve	15	50.0
Vesicle		
+ve	24	80.0
-ve	6	20.0
Oral lesion		
+ve	16	53.3
-ve	14	46.7

Table (3): Histopathological classification of the autoimmune bullous dermatoses studied cases:

Classification	All patients (N=30)	
	No	%
Pemphigus vulgaris	18	60.0
Pemphigus foliaceus	3	10
Drug induced pemphigus	3	10
Bullous pemphigoid	6	20

Table (4): Immunohistochemical classification of the autoimmune bullous dermatoses studied cases:

Classification	All patients (N=29)	
	No	%
Pemphigus vulgaris	18	62.1
Pemphigus foliaceus	3	10.3
Drug induced pemphigus	3	10.3
Bullous pemphigoid	5	17.3

Table (5) Comparison between Immunohistochemical & Histopathological findings in detecting the autoimmune bullous dermatoses types:

Variable	Histopathological (N=30)		Immunohistochemical (N=29)		χ^2	p-value
	No.	%	No.	%		
Pemphigus vulgaris	18	60.0	18	62.1	0.03	0.99 (NS)
Bullous pemphigoid	6	20.0	5	17.3		
Drug induced pemphigus	3	10.0	3	10.3		
Pemphigus foliaceus	3	10.0	3	10.3		

$\chi 2$ = chi square P value significant if <0.05.

Table (6): Diagnostic performance of Immunohistochemical versus Histopathological findings in detecting the autoimmune bullous dermatoses (N=35):

		Histopathological findings		Total
		Present	Absent	
Immunohistochemical Findings	**Present**	(true +ve) 29	(false +ve) 0	29
	Absent	(false –ve) 1	(True –ve) 5	6
Total		30	5	35

Table (7) Degree of agreement between Immunohistochemical & Histopathological findings:

	Immunohistochemistry (N=35)	Histopathology (N=35)
Cases	%	%
+ve cases	82.9	85.7
-ve cases	17.1	14.3
Degree of agreement (Kappa agreement)	89.2%	

Table (8): Family history of the autoimmune bullous dermatoses studied cases:

Classification	All patients (N=30)		χ^2	P value
	No	%	10.8	**0.001 (S)**
+ve family history	6	20.0		
-ve family history	24	80.0		

$\chi 2$ =single sample chi square P value significant if <0.05

This table shows that 80% of the studied group has –ve family history with statistically significant difference (p<0.001) in comparison with those with +ve family history (20%).

Table (9): Drug history among the autoimmune bullous dermatoses studied cases:

Drug history among studied group	All patients (N=30)		χ^2	P value
	No	%	19.2	**<0.001 (HS)**
+ve drug history	3	10		
-ve drug history	27	90		

This table shows that 90% of the studied group has –ve drug history with statistically significant difference (p<0.001) in comparison with those with +ve drug history (10%).

Table 10: Validity of Immunohistochemistry in detecting AIBD.

SN (%)	**96.7%**
SP (%)	100%
PPV (%)	100%
NPV (%)	83.3%
Acc (%)	97.1%

SN: Sensitivity SP: Specificity PPV: Positive Predictive Value.
NPV: Negative Predictive Value. Acc: Accuracy.

DISCUSSION

Autoimmune bullous diseases are rare, potentially distressing bullous dermatoses of the skin and mucous membranes characterized by the presence of tissue-bound and circulating antibodies directed against various structural components of the skin and mucous membranes. These autoantibodies lead to a

loss of skin adhesion which shows up clinically as the formation of blisters or erosions. The classification of autoimmune bullous dermatoses still relies on histologic criteria, primarily according to the localization of bullae, so intraepidermal and subepidermal bullous dermatoses are distinguished**[8]**.

The autoimmune **intraepidermal** bullous diseases include the **pemphigus family**: pemphigus vulgaris (PV), pemphigus foliaceus (PF), IgA pemphigus, paraneoplastic pemphigus (PNP), and drug-induced pemphigus**[9]**.

The autoimmune **subepidermal** bullous dermatoses include: bullous pemphigoid (BP), cicatricial pemphigoid, pemphigoid gestationis (PG), dermatitis herpetiformis (DH), linear IgA disease (LAD), epidermolysis bullosa aquisita (EBA) and bullous systemic lupus erythematosus (SLE) **[10]**.

