EVALUATION OF THE MOSAIC 7 TEST FOR THE DIAGNOSIS OF IMMUNOBULLOUS SKIN DISEASE

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ABSTRACT

Objectives: To determine the positive rate of the Mosaic 7 test in autoimmune bullous diseases at the National Hospital of Dermatology and Venereology.

Materials and methods: A cross-sectional retrospective study was conducted on 44 medical records and serology of patients with autoimmune bullous diseases at the National Hospital of Dermatology and Venereology from October 2021 to September 2022. Data collected included gender, age, diagnosis, histopathology results, indirect immunofluorescence, direct immunofluorescence, and Mosaic 7 test results.

Results: Of the 44 patients with autoimmune bullous diseases, 22 had pemphigus vulgaris, 5 had pemphigus foliaceus, and 17 had bullous pemphigoid. The Mosaic 7 test results varied among the different types of bullous diseases. In pemphigus vulgaris patients, the Mosaic 7 test showed a high positive rate on the esophageal well (90.9%), Desmoglein 1 well (95.5%), and Desmoglein 3 well (86.4%). In pemphigus foliaceus patients, the test showed a 100% positive rate on the esophageal and Desmoglein 1 wells, but 0% on the Desmoglein 3 well. In bullous pemphigoid patients, the positive rates varied across different wells of the Mosaic 7 test.

Conclusions: The Mosaic 7 test demonstrated high utility in diagnosing autoimmune bullous skin diseases, particularly in identifying Desmoglein 1 and Desmoglein 3 in pemphigus vulgaris. However, further studies with larger sample sizes are warranted to validate and refine the test's accuracy.

Keywords: *Autoimmune bullous diseases, Mosaic 7.*

1. INTRODUCTION

Autoimmune blistering skin diseases are characterized by autoantibodies targeting molecules responsible for cell adhesion or structural proteins in the skin and mucosa, leading to the formation of clinical blisters. Depending on the affected proteins and their location within the skin, these diseases are classified into three main groups: intraepidermal

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diseases, such as pemphigus; junctional diseases, like pemphigoid; and subepidermal diseases, such as acquired epidermolysis bullosa and dermatitis herpetiformis. Diagnosis is based on clinical features, histopathology, as well as direct and indirect immunofluorescence to detect circulating antibodies[1,2].

Indirect immunofluorescence testing aims to detect circulating antibodies using the principle that these antibodies in the patient's blood bind to antigen samples either present in tissue or affixed to wells[3-5]. At the National Hospital of Dermatology and Venereology, indirect immunofluorescence has traditionally been used to detect IgG antibodies on a normal skin sample. The Mosaic 7 test is a new method based on indirect immunofluorescence principles that can simultaneously detect IgG autoantibodies in six antigen wells. This method has not yet been studied or implemented in Vietnam. Therefore, we conducted this research to determine the positive rate of the Mosaic 7 test in patients with autoimmune blistering skin diseases at the National Hospital of Dermatology and Venereology.

2. SUBJECTS AND METHODS

2.1. Study subjects

Inclusion criteria involved patients with medical records and serum diagnosed with autoimmune blistering skin diseases based on clinical examination and tests, who were hospitalized in the Women's and Children's Dermatology Departments and the Men's Dermatology Department at the National Hospital of Dermatology and Venereology. Patients must have complete information regarding the Mosaic 7 test. Exclusion criteria included incomplete medical records and patients who did not undergo the Mosaic 7 test.

2.2. Study methods

Study design

This is a cross-sectional, retrospective study conducted from October 2021 to September 2022 at the National Hospital of Dermatology and Venereology. The sample size consisted of 44 patients. A total sampling approach was employed, collecting data from eligible medical records until the study period ended.

Study procedure

Medical records of patients diagnosed with autoimmune blistering skin diseases were reviewed. Patients must have had serum preserved and received results from the Mosaic 7 test. The research medical record was completed. The Mosaic 7 test is based on the principle of indirect immunofluorescence. If the sample is positive, specific antibodies in the patient's serum will bind to either tissue samples or antigens affixed to the wells. The fluorescent-labeled antibodies bound to the antigens are then observed under a fluorescence microscope for deposition images. The wells included esophageal wells, salt-split skin wells, pure BP180 antigen wells, and cells attached to BP230, Dsg1, and Dsg3 antigens. The advantage of this test is that it allows simultaneous testing across six antigen wells, enhancing convenience and cost-effectiveness.

