Dermatological Health

Editors-in-Chief

Shauna Higgins

University of Southern California, USA

Li He

Yunnan Dermatology Hospital, China

BIO-BYWORD SCIENTIFIC PUBLISHING PTY LTD
(619 649 400)
Level 10
50 Clarence Street
SYDNEY NSW 2000

Dermatological Health

Focus and Scope

Dermatological Health is a peer-reviewed, open access journal that publishes original research articles and review articles related to the prevention, diagnosis, and treatment of disorders of the skin, hair, and nails. The covered topics include, but are not limited to: clinical, investigative, and population-based studies, healthcare delivery and quality of care research, high quality, cost effective, and innovative treatments, new diagnostic techniques, and other topics related to the prevention, diagnosis, and treatment of disorders of the skin, hair, and nails. Each issue includes continuing medical education articles designed to fill practice and knowledge gaps in the delivery of dermatologic care.

About Publisher

Bio-Byword Scientific Publishing is a fast-growing, peer-reviewed and open access journal publisher, which is located in Sydney, Australia. As a dependable and credible corporation, it promotes and serves a broad range of subject areas for the benefit of humanity. By informing and educating a global community of scholars, practitioners, researchers and students, it endeavors to be the world's leading independent academic and professional publisher. To realize it, it keeps creative and innovative to meet the range of the authors' needs and publish the best of their work.

By cooperating with University of Sydney, University of New South Wales and other world-famous universities, Bio-Byword Scientific Publishing has established a huge publishing system based on hundreds of academic programs, and with a variety of journals in the subjects of medicine, construction, education and electronics.

Publisher Headquarter

BIO-BYWORD SCIENTIFIC PUBLISHING PTY LTD
Level 10
50 Clarence Street
Sydney NSW 2000
Website: www.bbwpublisher.com
Email: info@bbwpublisher.com

2024, Volume 2, Issue 1

Table of Contents

Dermatological Health, 2024, Volume 2, Issue 1
http://ojs.bbwpublisher.com/index.php/DH

Diagnostic Value of Anti-Desmoglein 1 and 3 Antibodies and Anti-BP180 and 230 Antibodies in Autoimmune Bullous Dermatoses

Caihong Li*, Dongyun Jing, Xinyong Liu

Department of Clinical Lab, Dalian Dermatosis Hospital, Dalian 116021, Liaoning Province, China

***Corresponding author:** Caihong Li, rake2007@163.com

Abstract: *Objective:* To analyze and evaluate the value of the anti-epidermal intercellular desmosome antibodies, anti-desmoglein (Dsg) 1, and anti-Dsg3, as well as the anti-epidermal basement membrane hemidesmosomes antibodies, anti-BP180, and anti-BP230 in the diagnosis of pemphigus and bullous pemphigoid. *Methods:* Patients with pemphigus or bullous pemphigoid treated in the Department of Dermatology of Dalian Dermatosis Hospital from July 2019 to July 2021 were selected. They were clinically diagnosed with histopathological and indirect immunofluorescence methods. They were divided into the pemphigus group (n = 102) and the bullous pemphigoid group (n = 175). Additionally, 120 patients who were ruled out of pemphigus and bullous pemphigoid during the same period were selected as the control group. Enzyme-linked immunosorbent assay (ELISA) was used to detect anti-Dsg1, anti-Dsg3, anti-BP180, and anti-BP230 antibodies. Indirect immunofluorescence (IIF) was used to detect the IgG levels of the anti-epidermal intercellular desmosome antibodies and anti-epidermal basement membrane hemidesmosomes antibodies. The positive rate, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic rate of each antibody were evaluated and analyzed comprehensively. *Results:* In the pemphigus group, bullous pemphigoid group, and control group, the positive rates of anti-Dsg1 are respectively 83.3% (85/102), 0 (0/175), and 1.7% (2/120); the positive rates of anti-Dsg3 are 41.1% (42/102), 0 (0/175), and 0 (0/120), respectively; the positive rates of BP180 are 5.9% (6/102), 85.7% (150/175), and 5% (6/120), respectively; the positive rates of BP230 are 1.9% (2/102), 57.7% (101/175), and 1.7% (1/120), respectively. Meanwhile, the positive rates of anti-epidermal intercellular desmosome antibodies are 69.6% (71/102), 0 (0/175), and 0 (0/120), respectively; the positive rates of anti-epidermal basement membrane hemidesmosome antibodies are 0% (0/102), 51.4% (90/175), and 0 (0/120), respectively. Among the patients with pemphigus, the sensitivity, specificity, PPV, NPV, and diagnostic rates of the anti-epidermal intercellular desmosome antibody test were 65.1%, 100%, 100%, 85.2%, and 92.2%; for anti-Dsg1, respectively, 83.3%, 99.3%, 97.7%, 88.7%, and 95.2%; for anti-Dsg3, respectively, 41.1%, 100%, 100%, 83.1%, and 84.9%. Among the patients with bullous pemphigoid, the sensitivity, specificity, PPV, NPV, and diagnostic rates of the anti-epidermal basement membrane hemidesmosomes antibody test were 51.4%, 100%, 100%, 72.3%, and 78.6%; for anti-BP180, respectively, 85.7%, 94.6%, 92.6%, 89.4%, and 90.7%; for anti-BP230, respectively, 57.7%, 98.2%, 96.2%, 74.7%, and 80.1%. *Conclusion:* The detection of autoantibodies in serum and the confirmation of the specific target antigens could complement each other to reduce clinical missed diagnosis and increase the positive diagnostic rate if the two tests were conducted simultaneously. The positive result of the anti-epidermal intercellular desmosome antibody and the anti-epidermal basement membrane hemidesmosomes antibody has

better accuracy in diagnosing pemphigus and bullous pemphigoid. In contrast, the negative result is of great value in ruling out pemphigus and bullous pemphigoid.

Keywords: Pemphigus; Dsg1; Dsg3; Bullous pemphigoid; BP180; BP230

Online publication: March 29, 2024

1. Introduction

Pemphigus (P) and bullous pemphigoid (BP) are typical intraepidermal and subepidermal autoimmune bullous dermatoses (AIBD), respectively [1]. Autoantibodies mediate their onset, but the autoantibody profiles of the two diseases differ [2]. In patients with pemphigus, indirect immunofluorescence (IIF) can be used to detect the presence of antibodies against desmosomes between spiny cells in the patient's serum, and enzyme-linked immunosorbent assay (ELISA) can be used to detect autoantibodies such as anti-desmoglein (Dsg) 1 and anti-Dsg3. The IIF method in patients with bullous pemphigoid can detect the presence of hemidesmosomal antibodies against the epidermal basement membrane in the patient's serum. The ELISA method can detect specific anti-BP180 and anti-BP230 antibodies, and it is found that the serum of more than 90% of bullous pemphigoid patients contains anti-BP180 antibodies, which is related to the disease severity [3-5]. Previous literature shows that direct immunofluorescence (DIF) is the "gold standard" for diagnosing bullous dermatoses, but there are false negatives or false positives in clinical practice [3]. To explore the clinical application and significance of the detection of anti-Dsg1, anti-Dsg3, anti-BP180, and anti-BP230 antibodies in the serological diagnosis of bullous dermatoses, the IIF and ELISA test results of patients with bullous dermatoses in Dalian were collected and analyzed, providing the basis for the diagnosis and treatment of pemphigus and bullous pemphigoid.

2. Materials and methods

2.1. Clinical data

Patients diagnosed and treated from July 2019 to July 2021 at Dalian Dermatosis Hospital were selected. Based on clinical manifestations and pathological results, 102 cases of diagnosed pemphigus were recorded as group P; 175 patients diagnosed with bullous pemphigoid were recorded as group BP. During the same period, 120 patients who were clinically ruled out of having autoimmune bullous dermatoses were selected as the control group. The sera of all the patients were collected for testing. In the control group, there were 70 cases of eczema, 7 cases of dermatitis, 10 cases of prurigo nodularis, 4 cases of urticaria, 8 cases of psoriasis, 2 cases of epidermolysis bullosa, 3 cases of oral lichen planus, 1 case of erythroderma, 12 cases of erythema multiforme, 2 cases of fixed-drug eruption, and 1 case of folliculitis. There is no clinical subdivision of pemphigus, so the patients were classified into the pemphigus group (group P) in this study. Since epidermolysis bullosa has pathological manifestations on the dermal side and the general target antigen is collagen VII, the cases were classified in the control group instead of the BP group.

2.2. Methods

Serum anti-Dsg1, anti-Dsg3, anti-BP180, and anti-BP230 antibodies were measured using ELISA kits (Oumeng Medical Experimental Diagnostics AG, Germany) according to the kit's instructions. 100 μl of serum specimen, Dsg1, Dsg3, BP180, and BP230 antibody standards was each added to the reagent wells and incubated at

room temperature for 30 minutes. After washing three times, 100 μl enzyme-labeled antibodies was added to each well and incubated at room temperature for 30 minutes; washing was repeated for 3 times, 100 μl substrate solution was added to each well and incubated at room temperature for 15 minutes, and then 100 μl stop solution was added. A fully automated microplate reader (TECAN fully automated enzyme immunoassay instrument) was used for the measurement, with 450 nm as the detection wavelength and 620 nm as the reference wavelength. The absorbance value was recorded and data processing was performed according to the calculation method provided in the instruction manual. According to the kit's instructions, antibody quantitative value of ≥ 20 RU/ml is positive and < 20 RU/ml is negative.

The indirect immunofluorescence method was used to determine the serum antibody IgG level using the IIF kit (Oumeng Medical Experimental Diagnostics AG, Germany), and it was performed according to the kit's instructions. The anti-epidermal basement membrane hemidesmosome antibodies and anti-epidermal intercellular desmosome antibodies IgG subtypes in the patient's serum were detected using the monkey esophagus and tongue tissue sections as the detection matrix. Fluorescein-labeled goat anti-human IgG was used as the secondary antibody, and the results were observed under a fluorescence microscope. Positive and negative serum controls were set for each batch.

2.3. Statistical processing

The data were collected using the SPSS22.0 statistical software package. The positivity rate, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic rate were calculated for anti-Dsg1, anti-Dsg3, anti-BP180, anti-BP230, anti-epidermal intercellular desmosome antibodies, and anti-epidermal basement membrane hemidesmosome antibodies, and the four-grid table χ^2 test was used to determine the test level $\alpha = 0.05$.

3. Results

3.1. Overall detection status of anti-Dsg1, anti-Dsg3, anti-BP180, anti-BP230, anti-epidermal intercellular desmosome antibodies, and anti-epidermal basement membrane hemidesmosome antibodies

In the pemphigus group, bullous pemphigoid group, and control group, the positive rates of anti-Dsg1 antibodies were 83.3% (85/102), 0 (0/175), and 1.7% (2/120) respectively; the positive rates of anti-Dsg3 antibodies were 41.1% (42/102), 0 (0/175), and 0 (0/120) respectively; the positive rates of anti-BP180 antibodies were 5.9% (6/102), 85.7% (150/175), and 5% (6/120) respectively; the positive rates of anti-BP230 antibodies were 1.9% (2/102), 57.7% (101/175), and 1.7% (1/120) respectively; the positive rates of anti-epidermal intercellular desmosome antibodies were respectively 69.6% (71/102), 0 (0/175), and 0 (0/120); and the positive rates of anti-epidermal basement membrane hemidesmosome antibodies were respectively 0% (0/102), 51.4% (90/175), and 0 (0/120). In the pemphigus group, the positive rate of anti-Dsg1 and anti-Dsg3 antibodies, and anti-intercellular desmosome antibodies was statistically significant compared with the bullous pemphigoid and control groups ($P < 0.01$). In the pemphigoid group, the positive rate of anti-BP180 and anti-BP230 antibodies, and anti-epidermal basement membrane hemidesmosome antibodies was statistically significant compared to the pemphigoid and control groups ($P < 0.01$), as shown in **Table 1**.

Table 1. Results analysis of the pemphigus, bullous pemphigoid, and control groups

Antibody	Positive rate (%)		
	Group P (n = 102)	Group BP (n = 175)	Control group (n = 120)
Anti-Dsg1*	83.3 (85/102)	0 (0/175)	1.7 (2/120)
Anti-Dsg3*	41.1 (42/102)	0 (0/175)	0 (0/120)
Anti-epidermal intercellular desmosome antibodies*	69.6 (71/102)	0 (0/175)	0 (0/120)
Anti-BP180**	5.9 (6/102)	85.7 (150/175)	5 (6/120)
Anti-BP230**	1.9 (2/102)	57.7 (101/175)	1.7 (2/120)
Anti-epidermal basement membrane hemidesmosome antibodies**	0 (0/102)	51.4 (90/175)	0 (0/120)

*$P = 0.007$, $P < 0.01$; **$P = 0.004$, $P < 0.01$

3.2. Test evaluation of anti-Dsg1, anti-Dsg3, anti-BP180, anti-BP230, anti-epidermal intercellular desmosome antibodies, and anti-epidermal basement membrane hemidesmosome antibodies

The sensitivity, specificity, PPV, NPV, and diagnostic rate of anti-epidermal intercellular desmosome antibody detection test for pemphigus patients were 65.1%, 100%, 100%, 85.2%, and 92.2%, respectively; for anti-Dsg1, respectively, 83.3%, 99.3%, 97.7%, 88.7%, and 95.2%; and for anti-Dsg3, respectively, 41.1%, 100%, 100%, 83.1%, and 84.9%. The sensitivity, specificity, PPV, NPV, and diagnostic rate of the anti-epidermal basement membrane hemidesmosome antibody test for bullous pemphigoid patients were 51.4%, 100%, 100%, 72.3%, and 78.6%, respectively; for anti-BP180 antibody, 85.7%, 94.6%, 92.6%, 89.4%, and 90.7%, respectively; and for anti-BP230 antibody, 57.7%, 98.2%, 96.2%, 74.7%, and 80.1%, respectively (**Tables 2** and **3**).