Pemphigus (P) and bullous pemphigoid (BP) account for two of the most common autoimmune bullous dermatoses (AIBD) **[2]**.

Diagnosis of pemphigus (P), bullous pemphigoid (BP) and other autoimmune bullous dermatoses (AIBD) can be confirmed by direct immunofluorescence (DIF) testing of a snap-frozen (or fixed in Michel's medium before freezing) skin biopsy taken from peribullous skin, as well as indirect immunofluorescence (IIF) using the patient's serum for detection of auto-antibodies**[11]**.

Some studies found that the efficacy of H&E staining alone in diagnosing bullous diseases is probably greater than 90% and could be considered satisfactory. However, ambiguous cases and treatment planning remain challenging**[12]**.

Equivocal cases come from a number of conditions that are expressed as vesicles or bullae that rapidly rupture and result in erosions or ulcers. Such then the differential diagnosis became a group of wide scale. In such cases, DIF and IIF are important to distinguish between them, confirm diagnosis, and plan proper treatment. Limitations of these techniques are the availability of blood serum or fresh frozen tissue that require specific facilities to perform, and they do not always exist in all health services, especially in developing countries. In addition, it requires a pathologist skilled in its interpretation and it is costly thus not affordable for all patients. Moreover, pathologists usually receive patients' specimens that have already been fixed in formalin**[13]**.

Because of some difficulties that may face dermatologists and pathologists in diagnosis of some problematic cases of bullous diseases on the base of routine histological examination and the unavailability of fresh tissue or prepared frozen samples for immunofluorescence study, immunohistochemical expression of C4d performed on paraffin sections may be a useful diagnostic test for common AIBD**[14]**.

Complement fragment 4d (C4d) is an index of complement activation by the classical pathway. The utility of IHC and immunofluorescence studies to detect C4d has been demonstrated in immunoallograft biopsies, with C4d deposition indicating humoral allograft rejection**[15]**.

Recent studies have demonstrated the utility of IHC for C4d and C3d in the diagnosis of autoimmune vesiculobullous disorders including BP in routine FFPE skin biopsy specimens**[1]**.

On the other hand, the use of immunohistochemistry on paraffin-embedded tissue to show complement and immunoglobulin deposition in the skin is not well established. Only a few studies report the use of immunohistochemistry on formalin-fixed tissue in the assessment of inflammatory autoimmune skin diseases, demonstrating a comparatively high number of false-negative results **[16]**.

The present study is concerned with the role of C4d immunohistochemistry in diagnosis of some autoimmune bullous dermatoses (AIBD) and **included** 30 cases of AIBD (18 cases of pemphigus vulgaris, 6 cases of bullous pemphigoid, 3 cases of pemphigus foliaceus and 3 cases of drug induced pemphigus) representing 60%, 20%, 10% and 10% respectively.

Our results indicate that C4d immunohistochemistry proved to be a reliable method for detecting AIBD in 29 of the 30 cases already diagnosed by histopathology, with **a sensitivity** of **96.7%**. Also it was efficient in ruling out of the all 5 negative

cases already ruled out by histopathology with **a specificity** of **100%.**

It is unclear why one case of BP in our study failed to demonstrate immunoreactivity against C4d. It is possible that there was inadequate IgG autoantibody deposition to activate classical complement pathway. Multiple studies have shown that IgG4 subclass, which is the most consistent IgG subclass deposited at the BMZ in BP, does not activate complement pathway by the classical pathway and instead may participate in complement activation by the alternate pathway**[17]**.

One or more of the complement-fixing subclasses of IgG (IgG1, IgG2 and IgG3) is deposited at the basement membrane in a majority of, but not all, cases of BP**[18]**.

Our results are in agreement with *Chandler et al,* who observed that C4d immunohistochemical stain is a sensitive method to confirm immunoreactant deposition in formalin-fixed paraffin-embedded tissue in bullous pemphigoid and that in cases of pemphigus vulgaris and pemphigus foliaceus, C4d staining could be useful in supporting the diagnosis with formalin-fixed paraffin-embedded tissue **[7]**.

