EUROPEAN ACADEMIC RESEARCH
Vol. IV, Issue 2/ May 2016

Impact Factor: 3.4546 (UIF)
DRJI Value: 5.9 (B+)

ISSN 2286-4822
www.euacademic.org

Evaluation the role of direct immunofluorescence techniques and their relevance to the diagnosis of the blistering diseases

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Abstract:

Several studies have showed that the role of direct immunofluorescence techniques, and their relevance to the diagnosis of the blistering diseases. This descriptive cross-sectional study was conducted in Khartoum State –Sudan, in the period from January 2016 to April 2016. Aim of this study to evaluate the role of direct immunofluorescence techniques and their relevance to the diagnosis of the blistering diseases. The study includes sixty two (62) skin biopsies are placed on aluminium foil, fixed in tissue freezing media and immersed in liquid nitrogen.

From which 62 patients were included in this study. There were 16 (25.8 %) male and 46 (74.2%) female patients. The male to female ratio in bullous pemphigoid was 1:3.3. In pemphigus group was 1:4. In DH was 1:1.In CBC was 0:1

The age ranged from 0 -80 years. But the most common age is among the age group 31 to 40 n=18 (29%) then age group from 41 to 50

n=14 (22.6%) and the least common is in the age group 0 to 10 n=2 (3.2%). Diagnosis of blistering diseases can often made on the basis of clinical features but in some cases it may be possible to produce only differential diagnosis. Although clinical diagnoses of all the patient show concordance with final direct immunofluorescence diagnosis in present study (P value = 0.00).

When we compare the histological diagnosis with the immunofluorescence diagnosis there was no significant difference (P value = 0.00).

Key words: Anti-IgG anti-IgA, anti-IgM, and anti-C3, Skin biopsy

INTRODUCTION:

Autoimmune blistering skin diseases (ABSD) represent a group of heterogeneous mucocutaneous blistering diseases characterized by the deposition within the tissues of autoantibodies recognizing specific molecules involved in the cohesion between epidermal cells or between epidermis and the dermis. Intraepidermal ABSD include the various forms of pemphigus, pemphigus vulgaris (PV), pemphigus vegetans (PVE), pemphigus foliaceus (PF), drug-induced pemphigus (DIP), paraneoplastic pemphigus (PNP), and immunoglobulin A pemphigus (IgA-P) [1].

Diseases due to autoimmune dermal-epidermal dysadhesion (subepidermal ABSD) include mainly bullous pemphigoid (BP), pemphigoid gestationis (PG), cicatricial pemphigoid (CP), epidermolysis bullosa acquisita (EBA), and bullous systemic lupus erythematosus[2].

Immunofluorescence is a histochemical staining laboratory technique for demonstrating the presence of antibodies bound to antigens in tissue or circulating in body fluids. Fluorescein-labeled antibodies to human plasma components are used for the staining procedure [3].

Immunofluorescence was introduced in dermatology in the early 1960s, since 1963, the extensive used of immunofluorescence tests in immunopathologic studies of the skin had provided better understanding and classification of various disorders in which immune mechanisms are involve [4].

In recent years, a great knowledge has been learned about autoimmune blistering and connective tissue diseases. Much of this new information has been generated by the application of immunofluorescence in immunopathologic studies of the skin [5].

The results of Immunofluorescence tests are now considered obligatory criteria on which the diagnoses of various skin diseases are based [6].

MATERIALS AND METHODS:

This descriptive cross-sectional study was conducted in Khartoum State –Sudan, in the period from January 2016 to April 2016. Data are collected by questionnaire. Elliptical surgical biopsy is taken from skin or of recent fresh lesion.

Two skin biopsy samples were obtained containing a small intact bulla, for routine sectioning was placed in 10% formalin and perilesional skin for direct immunofluorescence staining placed on aluminium foil, fixed in tissue freezing media and immersed in liquid nitrogen.

The samples will be examined by direct Immunofluorescence technique, the steps are as following:

(1) Frozen 4 to 6 µm thick sections are cut in a cryostat at about –20°C, placed on glass slides, and air-dried for 15 min.

(2) Tissue sections must be wetted and rinsing in a phosphate-buffered saline (PBS) pH 7.0, 0.15 N, slides and overlaid in a moist chamber with (FITC) fluorescein isothiocyanate, which emits an apple green fluorescence,

conjugates with the following specificities: anti-IgG anti-IgA, anti-IgM, and anti-C3. Each reagent is tested on a separate slide for at least 30 min.