Table 2. The analysis and evaluation of anti-Dsg1 and anti-Dsg3 antibodies and anti-epidermal intercellular desmosome antibody in pemphigus group

Pemphigus group	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Diagnostic rate (%)
Anti-Dsg1	83.3	99.3	97.7	88.7	95.2
Anti-Dsg3	41.1	100	100	83.1	84.9
Anti-epidermal intercellular desmosome antibodies	65.1	100	100	85.2	92.2

Table 3. The analysis and evaluation of anti-BP180 and anti-BP230 antibodies and anti-epidermal basement membrane hemidesmosomes antibodies in bullous pemphigoid group

Bullous pemphigoid group	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Diagnostic rate (%)
Anti-BP180	85.7	94.6	92.6	89.4	90.7
Anti-BP230	57.7	98.2	96.2	74.7	80.1
Anti-epidermal basement membrane hemidesmosome antibodies	51.4	100	100	72.3	78.6

3.3. Evaluation of detection results of indirect immunofluorescence method and enzyme-linked immunosorbent assay

The tissues used for indirect immunofluorescence detection were monkey esophagus and tongue tissue sections

while the enzyme-linked immunosorbent assay used purified or genetically recombinant specific antigens as the detection matrix, detecting the antibody levels in the patient's serum from different aspects. This part analyzed the results of dual-method detection of pemphigus and bullous pemphigoid-related antibodies, as shown in **Tables 4** and **5**.

Table 4. The results analysis of pemphigus by the two methods

Test methods	Results		Total
	+	–	
ELISA	102	0	102
IIF	45	57	102

Among 102 patients with pemphigus, 102 were positive for anti-Dsg1 or anti-Dsg3 antibodies, with a positive rate of 100%. Among the 102 patients, 45 had positive IIF test results at the initial diagnosis, with a positive rate of 44.1%. The positive rate of the IIF method is lower than that of the ELISA method.

Table 5. The results analysis of bullous pemphigoid by the two methods

Test methods	Results		Total
	+	–	
ELISA	175	0	175
IIF	112	63	175

Among 175 patients with bullous pemphigoid, 175 were positive for anti-BP180 or anti-BP230 antibodies, with a positive rate of 100%. Among the 175 patients, 112 had positive IIF test results at the initial diagnosis, with a positive rate of 64.0%. The positive rate of the IIF method is lower than that of the ELISA method.

4. Discussion

Autoimmune bullous dermatoses (AIBD) are a group of organ-specific autoimmune diseases. The patient's serum has antibodies (desmosomes, hemidesmosomes) against structures of skin junctions. Autoantibodies damage the connections between epidermal cells and between the epidermis and the dermis, causing intraepidermal blisters or subepidermal blistering diseases. AIBD can be clinically divided into pemphigus and bullous pemphigoid. In addition to typical clinical manifestations, specific antibodies often appear in patients' serums. For example, anti-epidermal intercellular desmosome antibodies, anti-Dsg1 antibodies, and anti-Dsg3 antibodies are detected in the serum of patients with pemphigus; anti-epidermal basement membrane hemidesmosome antibodies, anti-BP180 antibodies, and anti-BP230 antibodies are detected in the serum of patients with bullous pemphigoid [6].

Clinical diagnosis of patients with bullous dermatoses mainly relies on skin pathology, and serological testing is not widely used. However, the detection of skin pathology is invasive, with slow wound healing when patients undergo immunosuppressive and hormonal treatments, and the location from which the skin specimen is collected, etc., can also easily lead to false negative results. Domestic expert recommendations [7,8] clearly stated that the detection of serum antibodies can be used as a diagnostic standard for these types of diseases and that the antibody titer is positively correlated with the severity assessment and treatment monitoring. The methods for the detection of antibodies in serum include IIF and ELISA, but most laboratories

do not fully utilize these two methodologies for detection, which may lead to certain deficiencies in diagnosis [9]. The indirect immunofluorescence method detects total antibodies and cannot classify antibody target antigens. Evaluating antibody titer changes is not as intuitive and sensitive as the ELISA method, and treatment monitoring is poor [10].

102 patients with clinically diagnosed pemphigus and 175 patients with bullous pemphigoid were selected for serum antibody tests, and 120 patients without bullous dermatoses were collected as a control group. The anti-epidermal intercellular desmosome antibodies, anti-epidermal basement membrane hemidesmosome antibodies, anti-Dsg1, anti-Dsg3, anti-BP180, and anti-BP230 antibodies in the patient's serum were statistically analyzed respectively, and the value of each antibody in disease diagnosis was evaluated. The research results showed that anti-epidermal intercellular desmosome antibodies, anti-Dsg1, and anti-Dsg3 antibodies have better specificity, sensitivity, PPV, NPV, and diagnostic rate in patients with pemphigus, among which the diagnostic rates are respectively 92.2%, 95.2%, and 84.9%, which are similar to the research results of Chen *et al.* [11]. The research results of Li *et al.* [12] demonstrated that the ELISA method detecting anti-Dsg antibodies can be used as a screening method for bullous diseases. No further testing is needed if the results are consistent with the clinical diagnosis. Further pathological and immunofluorescence testing can be performed if the results show discrepancy with the clinical diagnosis. Anti-epidermal basement membrane hemidesmosome antibodies, anti-BP180, and anti-BP230 antibodies also show good characteristics in patients with bullous pemphigoid, with diagnostic rates of 78.6%, 90.7%, and 80.1%, respectively. It can be seen from the data that there are differences in the specificity and sensitivity between the indirect immunofluorescence method and the enzyme-linked immunosorbent assay. The specificity of the indirect immunofluorescence method is almost 100%, but the sensitivity is lower than that of the enzyme-linked immunosorbent method. They play a complementary role in the diagnosis of diseases and avoid missed diagnoses due to a single methodology. We can see from the test results of the dual-method detection that the positive rate of the ELISA method is significantly higher than the IIF method. This test result is slightly different from the research results of Huang *et al.* [13,14]. From the matrix analysis of the test, the monkey esophageal or tongue tissues are used to detect anti-epidermal intercellular desmosome antibodies and anti-epidermal basement membrane hemidesmosome antibodies, the target antigens are anti-Dsg1, anti-Dsg3, anti-BP180, and anti-BP230 antibodies, and the consistency of the two methods should be better. From the perspective of human subjective interpretation of IIF, the difference between the results of this study and that of Huang *et al.* may be caused by fluorescence interpretation. Our laboratory will further standardize the interpretation and observe the consistency of the dual-method detection results to better inform clinical practice.

Disclosure statement

The authors declare no conflict of interest.

References

[1] Hammers CM, Stanley JR, 2016, Mechanisms of Disease: Pemphigus and Bullous Pemphigoid. Annu Rev Pathol, 11(11): 175–197.

[2] Giusti D, Jan SL, Gatouillat G, et al., 2017, Biomarkers Related to Bullous Pemphigoid Activity and Outcome. Exp Dermatol, 26(12): 1240–1247.

[3] Lapeyre-Lienard H, Joly P, 2010, Pemphigus. Presse Med, 39(10): 1066–1070.

[4] Grando SA, 2012, Pemphigus Autoimmunity: Hypotheses and Reality. Autoimmunity, 45(1): 7–35.

[5] Ishii K, Amagai M, 2013, *In Vitro* Pathogenicity Assay for Anti-Desmogle in Autoantibodies in Pemphigus. Methods Mol Biol, 2013(961): 219–225.

[6] Zhang D, Wei H, Huang Q, et al., 2011, Analysis of Immunofluorescence and Histopathological Examination Results of 53 Cases of Bullous Skin Disease. Chinese Journal of Dermatology and Venereology, 25(12): 944–945, 948.

[7] Autoimmune Disease Subprofessional Committee of the Dermatologist Branch of the Chinese Medical Doctor Association, 2016, Expert Recommendations on the Diagnosis and Treatment of Bullous Pemphigoid. Chinese Journal of Dermatology, 49(6): 384–387.

[8] Dermatology Branch of China Association for Promoting International Healthcare Exchange, 2020, Expert Recommendations on Diagnosing and Treating Pemphigus Vulgaris (2020). Chinese Journal of Dermatology, 53(1): 1–7.

[9] Xu W, 2007, Detection of Serum Anti-BP180 Autoantibodies in Patients with Bullous Pemphigoid Before and After Treatment. Chinese Journal of Leprosy and Dermatology, 23(4): 317–318.

[10] Yang B, Zhang F, 2000, Research Progress in Laboratory Diagnosis of Pemphigus. Chinese Journal of Leprosy and Dermatology, 16(4): 252–253.

[11] Chen Z, Wang X, Zheng Q, 2014, Diagnostic Value of Anti-Epidermal Acanthocyte Desmosome Antibodies and Anti-Desmoglein 1, 3 Antibodies in Pemphigus. Journal of Xinjiang Medical University, 37(2): 192–195.

[12] Li Y, Du Y, Shi Y, 2014, Evaluation of Enzyme-Linked Immunosorbent Assay in Diagnosing Bullous Skin Diseases. Chongqing Medicine, 43(25): 3305–3306 + 3310.

[13] Huang F, Jin Y, Feng Y, et al., 2015, Clinical Diagnostic Value of Anti-Desmoglein Antibodies for Pemphigus. Chinese Journal of Dermatology and Venereology, 29(3): 318–320.

[14] Liu B, 2014, Study on the Content Differences and Changing Trends of BP180NC16A IgG, IgE Antibodies, and BP230 IgG Antibodies in Serum and Blister Fluid of Patients with Bullous Pemphigoid, dissertation, Peking Union Medical College.

Dermatological Health, 2024, Volume 2, Issue 1
http://ojs.bbwpublisher.com/index.php/DH

Influence of *ABCB1 3435C>T* Polymorphism on Methotrexate Safety in Patients with Psoriasis — A Secondary Publication

Alexey A. Kubanov[1,2], Anastasiia V. Asoskova[1,2]*, Michael S. Zastrozhin[1,3], Zhannet A. Sozaeva[1], Dmitry A. Sychev[1]

[1]Russian Medical Academy of Continuous Professional Education, Moscow, Russia
[2]State Research Center of Dermatovenereology and Cosmetology, Moscow, Russia
[3]University of California, San Francisco, USA

***Corresponding author:** Anastasiia V. Asoskova, stasya.asoskova@mail.ru

Abstract: *Background:* Methotrexate is a highly effective systemic treatment for moderate to severe psoriasis, but drug toxicity may limit its use. Recent evidence suggests that it is necessary to take into account the individual characteristics of methotrexate pharmacokinetics, which are determined by the presence of polymorphisms of genes encoding methotrexate carrier proteins, to predict the risk of methotrexate-induced toxicity. *Aim:* The research aims to assess the associations of *ABCB1* rs1045642 (*3435C>T*) polymorphism with methotrexate safety for patients with moderate and severe psoriasis. *Methods:* The study included 75 psoriasis patients treated with methotrexate with 21 days of follow-up duration. Data on adverse drug reactions (ADR) were collected using a clinically structured questionnaire, and complete and biochemical blood tests, and urinalysis were performed. The severity of ADR was assessed using visual analog scales and the CTCAE toxicity scale. The severity of gastrointestinal ADR was assessed using the GSRS questionnaire. Genotyping was carried out by real-time PCR. *Results:* Gastrointestinal toxicity was detected in 38 patients (50.67%). The mean GSRS score was 7.97 ± 9.18. Analysis of differences in the ADR incidence showed the presence of statistically significant differences in the frequency of ADR in the gastrointestinal tract: the toxic effect of methotrexate was more often observed in carriers of the T allele of the *ABCB1* rs1045642 polymorphism (*3435C>T*), (CC: 2 (14.3%), TC: 18 (52.9%), TT: 18 (66.7%), $P = 0.006$). Binomial regression demonstrated the presence of a statistically significant effect of the rs1045642 single-nucleotide polymorphism of the *ABCB1* gene on the incidence of ADR from the gastrointestinal tract (OR = 8.64, $P = 0.008$). *Conclusion:* An association of *ABCB1* rs1045642 single-nucleotide polymorphism with the safety of methotrexate therapy in patients with moderate and severe psoriasis was revealed. The data obtained can be used to personalize the prescription of methotrexate to psoriasis patients.