Our results are also in agreement with *Velez et al* revealed that IHC is as reliable as DIF or IIF for the diagnosis of AIBD **[19]**. ***Zimmer et al*** revealed that C4d immunostaining might be helpful in the assessment of esophageal autoimmune bullous diseases **[20]**. Recently, ***Miyamoto et al*** revealed that IHC may serve as a reliable method to assess PF diagnosis **[21].**

On the other hand, there were some contradicting studies regarding diagnostic value of C4d IHC. ***Magro and Dyrsen*** studied 17 cases of BP with C3d and C4d IHC and found invariable concordance between C3d IHC and classical DIF but (somewhat unexpectedly) did not detect C4d positivity in BP cases **[18]**. **This contradicts with the results of our study** that showed 83.3% of BP cases to be C4d positive (5/6 cases). Moreover, ***Magro et al*** reported a lower sensitivity of C4d compared to C3d **[22]. This discrepancy could be attributed to** differences in specimen handling (type of fixative and fixation duration) and in immunostaining protocols.

Historically, investigators have not been able to use formalin-fixed paraffin-embedded tissue to effectively show immunoreactant deposition in cases of BP**[3]**.

In fact, what could be peculiar in our study is that it is one of the very few studies that were able to demonstrate the high efficacy of C4d IHC in diagnosis of some AIBD when correlated to conventional histopathological staining not immunofluorescence, thus solving a great issue regarding specimen costs, availability and pathological accessibility; reserving more complex immunofluorescence to the most ambiguous cases.

Despite small sample size, being a single centered study and lack of using immunoflorescence as limitations to our study, yet we were able to illustrate the potential utility of C4d IHC on FFPE tissue in distinguishing some different and important AIBD.

CONCLUSION

When correlated with the light microscopic and clinical findings, the C4d assay defines an important diagnostic adjunct in the evaluation of some autoimmune vesiculobullous dermatoses. It may prompt further DIF testing or, in some instances, may even define a reasonable substitute for DIF and/or add to the morphologic assessment of a biopsy specimen submitted for routine light microscopic assessment.

It is our opinion that in the future, formalin-fixed paraffin-embedded tissue can be evaluated for the presence of C4d, and in positive cases, it allows clinicians to proceed directly to serologic confirmation of the immunobullous process, without rebiopsy for DIF. Therefore, C4d immunohistochemistry may be a helpful and accessible technique to replace other time consuming methods such as immunofluorescence. So before doing DIF, reliable immunohistochemical detection of C4d on formalin-fixed tissue is advised to be done.

Further studies using different molecular methods on C4d on larger number of cases are recommended to clarify its correlation with the other clinicopathological data and confirm our findings.

Conflict of interest: No conflict of interest
Financial Disclosure: no final disclosure

