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Research Article

Comparison of Clinical Findings, Histological Findings and Findings on DIF Examination in Autoimmune Vesiculobullous Disorders

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Abstract: Autoimmune blistering disorders are characterized by presence of pathogenic autoantibodies directed against target antigens which are components of desmosome or adhesion complex of basement membrane zone. This leads to clevage at particular level and subsequent blister formation. The aim and objective of the work was to study the clinical, histopathological and immunofluorescence findings in autoimmune blistering disorders and to study correlation between them. The study was conducted in OPD of dermatology department. 38 patients presenting with clinical impression of autoimmune vesiculobullous disorder were included in the study. In all patients, detailed clinical history, examination & routine Investigations were carried out & findings were recorded. Biopsy for H & E and DIF was taken in all the cases. The maximum clinical, histopathological and immunological correlation was present in pemphigus vegetans, Herpes gestationis and paraneoplastic pemphigus (100% each), while the minimum correlation was seen in bullous pemphigoid 50%). In pemphigus vulgaris the correlation was present in 92% while in pemphigus foliaceous it was 66%. Out of 38 cases of autoimmune blistering disorder clinical, histopathological & immunological correlation was present in 21 cases (60%). It is preferable to correlate clinical, histopathological & immunofloroscence findings for more precise diagnosis & better patient management.
Keywords: Autoimmune blistering disorders, autoantibodies, Biopsy.

INTRODUCTION

Blistering disorders are known to man since ancient times. First recorded instance of pemphigus disease was by Hippocrates (460 - 370 B.C) who described pemphigoid fever as "*pemphigodes pyertoi"* and Galen (AD 131 to 201) named a pustular disease of the mouth as "*febris pemphigodes*" [1, 2].

The immunobullous diseases are characterized by pathogenic autoantibodies directed against target antigens [3] whose function is either cell-to-cell adhesion within the epidermis or adhesion of stratified squamous epithelium to dermis or mesenchyme. The target antigens are components of desmosomes or the functional unit of the basement membrane zone that are known as the adhesion complex [4].

The most important techniques for the investigation of patients with immunobullous disease are histopathology and direct-indirect immunofluorescence. Immunofluorescence testing is invaluable in the confirmation of a diagnosis that is suspected by clinical or histologic examination. This is especially true in subepidermal bullous diseases, often having overlap in the clinical and histologic findings [5]. Techniques such as immunoblotting and immunoelectron microscopy may refine the diagnosis in individual patient but do not replace the clinical diagnosis [6].

Histologic findings alone may not be sufficient to classify correctly the subtype of eruption [7].

AIM AND OBJECTIVES

- To study the clinical , histopathological and direct immunofluorescence findings in autoimmune blistering disorders.
- To study correlation between them.

MATERIALS AND METHODS

Study was conducted in OPD of dermatology. 38 patients with clinical impression of autoimmune vesiculobullous disorders were included. In all patients, detailed clinical history, clinical examination & all routine Investigations were obtained & recorded. Prior to skin biopsy written consent was taken.

For histopathological examination, intact fresh vesicle was chosen for excisional biopsy; while for DIF examination unblistered perilesional area between 1.5-2 cm was chosen for punch biopsy [8]. The site was anaesthetized by 2% lignocaine injection & biopsy was

performed. For HPE biopsy specimen was placed on a piece of small filter paper to prevent curling & was put in 10% formalin. For DIF, the specimen washed in normal saline in petridish and it was placed in michel's medium. Proper labeling of biopsy specimen was done & sent to laboratory accompanied by detailed clinical notes and our probable clinical diagnosis.

All specimens for HPE were stained with haematoxylin eosin. For DIF frozen section of specimen were incubated with antihuman antibodies to Ig G, Ig A, Ig M, c3 and fibrinogen. These antibodies are linked to a fluorescent label to allow visualization using fluorescent microscope. In this way clinical, histopathological & DIF examination was carried out and findings were recorded.