(3) antibodies treatments are for 2–4 h at room temperature in a humid container. The containers must be light-proof.
(4) Wash tissues in PBS pH 7 with three changes for a total of 20 min.
(5) Mount the specimen by adding one or tow drops of in anti-fading fluorescent mountanting medium.(Dako) onto tissue section To enhance visualization of specimens when viewed under a fluorescent microscope.
(6) Apply coverslipe over fluorescent mountanting medium.
(7) After rinsing in PBS, slides are mounted with buffered glycerine and examined under fluorescence microscope (Hund-Helmut hund .Type:H 600/12).

The samples will be examined by histopathology, the steps are as following:

The specimen is processed for light microscopic evaluation of sections from embedding, paraffin wax at 56°C.The block is cut by macrotom to 3 micro the section is stain by haematoxylin and eosin (H&E).

Statistical analysis: Will be conduct using SPSS-12, data was analyzed using SPSS software. The chi-square test was used to compare final outcomes. Confidence rate higher than 95% was considered as significant ($P < 0.05$).

RESULTS:

62 patients were included in this study. There were 16 (25.8 %) male and 46 (74.2%) female patients. Table (1). The male to female ratio in bullous pemphigoid was 1:3.3. In pemphigus group was 1:4. In DH was 1:1.In CBC was 0:1

The age ranged from 0 -80 years. But the most common age is among the age group 31 to 40 n=18 (29%) then age group from 41 to 50 n=14 (22.6%) and the least common is in the age group 0 to 10 n=2 (3.2%) Table (2)

The common age group in bullous pemphigoid was 50-79 years, the common age group in pemphigus group was 20-49 years, and the common age group in DH was 20-79 years. The common age group in chronic bullous diseases of childhood was 0-19 years; the common age group in herpes gestation was 20-49 years. Table (2)

The result of clinical diagnosis: Bullous pemphigoid was the most common diagnosis n=20 (32.2%) , two of them (10%) has negative immunofluorescence. Followed by pemphigus vulgaris n= 10 (16.1%), all of them have positive immunofluorescence. Then pemphigus foliaceus n=8 (12.9%) all of them have positive immunofluorescence, among 8 patient (12.9%)who diagnosed clinically as pemphigus vegetans tow (25%)of them have negative immunofluorescence and in tow of them (25%)the diagnosis of paraneoplastic pemphigus was made by immunofluorescence . Dermatitis herpetiformis have the same frequency n= 8 (12.9%) but four of them (50%)diagnosed by immunofluorescence as bullous pemphigoid and two of them(25%)as herpes gestation the remaining tow patient (25%) have negative immunofluorescence. The least frequent is the chronic bullous diseases of childhood n=4 (6.5%) but this diagnosis was not support by immunofluorescence, i.e have negative immunofluorescence. The other least frequent is the herpes gestation n=4 (6.5%) in tow of them (50%) the diagnosis was support by immunofluorescence, while in the other two (50%) the immunofluorescence is negative.

The result of histopathological diagnosis is that the most common disease is bullous pemphigoid n=22 (35.5%).

The pemphigus vulgaris n=14 (22.6%), in the 12 of them (85.7%) the diagnosis was support by immunofluorescence.

Dermatitis herpetiformis n= 4 (6.5%), tow (50%) of them had negative immunofluorescence.

Chronic bullous diseases of childhood n= 4 (6.5%) The diagnosis of non specific dermatitis is made in 8 patients (12.9%), in tow (33.3%) of them the diagnosis of pemphigus vulgaris was made by immunofluorescence and in tow (33.3%) of them the diagnosis of herpes gestation was made by immunofluorescence and in tow (33.3%) of them the immunofluorescence was negative.

Drug eruption in tow patients (3.2%) but the diagnosis of bullous pemphigoid was made by immunofluorescence.

Table (1) Fifth Distribution of immunobullous diseases in the study population:

Disease	Male	Female	Total	Male/ female Ratio
pemphigus group	6	20	26	1:3.3
Bullous pemphigoid	4	16	20	1:4
DH	4	4	8	1:1
CBC	4	0	4	1:0
HG	0	4	4	-

Table (2) Age Distribution of immunobullous diseases in the study population:

Disease	0-19	20-49	50-79	Total
pemphigus group	0	20	6	26
Bullous pemphigoid	0	6	14	20
DH	0	4	4	8
CBC	4	0	0	4
HG	0	4	0	4

Table (3) Clinical diagnosis

C.diagnosis	Percent	Frequency
pemphigus vulgaris	16.1	10
P.folicius	12.9	8
P.vegitant	12.9	8
bullous pemphigoid	32.3	20
Chronic boullus diseases of childhood	6.5	4
dermatitis herptiformis	12.9	8
Herpes gestation	6.5	4
Total	100.0	62