REFERENCES

1. Pfaltz K, Mertz K, Rose C, Scheidegger P, Pfaltz M, Kempf W. C3d immunohistochemistry on formalin-fixed tissue is a valuable tool in the diagnosis of bullous pemphigoid of the skin. J Cutan Pathol 2010; 37 (6): 654-8.
2. Villani AP, Chouvet B, Kanitakis J. Application of C4d Immunohistochemistry on Routinely Processed Tissue Sections for the Diagnosis of Autoimmune Bullous Dermatoses. Am J Dermatopathol 2016; 38 (3):186-8.
3. D'Ambra-Cabry K, Deng DH, Flynn KL, Magee KL, Deng JS. Antigen retrieval in immunofluorescent testing of bullous pemphigoid. Am J Dermatopathol 1995;17 (6): 560–3.
4. Al-Rifai I, Kanitakis J, Faure M, Claudy A. Immunofluorescence diagnosis of bullous dermatoses on formalin-fixed tissue sections after antigen retrieval. Am J Dermatopathol 1997; 19 (1):103–5.
5. Kwon EJ, Ntiamoah P, Shulman KJ. The utility of C4d immunohistochemistry on formalin-fixed paraffin-embedded tissue in the distinction of polymorphic eruption of pregnancy from pemphigoid gestationis. Am J Dermatopathol 2013; 35 (8): 787-91.
6. Troxell ML, Weintraub LA, Higgins JP, Kambham N. Comparison of C4d immunostaining methods in renal allograft biopsies. Clin J Am Soc Nephrol 2006; 1 (3): 583-91.
7. Chandler W, Zone J, Florell S. C4d immunohistochemical stain is a sensitive method to confirm immunoreactant deposition in formalin-fixed paraffin-embedded tissue in bullous pemphigoid. J Cutan Pathol 2009; 36 (6): 655-9.
8. Chams-Davatchi C, Valikhani M, Daneshpazhooh M, Esmaili N, Balighi K, Hallaji Z, et al. Pemphigus: analysis of 1209 cases. Int J Dermatol 2005; 44 (6): 470–6.
9. Hong W, Brandling-Bennett HA, Harrist TJ. Noninfectious vesiculobullous and vesiculopustular disease. In: Elder D, Elenitsas R, Aworosky C, Ohnson B, editors. Lever's Histopathology of the Skin, 10th edn. Philadelphia: Lippincott Raven; 2010: 246–67.
10. Olasz EB, Yancey KB. Bullous pemphigoid and related subepidermal autoimmune blistering diseases. Curr Dir Autoimmune 2008; 10: 141–66.
11. Schmidt E, Zillikens D. Modern diagnosis of autoimmune blistering skin diseases. Autoimmun Rev 2010; 10 (2): 84-9.
12. Suliman NM, Astrom AN, Ali RW, Salman H, Johannessen AC. Clinical and histological characterization of oral pemphigus lesions in patients with skin diseases: a cross sectional study from Sudan. BMC Oral Health 2013;13: 66–70.
13. Al-Shenawy HA. Can immunohistochemistry replace immunofluorescence in diagnosis of skin bullous diseases ?. APMIS 2017; 125 (2): 114-21.
14. Xuefeng Z, Elizabeth H, Keyoumars S, Vesna P, Christopher R. Immunohistochemistry for immunoglobulin G4 on paraffin sections for the Diagnosis of Pemphigus. Arch Pathol Lab Med 2012; 136 (11): 1402–7.
15. Haas M, Rahman MH, Racusen LC, Kraus ES, Bagnasco SM, Segev DL et al. C4d and C3d staining in biopsies of ABO- and HLA-incompatible renal allografts: correlation with histologic findings. Am J Transplant 2006; 6 (8): 1829-40.
16. Katrin P, Kirsten M, Christian R, Paul S, Madeleine P, Werner K. C3d immunohistochemistry on formalin- fixed tissue is a valuable tool in the diagnosis of bullous pemphigoid of the skin. J Cutan Pathol 2010; 37 (6): 654–8.
17. Ambros-Rudolph CM. Dermatoses of pregnancy—clues to diagnosis, fetal risk and therapy. Ann Dermatol 2011; 23 (3): 265–75.
18. Lamb PM, Patton T, Deng JS. The predominance of IgG4 in prodromal bullous pemphigoid. Int J Dermatol 2008; 47 (2): 150-3.
19. Velez AMA, Googe Jr PB, Howard MS. Immunohistochemistry versus immunofluoresence in the diagnosis of autoimmune blistering diseases. Our Dermatol Online 2013; 4 (3): 585-95.
20. Zimmer V, Muller CS, Juengling B, Vogt T, Lammert F. Esophageal intramural pseudodiverticulosis in mucous membrane pemphigoid: potential diagnostic utility of C4d immunohistochemistry. Dig Endosc 2012; 24 (6): 487.
21. Miyamoto D, Maruta C, Santi C, Zoroquiain P, Dias AB, Fukumori L et al. How can immunohistochemistry improve the diagnosis of pemphigus foliaceus?. Human Pathology: Case Reports. 2018; 12 (June): 1-8.
22. Magro CM, Abbas AE, Seilstad K, Pope-Harman AL, Nadasdy T, Ross PJ. C3d and the septal microvasculature as a predictor of chronic lung allograft dysfunction. Hum Immunol 2006; 67 (4-5): 274-83.

How to Cite
Mohammed Soliman, E., ElKashishy, K., Metwally, M., Hassan, A. The diagnostic roles of C4D expression in SOME AUTOIMMUNE BULLOUS DERMATOSES IN ZAGAZIG UNIVERSITY HOSPITALS; an immunohistochemical study. *Zagazig University Medical Journal*, 2021; (767-775): -. doi: 10.21608/zumj.2019.7305.1032