RESULTS

Total number of 38 patients of auto-immune blistering skin diseases was included and the results are as follows:

Table 1: Type of autoimmune blistering diseases on clinical examination

Disease	No. of cases	Percentage
Pemphigus vulgaris	16	42.11
Pemphigus foliaceous	3	7.90
Pemphigus vegetans	1	2.63
Senear-Usher syndrome	1	2.63
Bullous LSA	1	21.05
Bullous Pemphigoid	8	5.26
Herpes Gestationis	2	5.26
Id eruption	2	5.26
Paraneoplastic pemphigus	1	2.63
Dermatitis herpetiformis	2	5.26
EBS	1	2.63

On clinical examination, maximum patients were of Pemphigus vulgaris (16) F/b Bullous pemphigoid (8) F/b Pemphigus foliaceous (3). The mean age of presentation was 45.32 year.

Table 2: Types of autoimmune blistering diseases on histopathological examination

Type of disease	Number of cases	Percentage
Pemphigus vulgaris	13	37.14
Pemphigus foliaceous	3	8.57
Pemphigus vegetans	1	2.85
Senear Usher syndrome	1	2.85
Bullous LSA	1	2.85
Bullous pemphigoid	6	17.14
Herpes gestationis	1	2.85
Id eruption	1	2.85
Lichenoid eczema	1	2.85
Paraneoplastic pemphigus	1	2.85
Nonspecific inflammation	1	2.85
Dermatitis herpetiformis	1	2.85
EBS	1	2.85
VB disease	1	2.85
Suprabasal VB disease	1	2.85
Psoriasis	1	2.85

On histopathological examination, maximum cases were of pemphigus vulgaris (37.14%) followed by Bullous pemphigoid (17.14%). In 5.70% cases, biopsy findings were inconclusive.

In 5.70% cases biopsy findings were indicative of diseases other than autoimmune vesiculo-bullous disease.

Table 3: Comparison of clinical findings, histopathological findings and immunological (DIF) findings

Clinical diagnosis	Histopathological diagnosis	DIF findings
Pemphigus vulgaris(n=16)	Pemphigus vulgaris(n=13) Inconclusive(n=0)	Ig G positive(n=12) Inconclusive(n=1)
Pemphigus foliaceous (n=3)	Pemphigus foliaceous (n=3)	Ig G positive(n=3)
Pemphigus vegetans (n=1)	Pemphigus vegetan s(n=1)	Ig G positive(n=1)
Senear-Usher syndrome (n=1)	Bullous LE (n=1)	Inconclusive(n=1)
Bullous LSA (n=1)	Bullous LSA (n=1)	Inconclusive(n=1)
Bullous Pemphigoid(n=8)	Bullous Pemphigoid (n=6)	Ig G positive(n=4)
Herpes Gestationis(n=2)	Herpes Gestationis(n=1) Inconclusive(n=1)	Linear c3(n=1) Inconclusive(n=1)
Id eruption(n=2)	Id eruption(n=1) Inconclusive(n=1)	Inconclusive(n=2)
Paraneoplastic pemphigus(n=1)	Paraneoplastic pemphigus(n=1)	IgG+c3 positive(n=1)
Dermatitis herpetiformis(n=2)	Dermatitis herpetiformis(n=1) Inconclusive(n=1)	Inconclusive(n=2)
EBS(n=1)	EBS(n=1)	Inconclusive(n=0)

Out of 16 patients, with clinical diagnosis of pemphigus vulgaris , biopsy was performed in 13 patients from cutaneous lesions and HPE was suggestive of PV in all 13 cases ; while DIF was positive for PV in 12 cases.

In 3 patients with PF, biopsy and DIF was suggestive of the same in all 3 cases. In 8 cases of bullous pemphigoid, biopsy was suggestive of the same in 6 cases ; while DIF was positive in 4 cases.