Keywords: Pharmacogenetics; Biomarkers; Drug safety; Psoriasis; Methotrexate; Single-nucleotide polymorphism; Adverse reactions

Online publication: March 29, 2024

1. Introduction

Psoriasis is one of the most common skin diseases [1]. Psoriasis is a chronic relapsing disease whose therapeutic goal is to gain control of the disease course and minimize adverse drug reactions (ADRs) by using drugs that maintain long-term remission and are well tolerated by patients [2,3]. Methotrexate (4-deoxy-4-amino-10-methylfolic acid) is a highly effective drug for the systemic treatment of moderate to severe psoriasis, but the toxicity of the drug may limit its use [4]. According to the meta analysis conducted by West *et al.*, methotrexate has been reported in an average of 28.3% of patients. Gastrointestinal tract disorders are the most frequent ADR for methotrexate: 18.2% of patients have nausea and vomiting; 11.1% have ulcerations of the oral mucosa and other mucositis (gingivitis, ulcerative stomatitis, enteritis); 7.5% of patients reported the occurrence of abdominal pain; 6.6% of patients reported the occurrence of functional bowel disorders [5]. The quality of life of patients is severely affected, which in 13–28% of cases leads to forced interruption of treatment [6]. The main mechanism for the development of ADRs during methotrexate therapy is its cytotoxic effect on rapidly dividing cells, namely, inhibition of folate metabolism in tissues with high proliferation of cells with a high need for purines, thymidine, and methionine. Since the epithelium of the gastrointestinal tract is characterized by a high cell population renewal rate, folate deficiency is the main mechanism in the development of this group of ADRs [7]. Patients with psoriasis are often forced to use methotrexate for long periods of time, and there is currently no algorithm that can predict individual patient response to therapy; the study of safety issues of therapies is a highly urgent task. Due to the fact that low doses of methotrexate are used for psoriasis therapy, measuring the drug concentration in the plasma to predict its toxicity has no clinical significance. Recently, much attention has been paid to the role of genetic factors in individual tolerance to psoriasis therapy [8,9], they may be able to predict the development of about half of adverse responses to treatment [10].

The frequency of gastrointestinal tract ADRs may be influenced by genetic factors, namely the presence of polymorphisms in genes encoding methotrexate transporter proteins. According to current research, to predict the risk of methotrexate-induced toxicity, it is necessary to take into account individual characteristics of its pharmacokinetics, which are determined by the presence of polymorphisms in genes encoding proteins that carry methotrexate [7,9,11].

The bioavailability of methotrexate is dependent on ABC family transporters, which transport methotrexate molecules from enterocytes into the lumen of the gastrointestinal tract, as well as from P-glycoprotein (ABCB1 protein), a transporter protein that transports methotrexate molecules from enterocytes into the lumen of the gastrointestinal tract. The *ABCB1* gene, encoding P-glycoprotein, has a significant degree of polymorphism. The *3435C>T* polymorphism, which is a substitution of a cytosine nucleotide for a thymidine nucleotide at position 3435, has the highest clinical significance and prevalence [12]. It is proved that a low level of *ABCB1* expression in the intestine and kidneys leads to a decrease in P-glycoprotein content in these organs and consequently to more complete absorption and slower excretion of its substrates, which include methotrexate. As a result, the concentration of methotrexate in blood plasma is elevated, thus increasing the likelihood of developing ADRs [13].

This paper aims to identify associations of the single-nucleotide polymorphism *ABCB1* rs1045642 (*3435C>T*) with the safety of methotrexate therapy in patients with moderate to severe psoriasis. In particular, it aims to assess the frequency and severity of adverse drug reactions of the gastrointestinal tract and to analyze the relationship between the frequency and severity of adverse drug reactions and patients' genotypes.

2. Materials and methods

2.1. Study design

The prospective study was conducted in two phases: at the first stage, dynamic patients with moderate and

severe psoriasis were monitored, who were being treated in the 24-hour inpatient unit of the dermatology department, to identify adverse drug reactions that may be associated with methotrexate intake, their frequency, and severity.

From the moment of inclusion in the study, clinical, demographic, and laboratory examinations of the patients were carried out, and psoriasis severity was assessed using the Psoriasis Area and Severity Index (PASI) [14]. Data on adverse drug reactions were collected using a specially developed structured questionnaire, and complete and biochemistry blood tests, and urinalysis were performed. We analyzed the causal relationship between methotrexate intake and the development of ADRs using the Naranjo scale and the Liverpool causality assessment tool to assess the cause of adverse drug effects. The severity of adverse drug reactions was assessed using visual analog scales and the CTCAE (Common Toxicity Criteria for Adverse Event) scale.

In the second stage of the study, the frequency of alleles of the single-nucleotide polymorphism of the gene *ABCB1* (rs1045642) in this sample of patients and the association between the presence of genetic polymorphism and gastrointestinal safety parameters of methotrexate therapy were analyzed.

2.2. Eligibility criteria

75 patients diagnosed with psoriasis were included in the study. The patients were hospitalized in the Clinical Dermatology Department of the Russian Ministry of Health and were treated with methotrexate in the recommended therapeutic dosages.

The criteria for inclusion of patients in the study were:

(1) Presence of written informed consent of the patient to participate in the study.

(2) Patients with clinical forms of psoriasis: common psoriasis, pustular psoriasis (von Zumbusch pustular psoriasis, Barber's palms and soles), psoriatic erythroderma, arthropathic psoriasis.

(3) Patients receiving methotrexate during hospitalization.

The criteria for non-inclusion were:

(1) Severe somatic pathology.

(2) Psychotic state or history of severe psychotic illness.

(3) Concomitant use of drugs that affect the pharmacokinetics and/or pharmacodynamics of methotrexate.

The exclusion criteria were:

(1) Refusal of the patient to continue participation in the study.

Inclusion in the study occurred within the first 24 hours after the first methotrexate injection. Informed consent for inclusion in the study was obtained from each patient, and comprehensive information was given about the study, its aims, and results.

2.3. Research conditions

The work was carried out on the basis of the Federal State Budgetary Institution State Scientific Center for Children's Culture of the Ministry of Health of Russia (Director, Academician of the Russian Academy of Sciences, Doctor of Medical Sciences, Professor A.A. Kubanov). Pharmacogenetic studies were carried out at the Research Institute of Molecular and Personalized Medicine of the Ministry of Health.

2.4. Duration of the study

The study was conducted from 2019 through 2021, and the planned duration of follow-up for each patient was 21 days. There were no shifts in the planned time intervals during the study.

2.5. Medical intervention

2.5.1. Clinical research methods

The data on symptomatic adverse drug reactions were identified during the patient interview using a specially developed clinically structured questionnaire [15]. Questions about the tolerability of methotrexate therapy were asked to the patient daily from the moment the patient was included in the study throughout the hospitalization period. The principle of questionnaire development was based on the methodology of targeted detection of adverse drug reactions, the effectiveness of which was proved in the work of Tsvetova [16]: the patient was asked questions regarding the presence of each of the expected symptomatic adverse drug reactions across all organs and systems.

In case of a positive response, the severity of ADR was assessed by the patient independently using the visual-analog numerological evaluation scale graded from 1 to 10, they were asked to mark their perception of symptom severity. In addition, the severity of ADR was assessed using the CTCAE v5.0 Toxicity Severity Scale (Common Toxicity Criteria for Adverse Event, version 5.0, 2017) [17].

The severity of adverse drug reactions in the gastrointestinal tract was assessed using a specialized questionnaire to assess the quality of life of patients with regard to GI symptoms — GSRS (gastrointestinal symptoms rating scale) [18]. The questionnaire included 15 questions assessing patients' discomfort with symptoms of pain, reflux, dyspepsia, diarrhea, and constipation. The patient self-assessed the severity of the symptom constellation using targeted questions with a score from 1 to 7. Based on the sum, we calculated the toxicity in the gastrointestinal tract for each patient.

The Naranjo scale was used to assess the causal relationship between methotrexate intake and ADR [19]. Only those ADRs that had a definite, probable, possible degree of association were considered in the study.

2.5.2. Laboratory research methods

Biological material for genomic DNA extraction was obtained from 4 ml of venous blood collected from all patients after signing informed consent to participate in the clinical trial in the Department of Clinical Dermatology of FGBU GNTCDC Ministry of Health of Russia using a vacuum system VACUETTE (Greiner Bio-One, Austria) into tubes with 0.5 M EDTA. Blood was drawn regardless of food intake and duration of methotrexate therapy. Biological samples of whole blood were frozen at -70°C, then transported in thermocontainers to the Research Institute of Molecular and Personalized Medicine of the Russian Ministry of Health (Moscow) for genetic analysis. DNA extraction from whole venous blood samples was performed using the reagent kit "S-Sorb" (Syntol LLC, Russia) according to the manufacturer's protocol. Determination of allelic variants of *ABCB1* single-nucleotide polymorphism (C3435T, rs1045642) was performed using a commercial reagent kit (Syntol LLC, Russia) by the method of real-time PCR on the CFX96 Touch Real-Time System with CFX Manager software version 3.0 (BioRad, USA). The amplification program included incubation at 95°C for 3 minutes, followed by denaturation at 95°C for 15 seconds, and annealing at 63°C for 40 seconds for 39 cycles. The fluorescence signal was detected by the channel for the fluorophore FAM (carboxyfluorescein, absorption wavelength 492 nm, fluorescence wavelength 520 nm) and a channel for HEX fluorophore (hexachlorofluorescein, absorption wavelength 535 nm, fluorescence wavelength 556 nm).

2.6. Statistical analysis

Statistical analysis was performed using the methods of parametric and non-parametric statistics using the package of applied programs STATISTICA v10.0 (StatSoft Inc., USA). When selecting the method, the normality of sample distribution was assessed using the Shapiro-Wilk *W*-test, homogeneity of variance was assessed using the Fisher's *T*-test (when comparing two samples) and Levene's test (when comparing several samples). Differences were considered statistically significant at $P < 0.05$ (at statistical power > 80%). Student's

t-test or its nonparametric analog was used to compare two samples of quantitative data: Mann-Whitney *U*-test. When comparing quantitative data from several samples simultaneously, parametric single- and multivariate analysis of variance (and their non-parametric analogs) were used: Kruskal-Wallis analysis (ANOVA) and the Jonckheere-Terpstra criterion (when testing the shift hypothesis against ordering alternatives). Qualitative characteristics were compared using Fisher's chi-square test. The effect of one variable on the other was assessed by regression analysis.

2.7. Ethical review

The study was approved by the Local Ethical Committee of the "Russian Medical Academy of Continuing Professional Education" Ministry of Health of the Russian Federation, protocol No. 9 of November 13, 2018. The results of the review of the study protocol were found to be satisfactory, and the results of the study were recommended for publication.

3. Results

3.1. Participants of the study

Of the 75 patients included in the study, there were 47 males (62.7%) and 28 females (37.3%), aged between 19 and 84 years. The mean duration of hospitalization was 24.6 ± 8.2 days.

All patients included in the study had progressive stage of psoriasis. Patients received methotrexate in the form of a solution (trade name: Methotrexate-Ebeve; manufacturer: EbevePharma, Austria) administered intramuscularly once a week. The therapeutic dose of methotrexate was selected in accordance with the clinical guidelines of the 2019 Russian Society of Dermatovenerologists and Cosmetologists. The mean dose of methotrexate was 14.05 ± 3.49 mg. The average number of injections was 3.15 ± 1.00. All patients received methotrexate in combination with folic acid, which was taken at a dosage of 5 mg 12–24 hours after each methotrexate injection.

3.2. Gastrointestinal adverse drug reactions observed in patients with psoriasis during methotrexate therapy

Manifestations of toxic effects of methotrexate developed in 38 patients (50.67%). All patients who experienced one or another gastrointestinal ADRs were questioned using the GSRS Digestive Symptom Severity Questionnaire, which assesses the quality of life of patients with regard to complaints of various GI disorders. The most common intensity of symptoms was rated by patients as 1 (minor discomfort) to 4 points (relatively severe but tolerable discomfort). The maximum total score on the scale was 27, the minimum was 3. The mean value was 7.97 ± 9.18 points.

Analysis of gastrointestinal ADRs was carried out for each category of symptoms individually. Stomatitis was detected in 3 patients (4%). In two manifestations, one patient developed on the fourth to fifth day after the first methotrexate injection, and in one patient on the third day after the second injection of the drug. The average duration of symptoms was 10 days. Severity of manifestations according to the CTCAE scale in all patients was 1 point (mild toxicity, slightly or moderately expressed symptoms not requiring therapeutic intervention), as stomatitis was manifested by ulcers in the oral cavity and moderate soreness, while the patient's eating was not disturbed.

Diarrhea was noted in 17 patients (22.67%). Symptoms appeared, as a rule, a few hours after methotrexate injection, persisted for 2–3 days, and occurred again at the next injection. The severity of manifestations on the CTCAE scale in the majority of patients was 1 point, as the times of diarrhea were not more than 4 times

a day. Three patients had diarrhea up to 6 times a day, and the severity of manifestations on the CTCAE scale corresponded to 2 points.

Complaints of nausea were presented by 29 patients (38.67%). Symptoms occurred an average of 4.5 hours after methotrexate injection and lasted an average of about three days (2.96 days), recurring after repeated injections. Patients rated the severity of nausea in scores using a visual analog scale: The mean score was 6 [6,8]. The maximum score was 10 and the minimum score was 3. In five patients, nausea disrupted the usual eating pattern and was therefore rated as 2 on the CTCAE scale. In the other 24 patients, nausea only led to a decrease in appetite and corresponded to a score of 1. Two patients experienced vomiting while taking methotrexate that did not require special rehydration therapy (CTCAE score = 1).

Two patients noted the occurrence of heartburn 7–12 hours after methotrexate injection, lasting for 4–6 days. Heartburn manifested as an unpleasant burning or stinging sensation in the chest area.