Assessment

- Negative results.

- Positive results:

Esophageal wells: Staining along the basement membrane or intercellular spaces.

Salt-Split skin wells: Staining at the blister roof or base.

Desmoglein 1, 3, BP230, BP280 Wells: Fluorescent staining of cells.

- False positives:

Esophageal wells: General fluorescence throughout the esophagus, nuclear staining, or complete staining of the epidermis and basement membrane.

Salt-Split skin wells: Fluorescence observed at both the blister roof and base, with staining across the entire sample.

Desmoglein 1, 3, BP230, BP280 Wells: Fluorescence present, but with non-specific images for positive wells.

- Unsuccessful results: Complete staining of the wells without observable cells due to washout.

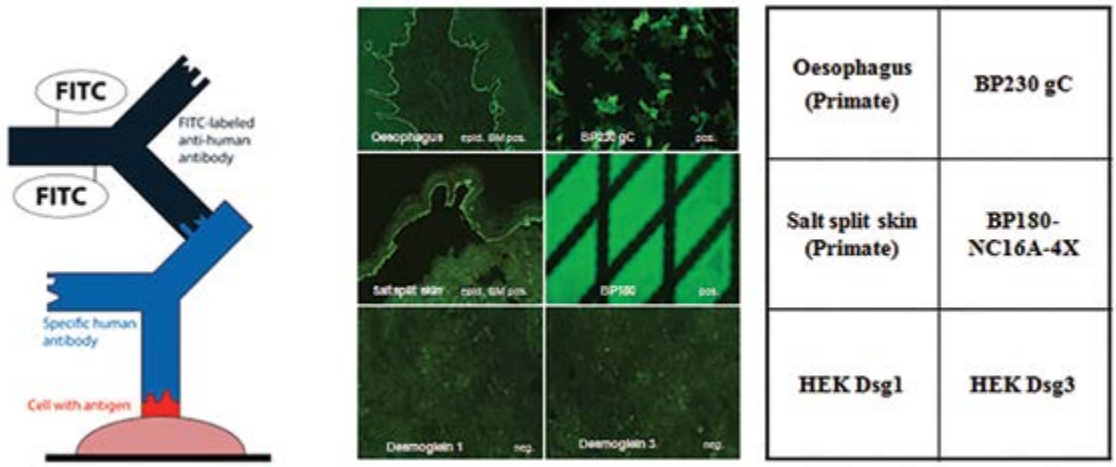

Figure 1. Illustration of the Mosaic 7 Immunofluorescence Test

Statistical analysis

Data was entered and processed using SPSS 20.0. Since the study relied on retrospective medical records, there may be potential errors. Therefore, the investigator directly collected the information, and the data collection tool was designed appropriately to minimize inaccuracies.

2.3. Ethics

Patients' personal information was kept confidential and used solely for the purpose of this study, in accordance with the Helsinki Declaration 2013. This research was approved by the National Hospital of Dermatology and Venereology, through scientific council decision number 1680/QĐ-BVDLTW.

3. RESULTS

3.1. General characteristics of study subjects

Table 1. Age and gender characteristics of patients with autoimmune blistering skin diseases (N = 44)

Characteristics / Condition	Age (X ± SD)	Male		Female	
		n	%	n	%
Pemphigus vulgaris (n = 22)	51.2 ± 15.6	10	45.5	12	54.5
Pemphigus foliaceus (n = 5)	50.6 ± 13.9	0	0	5	100
Bullous pemphigoid (n = 17)	70.8 ± 18.3	12	70.6	5	29.4
Total (n = 44)	58.7 ± 18.9	22	50	22	50

The average age of patients with autoimmune blistering diseases in this study was 58.7 ± 18.9 years, ranging from 16 to 96 years. Among the 22 patients with pemphigus vulgaris, the average age was 51.2 ± 15.6 years, with 12 female patients (54.5%). The 5 patients with pemphigus foliaceus had an average age of 50.6 ± 13.9 years, all of whom were female. There were 17 patients with bullous pemphigoid, with an average age of 70.8 ± 18.3 years; males constituted 70.6% (12 patients) (Table 1).