Table (4) Histopathological diagnosis

H.diagnosis	Percent	Frequency
Seb. Dermatitis	3.2	2
P.vulgaris	22.6	14
P.folicious	6.5	4
P.vegitant	6.5	4
Bullous pemphigoid	35.5	22
chronic bullous disease of childhood	6.5	4
dermatitis herpitiformis	6.5	4
Non-specific dermatitis	9.7	6
drug eruption	3.2	2
Total	100.0	62

DISCUSSION:

Autoimmune acquired blistering diseases constitute an important group of disease in dermatology. Those include the various forms of pemphigus: pemphigus vulgaris (PV), pemphigus vegetans (PVE), pemphigus foliaceus (PF), drug-induced pemphigus (DIP), paraneoplastic pemphigus (PNP), and immunoglobulin a pemphigus (IgA-P), bullous pemphigoid (BP), pemphigoid gestationis (PG), cicatricial pemphigoid (CP), and dermatitis herptiforms (DH) [7].

The differential diagnosis of these conditions had long relied on the clinico-pathological attributes; however the main differentiating point was the topographical location of the blister and presence of acantholysis. A blister lying above the

basement membrane was diagnosed as pemphigus group while the sub-basal blisters were considered either bullous pemphigoid or dermatitis herptiforms.

The introduction of immunofluorescence techniques based on the immunological target localization has revolutionized the diagnostic criteria for those diseases. The histological method of diagnosis was relegated to a secondary seat. Now internationally a diagnosis of any of those diseases without immunofluorescence evidence is not acceptable. However in countries like Sudan, the facilities available and cost entailed do not allow for such a conclusion. Still in dermatological circles the diagnosis of any of these conditions is made clinically and confirmed histologically.

It is this simple feat which has led us to try and validate such situation. We wanted to find out how reliable is the clinical and histological way of diagnosing those conditions by comparing the clinico-pathological data with those obtained by direct immunofluorescence. This study also streams to characterize different immunofluorescence finding.

In the present study which includes 62 patients, we find that:

The maximum numbers of subjects were female 74.2% were only 25.8% were male, ratio 3:1.Although in another study done in 2008 in Pakistan found that both sexes are affected equally [8].

The result of clinical diagnosis: bullous pemphigoid was the most common diagnosis n=20 (32.2%), the male to female ratio is 1:4 .The most common age was 50-79 years. In 11 of them the DIF result confirmed the clinicopathological diagnosis of bullous pemphigoid, while in two patents the DIF being negative.

In another two patients the clinical diagnosis was bullous pemphigoid the histopathological diagnosis was pemphigus vulgaris and the DIF pattern carried picture of

paraneoplastic pemphigus. This reflects confusion that may occur and the important of DIF in such situation.

Two cases diagnosed clinically as bullous pemphigoid and DIF confirm the diagnosis, but histopathologically diagnosed as bullous drug eruption. A satiation which again illustrated the important of DIF in differential diagnosis, and how it can resolve the different between the clinician and histopathologist.

One interesting case diagnosed clinically and histopathologically as bullous pemphigoid while DIF show deposition of C3 only reflects a case of herpes gestation. On requisitioning the patient it become clear that the patient was in purpurium period.

Two cases clinical diagnosis was bullous pemphigoid the histopathological diagnosis was pemphigus pemphigoid and the DIF give a picture of DH.

So when we compare the clinical diagnosis with the DIF diagnosis the P value is 0.05. And when we compare the histopathological diagnosis with the DIF diagnosis the P value is 0.007.

The pattern of DIF was as following: A continuous, thin, linear deposition of IgG fluorescence was observed at the dermo-epidermal junction in 18 (100%) patients who diagnosed by DIF. While there was deposition of both IgG and C3, in 14 (77.8%), this finding is consistent with study done by Mahmood and Haroon in Which IgG was observed in 100% of patients and C3 in 10 (71.4%) patients [9]. Generally, patterns were the same as seen in other studies.[86] Common deposit in direct immunofluorescence was C3 in study done by Kabir1 et al [8]. Provost et al. showed that deposition of C3 was present in all (100%) of 11 cases, they studied [10].

Two cases (11.1%) showed deposition of all C3, IgG and IgM along the basement membrane zone. And tow cases (11.1%) showed deposition of IgG only. Maurice et al study report that

the presence of a second immunoglobulin at the DEJ (in addition to IgG) in patients with bullous pemphigoid was associated with more severe disease [11].

Pemphigus group: The female to male ratio was 3.3:1. The common age group was between 20-49 years.

Pemphigus vulgaris n= 10 (16.1%). In all of them the diagnosis was supported histopathologically and by DIF.

Pemphigus foliaceus n=8 (12.9%) all of them have positive immunofluorescence, but in 4 of them the diagnosis is non specific dermatitis by histopathology but DIF confirmed the diagnosis of pemphigus vulgaris.