12 patients (16%) complained of abdominal pain of different nature: tingling, aching, pulling. One patient experienced pain in the right hypochondrium. They developed within 1–2 days after injection and were short-lived: the duration of no more than 24 hours. The severity of abdominal pain rated by patients on a visual analog scale was 1.05 ± 1.54 points on average. On the CTCAE scale, the pain was rated as 1 point, as it was moderate.

In addition to the adverse drug reactions described, 33 patients (44%) described other symptoms that were grouped under the category of dyspepsia. Patients complained of abdominal rumbling, a feeling of air in the abdomen, belching, which patients described as the release of air out of the stomach through the mouth, and flatulence. Patients complained of discomfort in the upper abdomen and the stomach area, a feeling of sour or bitter liquid from the stomach flowing down the throat, and a feeling of unpleasant emptiness in the stomach. Some patients complained of a reduced, compared to normal, ability to empty the bowels; others complained of alternating liquid and too hard stools, with liquid stools predominating, and a sudden need to empty the bowels. Dyspeptic symptoms occurred within the first 24 hours after methotrexate injection and persisted for several days. After subsequent injections, the symptoms recurred. GSRS assessment of the gastrointestinal tract also revealed the predominance of dyspeptic syndrome over abdominal pain syndrome, reflux syndrome, and diarrhea syndrome.

The structure and severity of ADRs in the gastrointestinal tract are presented in **Table 1**.

Table 1. Structure and severity of gastrointestinal toxicity of methotrexate during psoriasis therapy

Adverse drug reactions	Number of patients, abs. (relative %)	Method for assessing severity	Degree of severity/ average score*
Dyspepsia	33 (44%)	CTCAE	1–2
Nausea	29 (38, 67%)	CTCAE	1–2
		VAS	2.78 ± 3.55
Vomiting	2 (2, 67%)	CTCAE	1
Diarrhea	17 (22, 67%)	CTCAE	1–2
Abdominal pain	12 (16%)	CTCAE	1
		VAS	1.05 ± 1.54
Pain in the right hypochondrium	4 (5, 33%)	CTCAE	1
Stomatitis	3 (4%)	CTCAE	1
Heartburn	2 (2, 67%)	–	–
All adverse drug reactions in the gastrointestinal tract	38 (50, 67%)	GSRS	7.97 ± 9.18

*The severity was assessed in degrees of severity according to the CTCAE scale. The severity was assessed in points according to the GSRS and visual analogue scales (VAS).

3.3. Frequency analysis of allele and genotype distribution at polymorphic marker *3435C>T* of *ABCB1* gene (rs1045642) in psoriasis patients treated with methotrexate

The distribution of allelic variants of single-nucleotide polymorphism *3435C>T* of the *ABCB1* gene (rs1045642) and their distribution conformity to the Hardy-Weinberg law in patients with psoriasis treated with methotrexate were analyzed.

According to the results of *ABCB1* genotyping by polymorph marker rs1045642 in 75 patients with psoriasis treated with methotrexate, the following results were obtained (**Table 2**).

(1) The number of patients who are carriers of the all-allelic variant of the wild-type genotype *ABCB1* rs1045642 (CC genotype) amounted to 14 patients (18.67%);

(2) The number of patients who are heterozygous carriers of the C3435T polymorphism of the *ABCB1* gene (CT genotype), amounted to 34 patients (45.33%);

(3) The number of patients who were homozygous carriers of C3435T polymorphism of *ABCB1* gene (TT genotype) amounted to 27 patients (36.00%).

The distribution of genotypes followed the Hardy-Weinberg law (Fisher's exact test $\chi^2 = 0.32$; $P = 0.572$).

Table 2. The analysis of differences in the incidence of gastrointestinal toxicity of methotrexate in *ABCB1* rs1045642 CC, CT, and TT patients

Indicator	CC (0), n = 14	CT (1), n = 34	TT (2), n = 27	Reliability differences (*P* value)
Adverse drug reactions in the gastrointestinal tract	2 (14.3%)	18 (52.9%)	18 (66.7%)	0.006
Stomatitis	1 (7.1%)	2 (5.9%)	0 (0.0%)	0.407
Diarrhea	1 (7.1%)	8 (23.5%)	8 (29.6%)	0.261
Nausea	2 (14.3%)	13 (38.2%)	14 (51.9%)	0.064
Vomiting	0 (0.0%)	2 (5.9%)	0 (0.0%)	0.290
Heartburn	0 (0.0%)	0 (0.0%)	2 (7.4%)	0.161
Dyspepsia	1 (7.1%)	17 (50.0%)	15 (55.6%)	0.008
Abdominal pain	1 (7.1%)	11 (32.4%)	14 (51.9%)	0.016
Pain in the right hypochondrium	0 (0.0%)	2 (5.9%)	2 (7.4%)	0.595

3.4. Analysis of the possible association between *ABCB1* rs1045642 polymorphism and the occurrence of adverse drug reactions in the gastrointestinal tract

Results of the analysis of differences in the incidence of adverse drug reactions in the gastrointestinal tract between group of patients with genotypes rs1045642 CC, CT, and TT are presented in **Table 2**.

The comparison between the group of patients with the rs1045642 CC homozygote and the group of patients with the remaining genotypes is presented in **Table 3**. To calculate the statistical significance of the presence of minor allelic variant T rs1045642 with the incidence of ADRs when using methotrexate for psoriasis treatment, the number of T allele in the patient's genotype was denoted by 0, 1 or 2, where 0 is the CC genotype, 1 is the CT genotype, 2 is the TT genotype. The analysis of the frequency of gastrointestinal disorders revealed significant differences between groups of patients with CC, CT, and TT genotypes rs1045642: methotrexate toxicity was more frequent in carriers of CT and TT genotypes ($P = 0.006$).

However, when comparing groups with the CC genotype and other genotypes (CT and TT), the strength of the association remained unchanged ($P = 0.006$). The result of binomial regression showed a statistically significant effect of *ABCB1* gene rs1045642 polymorphism on the incidence of ADR development in the

gastrointestinal tract: estimation –2.16, OR = 8.64, 95% CI OR: 1.78–42,01, $P = 0.008$.

Table 3. The analysis of differences in the incidence of gastrointestinal toxicity of methotrexate in *ABCB1* rs1045642 CC and CT + TT patients

Indicator	CC (n = 14)	CT + TT (n = 61)	Reliability of differences (*P* value)
Adverse drug reactions in the gastrointestinal tract	2 (14.3%)	136 (59.0%)	0.006
Stomatitis	1 (7.1%)	2 (3.3%)	0.467
Diarrhea	1 (7.1%)	16 (26.2%)	0.168
CTCAE-1 diarrhea	0 (0.0%)	14 (23.7%)	0.059
Nausea	2 (14.3%)	27 (44.3%)	0.053
Vomiting	0 (0.0%)	2 (3.3%)	1.100
Heartburn	0 (0.0%)	2 (3.3%)	1.100
Dyspepsia	1 (7.1%)	32 (52.5%)	0.005
Abdominal pain	1 (7.1%)	25 (41.0%)	0.026
Pain in the right hypochondrium	0 (0.0%)	4 (6.6%)	1.000

When analyzing the frequency of occurrence of individual types of ADRs, statistically significant patterns were also identified. Dyspepsia was characteristic for carriers of genotypes CT and TT ($P = 0.008$), nausea was also detected predominantly in carriers of these genotypes ($P = 0.05$).

However, when comparing patients homozygous for the C allele rs1045642, with others, the relationship between carriage of CT and TT genotypes and the occurrence of dyspeptic events became more pronounced ($P = 0.005$), as well as the relationship between carriage of CT and TT genotypes and the occurrence of nausea ($P = 0.053$).

The result of binomial regression demonstrated the presence of statistically significant influence of *ABCB1* gene rs1045642 polymorphism on the frequency of nausea: estimate -1.56, OR = 4.76, 95% CI OR: 0.98–23.13, $P = 0.05$, as well as the development of dyspepsia: estimation -2.66, OR = 14.34, 95% CI OR: 1.76–116.57, $P = 0.013$.

No significant association was found between groups of patients with different genotypes ($P = 0.261$). However, when patients carrying the T allele were combined into the group with the CT and TT genotypes, the reliability of the differences between the frequency of diarrhea of the 1st degree of severity and carriage of the T allele was at a probability level of 0.06 ($P = 0.059$).

Abdominal pain was more frequently reported by carriers of the allele T: CC: 1 (7.1%), CT: 11 (32.4%), TT: 14 (51.9%), $P = 0.016$. Combining carriers of the T allele into one group confirmed the established pattern: CC: 1 (7.1%), CT + TT: 25 (41.0%), $P = 0.026$. The result of binomial regression construction demonstrated a statistically significant effect of alleles of rs1045642 polymorphism of *ABCB1* gene on the occurrence of pain in the abdomen: estimation –2.2, OR = 9.03, 95% CI OR: 1.109–73.5, $P = 0.04$.

Analysis of symptoms such as stomatitis, vomiting, heartburn, and right hypochondrium pain also revealed no statistically significant differences in patients with different genotypes for *ABCB1* rs1045642 polymorphic marker.

4. Discussion

The high efficacy of methotrexate and the possibility of long-term therapy allow methotrexate to be considered as one of the drugs of choice in the treatment of severe psoriasis. However, the development of adverse drug reactions significantly reduces patients' quality of life and adherence to treatment. In this regard, it is relevant to predict the safety of methotrexate therapy, including the use of pharmacogenetic studies.

Results of the study of the influence of the presence of polymorphisms of the methotrexate transporter protein gene demonstrate that carriage of the T allele rs1045642 in psoriasis patients on methotrexate therapy is associated with a higher incidence of adverse drug reactions in the gastrointestinal tract: carriers of the mutant T allele (genotypes CT and TT) more often reported toxic effects of methotrexate.

A possible explanation for this pattern may be the accumulation of methotrexate in the body due to its delayed excretion in carriers of the minor T allele *ABCB1* (rs1045642). Presumably, this is due to the effect of gene polymorphism on the functioning of the transporter protein encoded by the gene. The *ABCB1* gene encodes P-glycoprotein: it is likely that carriers of the minor T allele have reduced activity of this protein, whose substrate is methotrexate. This can cause methotrexate to be eliminated from the body more slowly. As a consequence, methotrexate reaches target receptors in greater quantities, its cytotoxic effect is realized, which manifests itself as an increased risk of adverse reactions.

5. Conclusion

The results of the pharmacogenetic study *3435C>T* polymorphism of the *ABCB1* gene may be taken into account to improve the safety of therapy for psoriasis patients receiving methotrexate, as carriers of the minor T allele have an increased risk of adverse drug reactions, possibly related to genetically determined slowing of methotrexate excretion. The data obtained may form the basis for an algorithm for predicting the safety of methotrexate therapy in patients with moderate to severe psoriasis. However, further research is needed to increase the accuracy and reliability of the prediction to evaluate the different manifestations of methotrexate toxicity and to identify other biomarkers for the safety of methotrexate therapy.

Funding

The study was conducted with the financial support of the Russian Ministry of Health
as part of the implementation of state task No. 056-00102-22-00 dated November 1, 2022.

Disclosure statement

The authors declare no conflict of interest.

Author contributions

Conceptualization and design of the study: Alexey A. Kubanov, Dmitry A. Sychev
Collection and processing of material, statistical analysis: Anastasiia V. Asoskova,
Michael S. Zastrozhin, Zhannet A. Sozaeva
Data analysis and writing: Anastasiia V. Asoskova
Writing – editing: Alexey A. Kubanov, Dmitry A. Sychev.
All authors approved of the final version of the article and took responsibility for the integrity of all parts of the

article.

References

[1] Psoriasis. Clinical recommendations, 2020, Approved by the All-Russian Public Organization "Russian Society of Dermatovenerologists and Cosmetologists" January 15, 2020, Approved by the Scientific and Practical Council of the Ministry of Health of the Russian Federation.

[2] Parisi R, Iskandar IYK, Kontopantelis E, 2020, National, Regional, and Worldwide Epidemiology of Psoriasis: Systematic Analysis and Modelling Study. BMJ, 2020(369): 1–15. http://doi.org/10.1136/bmj.m1590

[3] Lebwohl M, 2005, Clinician's Paradigm in the Treatment of Psoriasis. J Am Acad Dermatol, 53(1): S59–69. http://doi.org/10.1016/j.jaad.2005.04.031

[4] Chikin VV, Znamenskaya LF, Minaeva AA, 2014, Pathogenic Aspects of Treatment of Psoriatic Patients. Vestnik Dermatologii Ivenerologii, 2014(5): 86–90.