3.2. Mosaic 7 test results in patients with Pemphigus vulgaris

Table 2. Mosaic 7 test results in patients with Pemphigus vulgaris (N = 22)

Wells / Results	Esophagus		Salt-Split		BP230		BP180		Dsg1		Dsg3	
	n	%	n	%	n	%	n	%	n	%	n	%
Positive	20	90.9	0	0	0	0	1	4.5	21	95.5	19	86.4
Negative	0	0	20	90.9	22	100	21	95.5	1	4.5	3	13.6
Unsuccessful	2	9.1	2	9,1	0	0	0	0	0	0	0	0
Total	22	100	22	100	22	100	22	100	22	100	22	100

Among the 22 patients with pemphigus vulgaris tested using Mosaic 7, the intercellular positive rate on the esophageal well was 90.9% (20 patients), on the Dsg1 well was 95.5% (21 patients), and on the Dsg3 well was 86.4% (19 patients). The positive rate for the BP230 well was 0%, while for the BP180 well, it was 4.5% (1 patient) (Table 2).

3.3. Mosaic 7 test results in patients with Pemphigus foliaceus

Table 3. Mosaic 7 test results in patients with Pemphigus foliaceus (N = 5)

Wells / Results	Esophagus		Salt-Split		BP230		BP180		Dsg1		Dsg3	
	n	%	n	%	n	%	n	%	n	%	n	%
Positive	5	100	0	0	0	0	0	0	5	100	0	0
Negative	0	0	4	80	5	100	5	100	0	0	5	100
Unsuccessful	0	0	1	20	0	0	0	0	0	0	0	0
Total	5	100	5	100	5	100	5	100	5	100	5	100

In the 5 patients with pemphigus foliaceus tested using Mosaic 7, the positive rate on the esophageal well was 100% (5 patients), and on the Dsg1 well was also 100% (5 patients), while the Dsg3 well showed 0% positivity (Table 3).

3.4. Mosaic 7 Test Results in Patients with Bullous Pemphigoid

Table 4. Mosaic 7 test results in patients with bullous pemphigoid (N = 17)

Wells / Total	Esophagus		Salt-Split		BP230		BP180		Dsg1		Dsg3	
	n	%	n	%	n	%	n	%	n	%	n	%
False positive	5	29.4	1	5.9	0	0	0	0	0	0	0	0
Positive	10	58.8	9	52.9	5	29.4	8	47.1	0	0	0	0
Negative	1	5.9	6	35.3	9	52.9	9	52.9	17	100	16	94.1
Unsuccessful	1	5.9	1	5.9	3	17.6	0	0	0	0	1	5.9
Total	17	100	17	100	17	100	17	100	17	100	17	100

In the 17 patients with bullous pemphigoid tested using Mosaic 7, the rate of linear positivity along the basement membrane on the esophageal well was 58.8% (10 patients), with 29.4% (5 patients) showing false positivity. The salt-split skin well had a positivity rate of 52.9% (9 patients) at the blister roof, with 1 case of positivity at the blister base (5.9%). The BP230 well showed a positivity rate of 29.4% (5 patients), while the BP180 well had a positivity rate of 47.1% (8 patients) (Table 4).

4. DISCUSSION

In our study of 44 patients, 22 had pemphigus vulgaris, accounting for the highest proportion at 50%. Seventeen patients had bullous pemphigoid, representing 38.6%, while the remaining patients had pemphigus foliaceus, comprising 11.4%. The literature indicates that pemphigus vulgaris is more frequently encountered than other blistering skin diseases, with annual incidence rates ranging from 0.1 to 0.5 per 100,000 people worldwide. The highest risk areas include Jewish populations, India, Southeastern Europe, and the Middle East.

In contrast, bullous pemphigoid is rarer, with European reports indicating an incidence of 4 to 22 cases per million people annually[1].