A disadvantage of immunofluorescence methods is the difficulty to distinguishing pemphigus vulgaris from pemphigus foliaceus on the staining pattern [7].

Among 8 patient (12.9%) who were diagnosed clinically and histopathologically as pemphigus vegetans two of them have negative immunofluorescence and in tow of them the diagnosis of paraneoplastic pemphigus was made by immunofluorescence. This a gain showed the important of DIF, as early diagnosis of such conditions has prognostic value.

So when we compare the clinical diagnosis with the DIF diagnosis the P value is 0.031. And when we compare the histopathological diagnosis with the DIF diagnosis the P value is 0.036.

As is generally recognized in the literature the direct immunofluorescence in pemphigus group must reveal intercellular deposition of IgG in a pattern known as fishnet .We did this test on the patient diagnosed by DIF as pemphigus vulgaris and found that all our patient gave this pattern of IgG deposition. Similar results were observed by Judd and Lever [12].

In 12 (54.5%) there was deposition of both IgG and C3; in 10 (45.5%) there was an intercellular deposition of IgG only. This also found by Maurice et al in there review study of 279

cases in 2006 [11]. Complement components (C3), IgM and IgA are present less frequently than IgG [13].

Two patterns of pemphigus antibody deposition have been described. In most cases, there is full-thickness squamous intercellular substance deposition of IgG. Rarely, IgG may be localized only to the superficial portion of the epidermis [14].

Eight of the patient (12.9%) diagnosed clinically as dermatitis herpetiformis female to male ratio was 1:1.The common age group was 20-79.

Two of them the diagnosis is supported by histopathology but the diagnosed by immunofluorescence was bullous pemphigoid and in another two of them who again the histopathology confirm the diagnosis they have negative immunofluorescence.

So when we compare the clinical diagnosis with the DIF diagnosis the P value is 0.180. And when we compare the histopathological diagnosis with the DIF diagnosis the P value is 0.078. Both are significant differences.

The pattern of DIF was as following: half of them n=2 (50%) of them demonstrated granular deposits of IgA and IgM along the basement membrane zone. The other half showed granular deposition of IgG and C3 along the basement membrane zone.

This is not match with the study done by Mahmood and Haroon in which the diagnosis of DH was made in 4 (15.4%) patients. All of them demonstrated granular deposits of IgA exclusively in the dermal papillae. No other immunoreactant including IgM, IgG or C3 was detected [12].

The herpes gestation n=4 in one of them the histopathology diagnosed as bullous pemphigoid while in it the immunofluorescence is negative.

All of them (100%) had deposition of C3 at the dermo-epidermal junction in a thin, continuous, linear pattern. The same result found by Hililog et al in there study they found

that the complement component C3 is deposited along the basement membrane zone (BMZ) in almost all active cases [15]. Mahmood and Haroon in there study found that the deposition of C3 was more prominent than that of IgG [9].

The chronic bullous diseases of childhood n=4 (6.5%) this diagnosis was support by histopathology but not by immunofluorescence, tow (50%) of them the diagnosis of bullous pemphigoid was made by immunofluorescence and tow (50%) of them the diagnosis of dermatitis herpetiformis was made by immunofluorescence.

The paraneoplastic pemphigus pattern was found as with weak cell surface (intercellular space) pattern and linear basement membrane zone of C3 with strong IgG intercellular space pattern deposition. Cohen et al mentioned a case of mixed bullous disease with B-cell lymphoma diagnosed as paraneoplastic pemphigus, where the depositions were intercellular IgM and IgG along basement membrane zone [16].

Diagnosis of blistering diseases can often made on the basis of clinical features but in some cases it may be possible to produce only differential diagnosis. Although clinical diagnoses of all the patient show concordance with final direct immunofluorescence diagnosis in present study (P value = 0.00).

When we compare the histological diagnosis with the immunofluorescence diagnosis there was no significant difference (P value = 0.00).

CONCLUSION:

Clinical diagnoses of all the patients show concordance with final direct immunofluorescence diagnosis in present study (P value = 0.00).

Histological diagnosis with the immunofluorescence diagnosis there was no significant difference (P value = 0.00).

Immunofluorescence if available is necessary to obligatory the diagnosis of acquired autoimmune diseases.

Direct immunofluorescence is very important in diagnosis of different between pemphigus vulgaris and paraneoplastic pemphigus and it is application will have prognostic value.

RECOMMENDATION:

1. As direct immunofluorescence is a sensitive diagnostic tool for the diagnosis of autoimmune blistering diseases we recommended the use of immunofluorescence in finalized the diagnosis of autoimmune blistering diseases.
2. Since the histopathology is very crucial in diagnosis of autoimmune blistering diseases and since all the report was done by general histopathologist we recommended training more dermatopathologist.

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