[5] West J, Ogston S, Foerster J, 2016, Safety and Efficacy of Methotrexate in Psoriasis: A Meta-Analysis of Published Trials. PLoS One, 11(5): e0153740. http://doi.org/10.1371/journal.pone.0153740

[6] Yazici Y, Sokka T, Kautiainen H, et al., 2005, Long Term Safety of Methotrexate in Routine Clinical Care: Discontinuation is Unusual and Rarely the Result of Laboratory Abnormalities. Ann Rheum Dis, 64(2): 207–211. http://doi.org/10.1136/ard.2004.023408

[7] Bedoui Y, Guillot X, Sélambarom J, et al., 2019, Methotrexate an Old Drug with New Tricks. Int J Mol Sci, 20(20): 5023. http://doi.org/10.3390/ijms20205023

[8] Ray-Jones H, Eyre S, Barton A, et al., 2016, One SNP at a Time: Moving Beyond GWAS in Psoriasis. J Invest Dermatol, 136(3): 567–573. http://doi.org/10.1016/j.jid.2015.11.025

[9] Sutherland A, Power RJ, Rahman P, et al., 2016, Pharmacogenetics and Pharmacogenomics in Psoriasis Treatment: Current Challenges and Future Prospects. Expert Opin Drug Metab Toxicol, 12(8): 923–935. http://doi.org/10.1080/17425255.2016.1194394

[10] Sychjov DA, 2011, Recommendations for the Use of Pharmacogenetic Testing in Clinical. Kachestvennaya Klinicheskaya Praktika, 2011(1): 3–10.

[11] Lima A, Bernardes M, Azevedo R, et al., 2014, *SLC19A1*, *SLC46A1* and *SLCO1B1* Polymorphisms as Predictors of Methotrexate-Related Toxicity in Portuguese Rheumatoid Arthritis Patients. Toxicological Sciences, 142(1): 196–209. http://doi.org/10.1093/toxsci/kfu162

[12] Whetstine JR, Gifford AJ, Witt T, et al., 2001, Single Nucleotide Polymorphisms in the Human Reduced Folate Carrier: Characterization of a High-Frequency G/A Variant at Position 80 and Transport Properties of the His(27) and Arg(27) Carriers. Clin Cancer Res, 7(11): 3416–3422.

[13] Marzolini C, Paus E, Buclin T, et al., 2004, Polymorphisms in Human *MDR1* (P-Glycoprotein): recent Advances and Clinical Relevance. Clin Pharmacol Ther, 75(1): 13–33. http://doi.org/10.1016/j.clpt.2003.09.012

[14] Hallas J, Harvald B, Gram LF, et al., 1990, Drug Related Hospital Admissions: The Role of Definitions and Intensity of Data Collection, and the Possibility of Prevention. J Intern Med, 228(2): 83–90. http://doi.org/10.1111/j.1365-2796.1990.tb00199.x

[15] Stockwell DC, Slonim AD, 2006, Quality and Safety in the Intensive Care Unit. J Intensive Care Med, 21(4): 199–210. http://doi.org/10.1177/0885066606287079

[16] Cvetov VM, 2007, Monitoring of Adverse Reactions of Drugs in an Outpatient Clinic at the Present Stage, dissertation, Chelyabinsk, 130.

[17] US Department of Health and Human Services, National Institutes of Health, National Cancer Institute, 2017,

Common Terminology Criteria for Adverse Events (CTCAE) Version 5, viewed January 6, 2022, https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf

[18] Svedlund J, Sjödin I, Dotevall G, 1988, GSRS-A Clinical Rating Scale for Gastrointestinal Symptoms in Patients with Irritable Bowel Syndrome and Peptic Ulcer Disease. Dig Dis Sci, 33(2): 129–134. http://doi.org/10.1007/BF01535722

[19] Busto U, Naranjo CA, Sellers EM, 1982, Comparison of Two Recently Published Algorithms for Assessing the Probability of Adverse Drug Reactions. Br J Clin Pharmacol, 13(2): 223–227. http://doi.org/10.1111/j.1365-2125.1982.tb01361.x

Dermatological Health, 2024, Volume 2, Issue 1
http://ojs.bbwpublisher.com/index.php/DH

Topical Antifungal Therapy Combined with Nail Abrasion for the Treatment of Dermatophytoma: Single-Center Results — A Secondary Publication

Kunitaro Fukuyama*

Department of Dermatology, Kansai Rosai Hospital, Amagasaki, Japan

***Corresponding author:** Kunitaro Fukuyama, postmaster@kanrou.net

Abstract: *Objective:* Recently, topical antifungal agents have become available for the treatment of dermatophytoma. However, there are few reports of topical onychomycotic agents in combination with debridement of dermatophytoma. *Methods:* Twelve patients with dermatophytoma diagnosed and treated between April 2017 and March 2020 at the Department of Dermatology, Kansai Rosai Hospital were selected, including two patients with lesions on both toe nails and 14 nails. At the initial visit, the affected nail plate was removed and topical treatment was administered. The observation was terminated when the lesions were completely healed within 1 year. If the patients were not completely healed, the initial efficacy was determined at the date of the first visit closest to 1 year after the start of treatment. The second efficacy evaluation was conducted at the last visit or when the concomitant drug was changed. *Results:* Three patients dropped out of the study before the time of the initial efficacy evaluation. In the second efficacy evaluation, eight nails were completely healed, one nail was significantly healed, and one nail was effective. *Conclusions:* Dermatophytoma can be treated with topical onychomycotic agents in combination with surgical debridement to improve efficacy.

Keywords: Onychomycosis; Dermatophytoma; Nail abrasion; Topical antifungal drugs

Online publication: March 29, 2024

1. Introduction

Dermatophytomas are characterized by the melting of the nail plate and the formation of a cavity in which keratin, mycelium, and fungi are clustered. Clinically, the lesions are white to yellowish brown, linear or mottled, including wedge-shaped lesions [1]. The disease is resistant to treatment, with debridement as the basic treatment. The reason for resistance to treatment is thought to be that the fungus forms cavities that do not allow sufficient concentrations of drugs to reach the fungus [1], that the fungal colonies form biofilms, and that the spore-forming elements are dormant [2]. Although the treatment of tinea onychomycosis has advanced with the availability of new antifungal agents, patients may still experience refractory dermatophytoma even with these drugs. Debridement is recommended as adjunctive therapy for dermatophytoma [3], but there are few studies on the effectiveness of combined treatment with cutting in multiple cases using topical onychomycosis treatments.

2. Methods

2.1. Subjects

Twelve patients (8 males, 4 females, mean age 73 years) diagnosed with dermatophytoma who visited the Department of Dermatology, Kansai Rosai Hospital, between April 2017 and March 2020 underwent cutting treatment, and a total of 14 nails were cut. The diagnosis was confirmed by cutting the nail surface, observing the formation of a cavity in the nail surface, and confirming the presence of a fungal colony containing spore-shaped elements (**Figure 1**) by direct KOH microscopy of the internal keratinocyte.

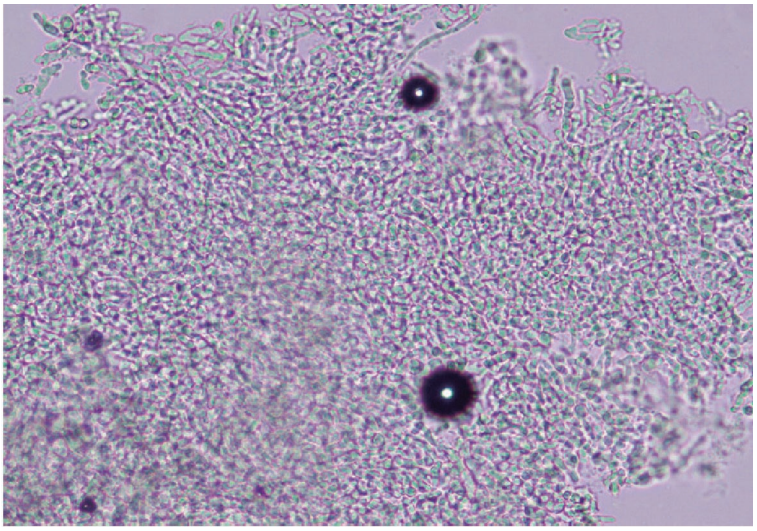


Figure 1. Direct specimen of dermatophytoma (×200) showing clumps of target nail No. 1: Short, thick mycelial and sporophytic fungal elements are clumped together

2.2. Usage of onychomycosis treatment

Topical therapy:

(1) 10% efinaconazole topical nail solution once a day

(2) 5% luliconazole topical nail solution once a day

2.3. Cutting of the affected nail bed

At the initial visit, the affected nail plate was removed as much as possible using a grinder (Urawa Industries G3, b-2, b-3), and the lesion was opened. Cutting was also performed during the course of the treatment when it was considered necessary.

Clinical efficacy was determined by taking photographs at the time of examination and quantifying the entire nail surface area and the area of the opacity using ImageJ, and using the following efficacy criteria.

2.4. Clinical efficacy criteria

(1) Ineffective: Change in the area ratio of the nail plate (opacity area of the nail plate/area of the nail plate) decreased or increased by less than 30%.

(2) Effective: Decrease of more than 30% and less than 60% in the area ratio of nail plate opacity.

(3) Markedly effective: Change in the area ratio of nail plate opacity is greater than 60% and less than 60% opacity disappearance.

(4) Healed: The nail plate opacity area disappeared and no fungal elements were observed by direct microscopic examination.

The efficacy was evaluated on the date of the visit closest to 1 year after the start of treatment and on the

date of the last visit or change of concomitant medication. If the patient was cured, the duration of the cure was also noted.

3. Results

Three patients dropped out of the study before the time of the initial efficacy evaluation. Among the ten nails, seven were completely healed and three were inoperable at the time of the initial efficacy evaluation. The results of concomitant drug treatment showed that efinaconazole cured five nails, luliconazole cured two nails and failed to cure three nails, and the second efficacy evaluation showed that eight nails were completely cured, one nail was markedly effective, and one nail was effective (**Table 1** and **Figure 2**). Photographs of representative cases are shown in **Figure 3**.

Table 1. All target nail progress

No.	Age and sex	Claw	Cultivation result	Antifungal	Evaluation of the initial clinical efficacy (period)	Progress (period)
1	77 years old male	Left big toe	T. interdigitale	EFCZ	Healed (11 months)	
2	70 years old male	Left big toe	T. interdigitale	EFCZ	Healed (5 months)	
3	52 years old female*	Left big toe	T. interdigitale	EFCZ	Hospital visits discontinued (0 months)	
4	52 years old female*	Right big toe	T. interdigitale	EFCZ	Hospital visits discontinued (0 months)	
5	71 years old female	Right big toe	Negative	EFCZ	Healed (11 months)	
6	76 years old male	Right big toe	Unassessed	EFCZ	Healed (5 months)	
7	72 years old female	Right big toe	Contamination	EFCZ	58% decrease in hospital visit discontinuation (3 months)	
8	70 years old female	Right big toe	Negative	EFCZ	Healed (8 months)	
9	89 years old male	Left big toe	T. interdigitale	LLCZ	Hospital visits discontinued (0 months)	
10	71 years old male	Right big toe	Unassessed	LLCZ	Ineffective (13 months)	Healed (22 months)
11	72 years old male**	Left big toe	Unassessed	LLCZ	Healed (6 months)	
12	72 years old male**	Right big toe	Unassessed	LLCZ	Ineffective (13 months)	Markedly effective (41 months)
13	81years old male	Right big toe	Unassessed	LLCZ	Healed (10 months)	
14	70 years old male	Right big toe	Unassessed	LLCZ	Ineffective (13 months)	Effective (32 months) then healed with EFCZ (36 months)

Abbreviation: EFCZ, efinaconazole; LLCZ, luliconazole; *,**same patient

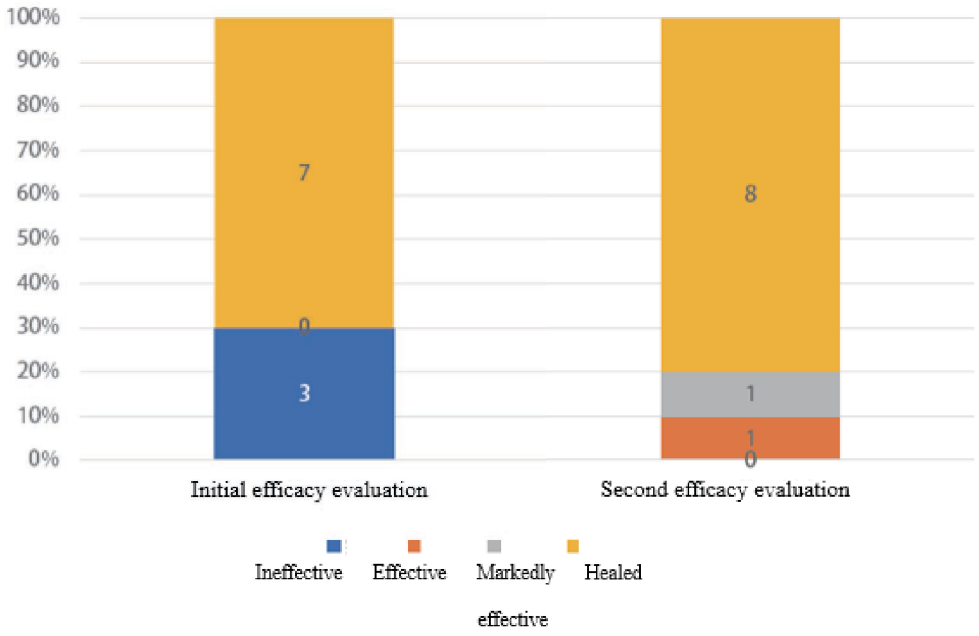


Figure 2. Target nail evaluation of first (approximately 1 year) and second efficacy evaluation (n = 10)