The average age of our study group was 58.7 ± 18.9 years, with a higher proportion of females (63%) in the pemphigus group. A study by Mimoun et al. (2008)[6] involving 155 pemphigus patients in Israel found that the most common age group was 40 - 60 years, with a female-to-male ratio of 1.54 : 1. This may be explained by changes in the immune and endocrine systems that begin in this age group, particularly in women, as some studies indicate a higher prevalence of the disease in women during the perimenopausal stage. The majority of bullous pemphigoid patients in our study were male (70.6%). Our findings align with observations from both Vietnam and abroad. According to Hywel C. Williams et al. (2017), a study of 253 bullous pemphigoid patients noted a mean age of 77.7 years, with varying male-to-female ratios depending on the study[7].

In our research, among the 22 pemphigus vulgaris patients tested with Mosaic 7, 20 (90.9%) showed intercellular positivity on the esophageal well. The positivity rates for Dsg1 and Dsg3 were 95.5% and 86.4%, respectively, while 4.5% tested positive for BP180. Two patients (9.1%) had inconclusive tests due to sample issues such as substrate interference and loss of cells, necessitating retesting to confirm positive rates. Yang et al. (2019) reported a 62.2% intercellular positivity rate on the esophageal well for 23 pemphigus vulgaris patients, with positivity for Dsg1 and Dsg3 at 13.0% and 60.9%, respectively[8]. The lower rates in their study may be due to differences in sample selection and disease severity. Another study by Kate Simpson et al. (2021) involving 10 pemphigus vulgaris patients found positivity rates for Dsg1 and Dsg3 at 70% and 100%, respectively[9].

Among the 5 patients with pemphigus foliaceus tested with Mosaic 7, all showed intercellular positivity on the esophageal well, with 100% positive for Dsg1 and none positive for Dsg3. None tested positive for BP230 or BP180. Yang et al. (2019)[8] found a high positivity rate in their study of 8 pemphigus foliaceus patients, with 87.5% showing intercellular positivity on the esophageal well, 75% positive for Dsg1, and 62.9% positive for Dsg3. In our study, the positivity rates on the esophageal well, Dsg1, and Dsg3 for pemphigus foliaceus patients were very high, closely aligning with several other global studies. However, some studies reported lower positivity rates, which may be related to disease activity; patients in milder or regressive stages may exhibit lower antibody positivity. Thus, more sensitive and specific tests, such as ELISA, are needed for antibody detection and quantification.

Seventeen patients with bullous pemphigoid were tested with Mosaic 7, and 58.8% showed linear positivity along the basement membrane on the esophageal well, while 52.9% were positive at the blister roof on the salt-split skin well. Positivity rates for BP230 and BP180 were 29.4% and 47.1%, respectively. However, 5 patients (29.4%) showed false positivity in the intercellular space and did not exhibit positivity along the basement membrane. Clinical characteristics and histopathological examination confirmed these 5 patients had bullous pemphigoid, indicating that the intercellular positive results in the Mosaic 7 test were false positives, requiring retesting with proper positive and negative controls. One test was inconclusive (5.9%) due to substrate interference preventing observation of results.

According to Simpson et al. (2021)[9], in their study of 8 bullous pemphigoid patients, 62.5% showed linear positivity along the basement membrane on the esophageal well, with 62.5% positive for BP180 and 50% positive for BP230. Compared to their study, our findings revealed lower rates of positivity along the basement membrane and a higher rate of false positives (29.4%) in the intercellular space. This raises concerns about the testing process from sample preparation to the execution of tests, highlighting the need for more rigorous testing with expert oversight to ensure quality and accuracy. Among the 5 false-positive cases in the intercellular space, all tested positive for BP180 or BP230, suggesting that errors occurred specifically with the esophageal well, potentially indicating issues related to test quality.

5. CONCLUSIONS

The positivity rates in the Mosaic 7 test for autoimmune blistering diseases are notably high. In pemphigus vulgaris, both the Desmoglein 1 and Desmoglein 3 wells showed positivity rates exceeding 85%. However, further studies with larger sample sizes are necessary to confirm the sensitivity and specificity of this method. This test holds promise as a valuable tool for diagnosing autoimmune blistering skin diseases.

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