Table 4: Comparison of clinical findings, histopathological findings and immunological (DIF) findings

Diagnosis	Clinical + Histological	Clinical + immunological	Histological + Immunological	Clinical + Histological + Immunological
P. vulgaris (n=16)	13/13=100%	12/13=92%	12/13=92%	12/13=92%
P. foliaceous (n=3)	3/3 =100%	3/3 =100%	3/3 =100%	2/3 =66%
Pemphigus vegetans (n=1)	1/1 =100%	1/1 =100%	1/1 =100%	1/1 =100%
Senear Usher syndrome(n=1)	1/1 =100%	0	0	0
Bullous LSA (n=1)	1/1 =100%	0	0	0
Bullous Pemphigoid (n=8)	6/8=75%	4/8 =50%	4/8 =50%	4/8 =50%
Herpes Gestationis (n=1)	1/1 =100%	1/1 =100%	1/1 =100%	1/1 =100%
Id eruption (n=2)	2/2	0	0	0
Paraneoplastic pemphigus(n=1)	1/1=100%	1/1 =100%	1/1 =100%	1/1 =100%
DH (n=2)	1/2= 50%	0	0	0
EBS (n=1)	1/1=100%	0	0	0

The maximum clinical, histopathological and immunological correlation was present in pemphigus vegetans, Herpes gestationis and paraneoplastic pemphigus (100% each), while the minimum correlation was seen in bullous pemphigoid (50%).

In pemphigus vulgaris the correlation was present in (92%); while in pemphigus foliaceous it was (66%).

Table 5: Correlation between clinical, histopathological and immunoflourescence (DIF) findings in auto-immune blistering diseases

Findings suggestive of VB diseases	No. of cases	Percentage	Correlation
Clinical alone	38/38	100	-
Clinical+histopathological	30/35	85	-
Clinical+immunological(DIF)	22/35	62	+
Histopathological+immunological(DIF)	21/35	60	+
Clinical+histopathological+immunological(DIF)	21/35	60	+

Out of total 38 cases of autoimmune blistering diseases; clinical, histopathological and immunological correlation was present in 21 cases (60%).

DISCUSSION

The adhesive structures of the skin include desmosomes, focal adhesions, hemidesmosomes and basement membrane. In autoimmune blistering diseases of the pemphigus or pemphigoid group and in epidermolysis bullosa the protein components of desmosomes, hemidesmosomes and epidermal basement membrane are targeted [9].

"A diagnosis is a clinical tool that assists in the process of codifying patients into disease groups that tend to share a common outcome and common set of responses to therapy" [10]. Sometimes however the histopathology can contribute by ruling out an important diagnosis, even though an exact diagnosis cannot be made.

Histolopathology reveals the location of blister formation and helps to classify the type of bullous disorder [11]. Microscopic study helps in detrmining the level of cleavage, mechanism of blister formation and the type of inflammatory infiltrate [12].

A major source of difficulty in making an exact diagnosis in pathology, as in clinical medicine, is that the information required to make the diagnosis is frequently incomplete at some or multiple levels. Another serious problem in histopathologic diagnosis results from the fact that specificity studies to determine the prevalence of the criteria in diagnostically challenging cases are infrequent.

Foucar has pointed out that the diagnostic process is an example of complex decision making that has intrinsic uncertainty usually resulting from one or more of the following: (a)The large number of variables, (b) One or more key variables lack clear definition, (c) One or more key variable is hidden from the problem solver [13], (d) The uncertainty of the specificity of the individual findings, (e)The uncertainty that results from deficiencies in the observer's ability to evaluate and categorize histological findings.

Immunofluorescence studies are considered the 'gold standard' for the diagnosis of autoimmune blistering diseases [14]. Fluorescent techniques involve the emission of light of one color/wavelength and a low energy level from a substance that is irradiated with light of a different wavelength. The antibody is linked with fluorescein isothiocyanate (FITC) through a thiocarbamide linkage without destroying its capacity in order to react with the corresponding antigen [15].

The differential diagnosis of a DIF test depends on 4 features: (a) The primary site of immune deposition, (b)The class of immunoglobulin or other type of immune deposit, (c)The number of immune deposits and , if multiple, the identity of the most intense deposits, (d) Deposition in other sites besides the main site [5].

Where applicable ultrastructural, immunohistochemical and molecular aids to diagnosis ; resulted in increased specificity for many diagnosis. Now days, Immunofluorescence testing is invaluable in confirming a dignosis that is suspected by clinical or histologic examination.

CONCLUSION

The best approximation to the goal of improving diagnostic specificity will be achieved by a detailed correlation of findings at the molecular, histological and gross anatomical levels with the physical finding and clinical history interpreted in the context of the whole patient and his or her environments, with long term follow up serving as the gold standard.

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