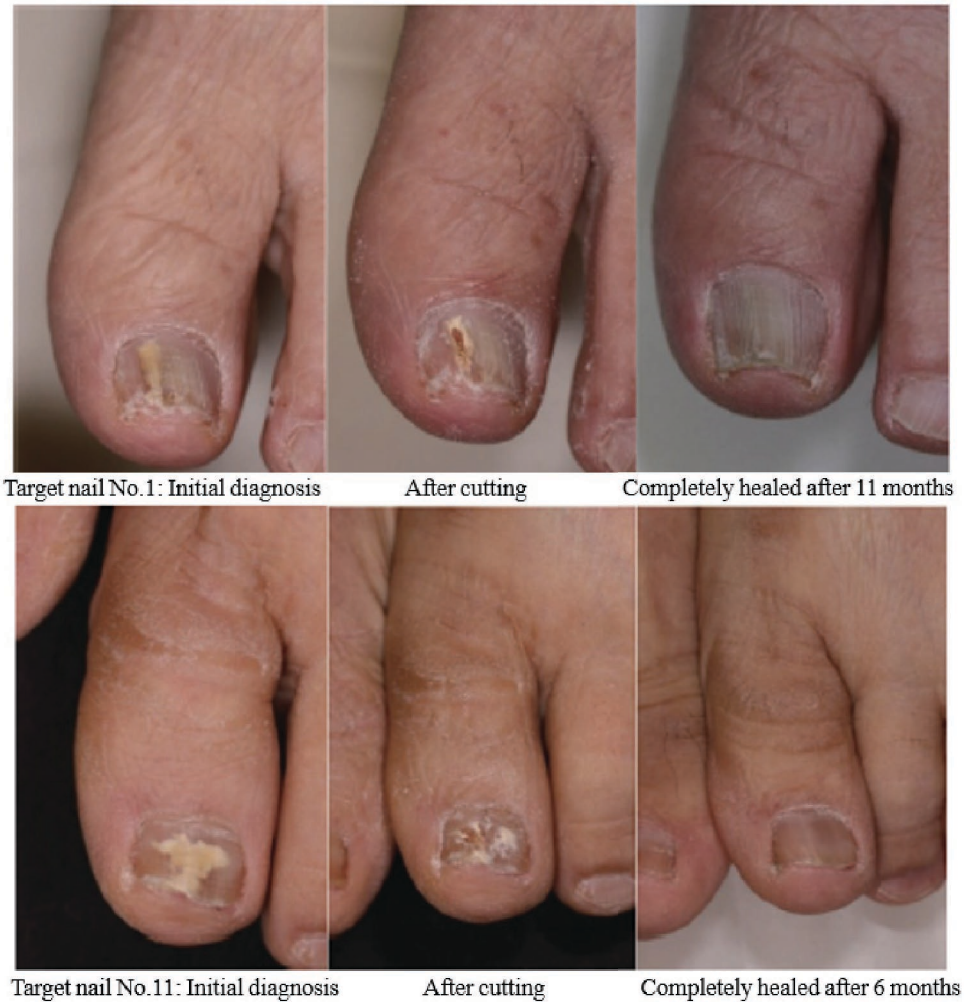


Figure 3. The clinical course of typical cases

4. Discussion

In 1998, Roberts and Evans first described dermatophytoma as a keratinous aggregate with thick walls and abnormal-looking mycelium and sporophytic elements forming clumps [1]. Clinically, it is an area of mottled or linear opacity that is clearly demarcated from the surrounding area. We consider that debridement may be necessary for antifungal drugs to be effective because they do not penetrate in sufficient concentrations. Dermatophytoma is a fungal mass that is less in contact with the nail bed than distal marginal subungual onychomycosis, and therefore, the drug does not reach the nail bed sufficiently [3]. It is also speculated that the clumps form biofilms and the spore-forming fungal elements are dormant, making them resistant to treatment [2]. A study of 199 patients treated with oral terbinafine [4] showed that dermatophytoma had a high odds ratio of not being cured (OR 3.453; 95% CI: 1.170–10.197). Thus, dermatophytoma is generally recognized as being difficult to cure, and debridement is recommended prior to treatment [5]. The purpose of debridement is to eliminate the causes of dermatophytoma refractoriness. There are few reports on whether the treatment of dermatophytoma is more effective when combined with debridement. In Japan, Ninomiya reported that nine patients with wedge-shaped dermatophytoma were completely cured by the combined use of itraconazole pulse therapy and surgical removal, with the exception of three patients who dropped out of the study [6]. In our study, all dermatophytoma lesions had disappeared by the 16th month except for the cases that had dropped out (**Figure 4**). However, there were cases in which other areas remained cloudy or worsened even after the dermatophytoma lesions disappeared, resulting in a low rate of complete healing and a time lag between the disappearance of dermatophytoma lesions and complete healing.

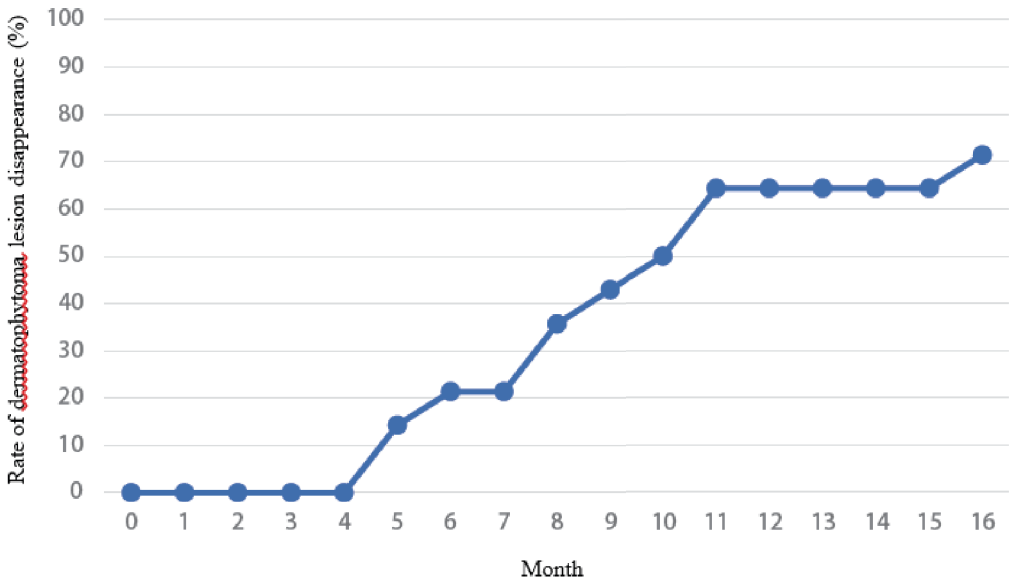


Figure 4. Change over time in the rate of dermatophytoma lesion disappearance (number of nails with dermatophytoma disappearance/total number of nails covered), n = 14

In recent years, new antifungal drugs have been launched one after another to treat onychomycosis. For example, 10% efinaconazole nail liquid for topical use in 2014, 5% luliconazole nail liquid for topical use in 2015, and oral fosravuconazole capsules in 2018 became available in Japan. Casey *et al.* reported the results of patients who used 10% efinaconazole topical solution for onychomycosis, excluding two dropout cases. It was reported that 13 of 19 patients with dermatophytoma (including one patient with two affected nails) were healed at 58 weeks (< 10% nail plate opacity and negative direct microscopy) and all dermatophytoma lesions had resolved [7]. Shimoyama *et al.* conducted a single-center, retrospective study of 109 patients with tinea onychomycosis treated with foslavuconazole. 12 of 21 patients with dermatophytoma were completely healed at

the last visit without special treatment (mean duration 34.3 ± 11.1 weeks). This is contrary to the conventional view that medical therapy without treatment for dermatophytoma is ineffective. Fosravuconazole may be effective against dermatophytosis without additional treatment.

The availability of highly effective tinea onychomycosis drugs has reduced the need for therapeutic innovations, but there is still room for innovations in the treatment of difficult-to-treat forms. The present study is a retrospective review of actual treatment and clinical practice in our department. The results showed that 50% (7/14) of the patients were completely cured in about 1 year, and 57.1% (8/14) were completely cured at the second evaluation. This indicates that the combination of cutting and topical onychomycosis treatment for dermatophytoma is as effective as oral fosravuconazole treatment without any special treatment. The efficacy of topical 10% efinaconazole solution without special treatment for longitudinal spikes, which are not completely identical to dermatophytoma but seem to overlap with the linear form of dermatophytoma for the most part, has also been reported [9]. 82 cases were studied and the spike lesions disappeared in about 80% of the patients at 72 weeks. The complete healing rate ranged from 3–40% at 48 weeks. Although not directly comparable, the complete healing rate was high in the autologous cases at the same time period, and the addition of cutting may increase the efficacy of the procedure.

There were many cases of treatment discontinuation, and one patient with two lesions did not come to the hospital after the initial treatment. We felt that adherence to treatment of tinea onychomycosis is a serious problem. In this study, we showed that the combination of cutting and topical onychomycosis treatment for dermatophytoma was effective in eradicating the lesions and was as effective as oral fosravuconazole therapy. With the availability of new antifungal agents, the effectiveness of adjuvant therapy should be reaffirmed, although the use of such therapy has become less common.

Disclosure statement

The author declares no conflict of interest.

References

[1] Roberts DT, Evans EG, 1998, Subungual Dermatophytoma Complicating Dermatophyte Onychomycosis. Br J Dermatol, 1998(138): 189–190.

[2] Craig N, Craig G, Aditya K, 2002, Dermatophytoma: Recalcitrance to Treatment Because of Existence of Fungal Biofilm. J Am Acad Dermatol, 2002(47): 629–631.

[3] Joël C, Ronald B, Aditya K, 2007, Multitherapy Approach to Onychomycosis Therapy. J Cutan Med Surg, 10(Suppl 2): S44–47.

[4] Sigurgeirsson B, 2010, Prognostic Factors for Cure Following Treatment of Onychomycosis. J Eur Acad Dermatol Venereol, 2010(24): 679–684.

[5] Sigurgeirsson B, 2018, Predicting the Outcome of Treatment: Prognostic Factors, in Rigopoulos D, Elewki B, Richert B, Onychomycosis: Diagnosis and Effective Management, 1st edition, John Wiley & Sons Ltd, Oxford, 83–102.

[6] Ninomiya J, 2006, Combination Therapy of Itraconazole Pulse Therapy, Removal of the Affected Nail Plate, and Topical Antifungal Agents for Wedge-Shaped Tinea Unguium. Skin Clinic, 2006(48): 1027–1031.

[7] Wang C, Cantrell W, Canavan T, et al., 2019, Successful Treatment of Dermatophytomas in 19 Patients Using Efinaconazole 10% Solution. Skin Appendage Disord, 5(5): 304–308.

[8] Shimoyama H, Yo A, Sei Y, et al., 2021, Treatment Outcome with Fosravuconazole for Onychomycosis. Mycopathologia, 186(2): 259–267.

[9] Watanabe S, Iozumi K, Abe M, et al., 2021, Clinical Effectiveness of Efinaconazole 10% Solution for Treatment of Onychomycosis with Longitudinal Spikes. J Dermatol, 48(10): 1474–1481.

Dermatological Health, 2024, Volume 2, Issue 1
http://ojs.bbwpublisher.com/index.php/DH

Research Progress on the Role of *Rubia cordifolia* in the Treatment of Lichen Planus

Baixue Liu[†], Yaqin Tan, Xuan Hu, Xu Gao, Youguang Ao*

College of Traditional Chinese Medicine, Inner Mongolia Medical University, Hohhot 010110, China
[†]***First author:*** Baixue Liu, lbxzgm@163.com

****Corresponding author:*** Youguang Ao, aoyouguang2008@126.com

Abstract: Lichen planus is a chronic inflammatory skin disease. Due to its unclear etiology, the treatment is slow and complicated. *Rubia cordifolia* is a drug with a long history, with the effects of cooling blood and removing blood stasis, promoting blood circulation, and dredging meridians. Modern research suggests that the components of *Rubia cordifolia* have the effects of immune regulation, anti-inflammation, liver protection, and antioxidation. It may be a potential drug for the treatment of lichen planus. However, the specific mechanism of *Rubia cordifolia* in the treatment of this disease is still under study. This article reviews the mechanism of *Rubia cordifolia* in the treatment of lichen planus, in order to provide references for the clinical application of the drug.

Keywords: *Rubia cordifolia*; Lichen planus; Immune regulation; Anti-inflammation; Liver protection; Antioxidation

Online publication: March 29, 2024

1. Introduction

Lichen planus (LP) is a chronic inflammatory skin disease of unknown cause, clinically manifested as small, discrete, irregularly shaped, flat-topped purplish-red papules, which may coalesce to form rough, scaly plaques, and are often accompanied by lesions of the oral cavity, fingernails/toenails, and genitalia. The etiology of the disease has not been clarified by modern medicine, but most scholars believe that LP is mainly a T-cell-mediated autoimmune reaction, in addition, there are other theories, such as liver function impairment and carcinoma, etc. LP has a global incidence, accounting for about 0.5–1.2% of new dermatologic cases, affecting more females than males, and is rare in children [1]. Currently, there is still no specific treatment for LP, the first choice for clinical treatment is topical corticosteroid drugs such as clobetasol and dieldrin, while oral drugs are mainly corticosteroids and retinoids. Therefore, clinical treatment of LP is characterized by long treatment, large differences in prognosis, and many adverse reactions, which bring great physical and mental harm and economic burden to patients and families. In the face of the difficulty in identifying the cause of the disease and the lack of effective treatments, TCM (traditional Chinese medicine) can recognize the pathogenesis of LP from another perspective and propose effective treatments. Chinese medicine believes that LP is mostly caused by

exogenous wind-dampness-heat-pathogen, liver and qi stagnation, and blood stasis. It is treated by the method of drying dampness and regulating qi, activating and cooling the blood, and *Rubia cordifolia* may be a potential therapeutic drug in the treatment of LP blood heat and blood stasis syndrome.

Rubia cordifolia, also known as Indian madder or blood-activating grass, is mainly produced in Anhui, Shandong, and other places; *Shennong Bencaojing* recorded "bitter flavor, cold; mainly used for treating wind-cold and dampness paralysis, jaundice, and tonifying the middle"; in *Chinese Materia Medica*, *Rubia cordifolia* can cool the blood, stop bleeding, activate blood circulation, and remove blood stasis, and it is suitable for treating vomiting and epistaxis, menstrual leakage and occlusion, bruises, rheumatism, paralysis, and pain. Modern research has concluded that *Rubia cordifolia* has immunosuppressive, leukocyte-boosting, anti-inflammatory, hepatoprotective, and antioxidant effects. In this paper, we reviewed the mechanism of *Rubia cordifolia* in the treatment of LP, with a view to providing references for the further clinical application of *Rubia cordifolia*.

2. Traditional Chinese medicine

Although there is no clear record of LP in Chinese medicine, according to clinical observation, the skin manifestation of LP is similar to the "purpura" described by Chinese medicine; and Chinese medicine believes that this kind of disease is mostly caused by exogenous wind-dampness-heat-pathogen, liver and qi stagnation, and blood stasis. Therefore, the treatment of this disease is mostly based on the drying of dampness, activating blood circulation, eliminating blood stasis, and dredging up the liver. In the *Yilin Gaicuo*, "purpura, blood stasis in the skin, treatment according to the vitiligo, the effect can be applied by hand," it can be seen that blood stasis is Qingren Wang's main understanding of the disease and thus put forward blood stasis as the treatment method. With continuous medical advancement, the knowledge and treatment of LP in Chinese medicine have also gradually improved. Xia and Wu [2] summarized the idea of treatment from the liver through clinical experience; Zhu *et al.* [3] put forward the treatment direction of "the disease is located in the liver, and its pathogen is dampness" according to the clinical observation of the onset site of LP, the color of the lesions, and analysis of the medication used. In addition, other scholars in China have also carried out detailed dialectical typing of LP, such as spleen and stomach heat type, liver stagnation and fire type, and blood stasis type, etc. [4,5], and these researches have far-reaching significance for the treatment of LP.

Rubia cordifolia is bitter and cold in nature, it can cool the blood and it has a more reliable potential therapeutic effect for LP with blood heat; *Rubia cordifolia* expels blood stasis, dredges channels, activates blood circulation, and removes blood stasis, it can be used to treat LP with blood stasis; *Rubia cordifolia* enters into the liver meridian, it can be used to carry out further clinical therapeutic research based on the idea of "treating from the liver."

3. Modern medicine

Although the etiology of LP has not been clarified by modern medicine, most scholars believe that the disease is caused by abnormal immune function and is accompanied by chronic inflammatory reactions. In addition, some scholars have found that lichen planus is related to liver function damage and cancer. In the following, we will discuss the four aspects of immune regulation, anti-inflammation, liver function regulation, and antioxidation in conjunction with the active ingredients of *Rubia cordifolia* and their effects (**Table 1**).

Table 1. Summary of active ingredients and therapeutic effects of *Rubia cordifolia*

	Active ingredients	Therapeutic effects
Immune regulation	Alizarin I Alizarin II *Rubia* diester Arborane triterpenes *Rubia cordifoli*a-containing serum	Regulation of immune function and increasing white blood cell levels
Anti-inflammation	Rubiacin *Rubia* total anthraquinone	Inhibition of some IL and TNF
Regulation of liver function	*Rubia* aqueous-methanol extract *Rubia cordifolia* essential oil Methylisorubicin	Liver protection
Antioxidation	*Rubia cordifolia* polysaccharide QC *Rubia cordifolia* polysaccharide QA2	Free radical scavenging, antioxidant

3.1. Immune regulation

LP is often accompanied by a T-cell-mediated process at the onset of the disease, with a large number of T cells infiltrating the locally diseased tissues. As the disease progresses, there is an autoimmune response with an increased number of T cells and a lowered level of B cells, which is accompanied by a decrease in the natural growth rate of the peripheral blood lymphocytes [6].

In response to this autoimmune response, *Rubia* diester, arborane triterpenes, and *Rubia cordifolia*-containing serum can effectively inhibit T cell proliferation. Yang and Liu [7] demonstrated that *Rubia* diester could reduce delayed hypersensitivity reaction and T cell proliferation by inhibiting phagocytosis of macrophages and neutrophils; He *et al.* [8] found that arborane triterpenoids could effectively inhibit the proliferation of T cells but had no effect on the proliferation of B cells when its concentration ranged from 30 to 100 μg/ml; Liu *et al.* [9] found that *Rubia cordifolia*-containing serum could significantly inhibit the expression of cell surface activation molecule CD69 and effectively downregulate the secretion of IFN-γ, IL-2, and TNF-α, and inhibit the proliferation of T cells. The immune regulation effect of *Rubia cordifolia* is also manifested in the elevation of leukocytes, alizarin I, II, and *Rubia* diester have a good effect on increasing the peripheral blood leukocyte level, as evidenced by Ma *et al.* [10]. *Rubia* diester also has an obvious effect of elevating leukocytes [11]; Song and Ding [12] gave oral administration of 2.5 mg of *Rubia* diester to each mouse, drew blood from the tail vein after drug administration to measure the leukocyte level, which was obviously elevated after 8 hours, reaching 151.9% of the control group, and then recovered to normal; when each dog was orally administered with 200 mg of *Rubia* diester, the peak was reached 18–24 hours after the drug administration, which was 196–209% of the pre-drug level. It indicates that *Rubia* diester can promote the proliferation and differentiation of hematopoietic stem cells and increase the peripheral blood leukocytes. Su and Zhou [13] obtained the water-extracted and alcohol-precipitated dry paste of *Rubia cordifolia* by extracting its active ingredients and found that the substance also had a high leukocyte-boosting effect. Therefore, *Rubia cordifolia* plays a therapeutic role in LP by regulating the immune system.

3.2. Anti-inflammation

As a chronic inflammatory skin disease, the pathogenesis of LP is characterized by the production of local cytokines that play an important role in the progression of the disease. Studies have shown that the development of LP is accompanied by elevated levels of IL-6 and granulocyte-macrophage colony-stimulating factor, and further studies have demonstrated that keratinocytes in oral LP tissues are capable of producing IFN-α, IL-6,

and TNF-α, and are positively correlated with the condition of skin lesions [14]. In recent years, it has also been found that the occurrence of LP may be related to IL-17 and IL-23 [15].

For the abnormal elevation of inflammatory mediators, *Rubia* total anthraquinone and rubiacin can effectively inhibit the release of inflammatory mediators, especially IL and TNF. Xu *et al.* [16] found experimentally that *Rubia* total anthraquinone could significantly reduce the levels of IL-1, IL-2, IL-6, and TNF in the serum of rats; Zhu and Jin [17] utilized rubiacin to interfere with lipopolysaccharide-treated mice and found that rubiacin could significantly inhibit the release of IL-1β and IL-6. The *Rubia cordifolia*-containing serum involved above can also effectively downregulate the secretion of relevant inflammatory mediators. It is thus clear that *Rubia cordifolia* can play a therapeutic role in LP by inhibiting the release of inflammatory mediators and reducing the inflammatory response.

3.3. Regulation of liver function

In recent years, some scholars have found a link between LP and liver disease, but the pathological mechanism has not yet been proven. Monk [18] suggested in 1985 that patients with LP had liver dysfunction along with the onset of the disease. In 1991, the occurrence of oral LP was first reported to be associated with HCV (hepatitis C virus) [19]. Another study showed that the risk of LP in patients with hepatitis B is twice as high compared to normal subjects [20].

It was found that *Rubia* aqueous-methanol extract, *Rubia cordifolia* essential oil, and methylisorubicin had good alleviating and therapeutic effects on liver injury. Experiments proved that oral administration of *Rubia* aqueous-methanol extract to mice had a significant alleviating effect on liver injury mediated by acetaminophen and CCl_4 [21]; Quan and Tian [22] also experimentally found that *Rubia cordifolia* essential oil significantly reduced hepatotoxicity induced by CCl_4; and Rao *et al.* [23] found that methylisorubicin had a strong therapeutic effect on liver injury induced by CCl_4 in mice. It can be seen that *Rubia cordifolia* may have a potential therapeutic role in the treatment of LP through the protection and regulation of liver function.

3.4. Antioxidation

Experiments have shown that *Rubia cordifolia* polysaccharides have significant antioxidant and free radical scavenging effects. Wang *et al.* extracted QA2 and QC from *Rubia cordifolia* and found that QA2 had a strong free radical scavenging effect with a scavenging rate of 94.59%, and the scavenging rate of QC was 93.24% [24,25]. Therefore, *Rubia cordifolia* can repair and regenerate skin and mucous membranes by scavenging free radicals and reducing oxidative damage. This effect can be applied during the recovery period of LP.

4. Conclusion

LP is a T-cell-mediated chronic inflammatory skin disease of unknown cause, which may be associated with liver function impairment, and most TCM diagnosis is based on the treatment from the liver. *Rubia cordifolia*, which enters the liver meridian, can cool the blood, dispel blood stasis, activate blood circulation, and dredge channels, and has great potential for the treatment of LP with blood heat and blood stasis. Modern medicine has proved that *Rubia cordifolia* can regulate immune function, inhibit inflammatory response, improve liver function, and reduce oxidative damage. However, the specific mechanism of action of *Rubia cordifolia* in the treatment of LP, especially from the perspective of modern medicine, is still being explored and developed.

Disclosure statement

The authors declare no conflict of interest.

References

[1] Hua H, Bai L, 2004, Research Progress on the Etiology and Pathogenesis of Lichen Planus. Shanxi Medical Journal, 2004(10): 855–857.

[2] Xia S, Wu J, 2008, Treatment of Oral Lichen Planus from Liver Theory. Shanxi Traditional Chinese Medicine, 2008(11): 14.

[3] Zhu S, Xu M, Guo D, et al., 2021, Preliminary Study of Treating Lichen Planus from 'the Location of Disease Lies in the Liver, Its Pathogen Lies in Dampness' Based on Theory of Integrated Chinese and Western Medicine. Chinese Journal of Traditional Chinese Medicine and Pharmacy, 36(03): 1573–1576.

[4] Wei J, Guo X, Mu H, et al., 2018, Study on the Correlation Between Peripheral Blood T-Lymphocyte Subpopulations and Polarization of Helper T-Lymphocyte 1/Helper T-Lymphocyte 2 in Patients with Oral Lichen Planus and Chinese Medicine Evidence. Hebei Traditional Chinese Medicine, 40(08): 1136–1141 + 1155.

[5] Zhao R, 1989, Combination of Chinese and Western Medicine in the Treatment of Oral Mucosal Lichen Planus in 50 Cases. Journal of the Fourth Military Medical University, 1989(03): 207–208.

[6] Yamamoto T, Osaki T, 1994, Cytokine Production by Keratinocytes and Mononuclear Infiltrates in Oral Lichen Planus. J Oral Pathol Med, 23(7): 309–315.

[7] Yang S, Liu F, 1996, Immunosuppressive Effects of *Rubia* Diester. Chinese Pharmacology Bulletin, 1996(05): 447.

[8] He L, Yang Z, Chen K, et al., 2002, Study on the Proliferative Response of Small Red Ginseng on Normal Human Peripheral Blood T Lymphocytes. Chinese Journal of Dermatology, 2002(02): 67.

[9] Liu X, Li P, Zhao J, et al., 2012, Effects of the Whole Formula and Split Formula of Blood-Cooling and Blood-Activating Capsule on the Proliferation, Activation and Cytokine Release of Jurkat T Lymphocytes. Chinese Journal of Experimental Formulas, 18(22): 198–202.

[10] Ma L, Sun Z, Liu Y, et al., 1979, Isolation and Observation of Properties of Rubiadic Acid Glycoside. Journal of the Academy of Military Medical Sciences of the Chinese People's Liberation Army, 1979(01): 46–53.

[11] Kang X, Fang Y, 1990, The Effects of Cycloheximide Bilipid and S-2-(3-Aminopropylamino)Ethyl Phosphorothioate on Chemiluminescence and Electron Paramagnetic Resonance in the Phagocytosis of Human Polymorphonuclear Leukocytes. Chinese Journal of Pharmacology and Toxicology, 1990(04): 251–254.

[12] Song S, Ding L, 1985, Studies on Hematopoietic Effect of Rubidate and Its Toxicity. Journal of Chinese and Western Medicine, 1985(10): 625–626 + 581.

[13] Su X, Zhou Y, 1992, Comparative Study on the Pharmacological Effects of Madder and Small Red Ginseng. Chinese Journal of Traditional Chinese Medicine, 1992(06): 377.

[14] Hua H, Bai L, 2004, Progress of Research on the Etiology and Pathogenesis of Lichen Planus. Shanxi Medical Journal, 2004(10): 855–857.

[15] Yao L, Bai L, 2014, Expression and Significance of IL-17, FoxP3 and Caspase-3 in Lichen Planus Lesions. China Contemporary Medicine, 21(05): 25–27.

[16] Xu L, Zhao S, Hu Q, et al., 2002, Effect of Total Anthraquinone of *Rubia cordifolia* L. on Adjuvant Arthritis of Rat and Its Cytokine. Journal of Weifang Medical College, 2002(01): 11–13.

[17] Zhu ZG, Jin H, 2013, Mollugin Inhibits the Inflammatory Response in Lipopolysaccharide-Stimulated RAW264.7 Macrophages by Blocking the Janus Kinase-Signal Transducers and Activators of Transcription Signaling Pathway. Biol Pharm Bull, 36(3): 399–406.

[18] Monk B, 1985, Lichen Planus and the Liver. J Am Acad Dermatol, 12(1 Pt 1): 122–124.

[19] Mokni M, Rybojad M, 1991, Lichen Planus and Hepatitis C Virus. J Am Acad Dermatol, 24(5 Pt 1): 792.

[20] Scelza G, Amato A, 2022, Effect of Hepatitis C Antiviral Therapy on Oral Lichen Planus and Hyposalivation in Inmates. Ann Gastroenterol, 35(1): 74–79.

[21] Sun B, 1996, Effect of *Rubia* Extract on Hepatotoxicity Caused by Carbon Tetrachloride and Acetaminophen. Overseas Medicine (Chinese Medicine and Traditional Chinese Medicine), 1996(05): 45.

[22] Quan M, Tian C, 2015, Hepatoprotective Effect of Essential Oil of *Rubia cordifolia*. Modern Food Science and Technology, 31(05): 12–17.

[23] Rao GMM, Rao CV, Pushpangadan P, et al., 2006, Hepatoprotective Effects of Rubiadin, a Major Constituent of *Rubia cordifolia* Linn. J Ethnopharmacol, 103(3): 484–490.

[24] Wang H, Wang B, 1998, Isolation, Purification and Structural Analysis of Polysaccharide QA2 from India Madder (Rubia cordifolia). Chinese Herbal Medicine, 1998(04): 219–221.

[25] Wang H, Wang B, Ma B, et al., 1998, Isolation, Purification and Structure Investigation of Glycoprotein QC from *Rubia cordifolia* L. Journal of the Academy of Military Medical Sciences, 1998(04): 38–41.

Dermatological Health, 2024, Volume 2, Issue 1
http://ojs.bbwpublisher.com/index.php/DH

Therapeutic Effect of Aminolevulinic Acid Photodynamic Two-Step Irradiation on Patients with Condyloma Acuminatum

Wenbin Yang, Li Li*

Department of Dermatology, Xi'an No.3 Hospital, the Affiliated Hospital of Northwest University, Xi'an 710018, Shaanxi Province, China

***Corresponding author:** Li Li, 570465937@qq.com

Abstract: *Objective:* To investigate the therapeutic effect of aminolevulinic acid photodynamic two-step irradiation on pain in patients with intraluminal condyloma acuminatum. *Methods:* 60 patients with intraluminal condyloma acuminatum treated with aminolevulinic acid photodynamic therapy (ALA-PDT) in the dermatology department of Xi'an Third Hospital from May 2019 to September 2021 were selected and randomly divided into the experimental group and the control group. The experimental group received aminolevulinic acid photodynamic two-step irradiation treatment while the control group was treated with conventional photodynamic irradiation. The experimental group and the control group were treated once every 7–10 days for four consecutive times. The treatment effect and pain score were recorded. *Results:* The efficacy of aminolevulinic acid photodynamic therapy was observed two weeks after the end of the treatment. The complete response rate of the experimental group was 66.7% (20/30), the partial response rate was 33.3% (10/30), and the ineffective rate was 0. In the control group, the complete response rate was 70% (21/30), the partial response rate was 30% (9/30), and the ineffective rate was 0. There was no significant difference in the complete response rate between the experimental group and the control group ($\chi^2 = 0.527$, $P = 0.706$). Pain scores were evaluated at 5 minutes and 20 minutes during photodynamic therapy, and 30 minutes after photodynamic therapy. The pain scores of patients in the experimental group were lower than those in the control group at 5 minutes, 20 minutes, and 30 minutes after treatment. There was no significant difference in adverse reactions between the experimental group and the control group. *Conclusion:* Aminolevulinic acid photodynamic two-step irradiation can effectively ensure the therapeutic effect of patients with intraluminal condyloma acuminatum, and can significantly reduce patients' pain during and after treatment.

Keywords: Aminolevulinic acid; Photodynamic; Two-step irradiation; Condyloma acuminatum; Pain

Online publication: March 29, 2024

1. Introduction

At present, aminolevulinic acid photodynamic therapy for the treatment of condyloma acuminatum has been a preferred treatment option with evidence-based medical evidence [1], especially for intraluminal (vaginal,

cervical, anal canal, urethral) infections of condyloma acuminatum, which not only reduces the local tissue damage but also effectively lowers the recurrence. However, conventional aminolevulinic acid photodynamic treatment may cause patients to suffer significant pain, or even be unable to tolerate the pain and fail to complete the treatment course, which seriously affects the application of photodynamic therapy in the treatment of condyloma acuminatum. Currently, some studies have found that the two-step method of aminolevulinic acid photodynamic therapy can effectively relieve patients' pain while guaranteeing therapeutic efficacy [2]. In this study, the aminolevulinic acid photodynamic two-step irradiation method is used to treat patients with intraluminal condyloma acuminatum, in order to clarify the relationship between the specific treatment parameters of the aminolevulinic acid photodynamic two-step irradiation method for intraluminal condyloma acuminatum and the clinical effect and pain.

2. Subjects and methods

2.1. Subjects

60 patients with intraluminal condyloma acuminatum treated in the Department of Dermatology of the Third Hospital of Xi'an City between May 2019 and September 2021 were selected. Inclusion criteria: cases of genital (urethra, cervix, vagina) and intraluminal condyloma acuminatum diagnosed by two dermatologists, and the diagnosis was confirmed by colposcopy, anoscopy, and acetic acid test; detailed records of disease duration, size, number, and distribution of lesions; inability or unwillingness to continue surgical, cryotherapy, laser, and other treatments. Exclusion criteria: patients with severe heart disease, liver disease, kidney disease, and other serious chronic diseases who cannot tolerate treatment; pregnant or breastfeeding women; patients with contraindications to the test drug or drug allergies; patients who have participated in other clinical trials in the last three months; patients who do not have the legal capacity or whose legal capacity is restricted; and any other conditions that are considered by the investigator to be unsuitable for participation in this trial. Patients who participated in this research trial had the right to be informed and were enrolled only after signing a protocol.

The patients were randomly and equally divided into the experimental group and the control group. The experimental group was treated with the aminolevulinic acid photodynamic two-step irradiation method while the control group was treated with the conventional photodynamic injection method. In the experimental group, there were five male patients (3 cases of urethral condyloma acuminatum, 2 cases of condyloma acuminatum in the anal canal) and 25 female patients (8 cases of cervical condyloma acuminatum, 10 cases of vaginal condyloma acuminatum, 4 cases of cervical and vaginal condyloma acuminatum, 2 cases of urethral condyloma acuminatum, and 1 case of condyloma acuminatum in the anal canal), with the age of 19–46 (32.45 ± 4.15) years old. In the control group, there were six male patients (4 cases of urethral condyloma acuminatum, 2 cases of condyloma acuminatum in anal canal) and 24 female patients (6 cases of cervical condyloma acuminatum, 13 cases of vaginal condyloma acuminatum, 3 cases of cervical and vaginal condyloma acuminatum, 2 cases of urethral condyloma acuminatum), with the age of 20–45 (31.65 ± 4.68) years old. There was no statistically significant difference in gender and age between the two groups of patients.

2.2. Methods

The vaginal and anal secretions were cleaned, the wart site and the number of warts were accurately determined through colposcopy and anoscopy, and image acquisition of the lesion site and calculation of the area of warts were performed (1 drug for every 3cm^2 lesion range). The topical aminolevulinic acid hydrochloride reagent (Fudan Zhangjiang) was configured into a 20% aminolevulinic acid hydrochloride solution, which was applied

to the warts locally with cotton wool and dressed and fixed. It was left for 3 hours to allow absorption, and the patient was asked to reduce his activity. Subsequently, the treatment site was identified again, the photodynamic therapy instrument fiber optic was used in the irradiation of the warts. The experimental group received aminolevulinic acid photodynamic two-step irradiation treatment: (1) the initial treatment power density was 50 mW/cm^2, irradiation time was 8 minutes, and the therapeutic energy reached 24 J/cm^2; (2) after the power density was adjusted to 80–100 mW/cm^2, irradiation was done for 15 minutes, and the total therapeutic energy reached 100 J/cm^2. The control group was treated with the conventional photodynamic irradiation treatment: power density 80–100 mW/cm^2, irradiation for 20 minutes, and total therapeutic energy 100 J/cm^2. Both the experimental group and the control group were treated once at an interval of 7–10 days, and continuously treated four times.

2.3. Observation indexes

(1) Efficacy indicators: Complete response (CR) is the complete disappearance of skin lesions and negative acetic acid test; partial response (PR) is the disappearance of more than 50% of warts (number or area); null response (NR) is the failure of treatment. Efficacy determination was performed and recorded by a dermatologist who was not involved in the clinical treatment. Patients were rechecked 2 weeks after the four photodynamic treatments for wart resolution.

(2) Pain score: The numeric rating scale (NRS) pain score was adopted. The specific method is as follows. A straight line was divided into 10 equal parts, each point was labeled with the number 0–10 points to indicate the degree of pain in order to assess the pain; 0 points for no pain, 10 points for severe pain; the patient was instructed to mark the number that best represents the degree of treatment pain. 0: no pain; 1–3: mild pain; 4–6 moderate pain; 7–10 severe pain. The pain scores of the two groups of patients were evaluated during photodynamic therapy for 5 minutes and 20 minutes, and 30 minutes after the end of photodynamic therapy, respectively.

(3) Observation of adverse reactions at the treatment site: Patients were observed for the presence of blisters, vesicles, exudation, and other adverse reactions at the photodynamic therapy site three days after the end of treatment.

2.4. Statistical methods

SPSS20.0 statistical software was applied to analyze the data, χ^2 test was used to compare the experimental group and the control group; the measurement data conforming to the normal distribution was expressed as mean ± standard deviation (SD), and the independent sample *t*-test was used to compare between the groups, and the difference of $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Comparison of the therapeutic efficacy between the two groups

The therapeutic efficacy was observed 2 weeks after the end of photodynamic therapy with aminolevulinic acid. As shown in **Table 1**, the complete response rate of the experimental group was 66.7% (20/30), the partial response rate was 33.3% (10/30), and the null response rate was 0. In the control group, the complete response rate was 70% (21/30), the partial response rate was 30% (9/30), and the null response rate was 0. There was no significant difference in the complete response rate of the experimental group compared with that of the control group ($\chi^2 = 0.527$, $P = 0.706$).

Table 1. Comparison of the therapeutic efficacy of aminolevulinic acid photodynamic therapy (cases)

Group	Cases	Complete response (CR)	Partial response (PR)	Null response (NR)
Experimental group	30	20	10	0
Control group	30	21	9	0
χ^2	-	0.527	-	-
P	-	0.706	-	-

3.2. Comparison of pain scores between the two groups

Pain scores were assessed during treatment at 5 minutes and 20 minutes, and 30 minutes after the end of photodynamic therapy, respectively. The pain scores of patients in the experimental group at 5 minutes and 20 minutes, and 30 minutes after the end of treatment were lower than those of patients in the control group (**Table 2**).

Table 2. Comparison of pain scores in aminolevulinic acid photodynamic therapy

Group	Cases	5 minutes	20 minutes	30 minutes after treatment
Experimental group	30	5.23 ± 0.62	4.03 ± 0.25	1.13 ± 0.16
Control group	30	7.67 ± 2.15	4.53 ± 0.38	2.67 ± 0.19
t	-	6.423	6.135	53.308
P	-	< 0.001	< 0.01	< 0.001

3.3. Comparison of adverse reactions between the two groups

The adverse reactions of patients' treatment sites were observed three days after the end of treatment. There was one case of localized erosion (male urethra) in the experimental group and one case of localized erosion and exudation (male urethra) in the control group. There was no significant difference in adverse reactions between the experimental group and the control group.

4. Discussion

Condyloma acuminatum has a high incidence rate among clinical sexually transmitted diseases, and the traditional methods of laser, freezing, surgery, and topical medication have a relatively long treatment cycle and a high recurrence rate, which puts great pressure on patients' lives. At present, aminolevulinic acid photodynamic therapy for condyloma acuminatum has become the first-line clinical treatment program [1], offering many advantages for the treatment of condyloma acuminatum in the cavity (vagina, urethra, cervical orifice, and in the anal canal). Photodynamic therapy can not only remove warts with little damage to local tissues, but also has a good therapeutic effect on latent human papillomavirus infections and subclinical infections, and significantly reduces the recurrence rate compared with other treatments [3]. However, the conventional photodynamic treatment method causes pain in the local skin and mucous membrane during the treatment and even leads to discontinuation of the photodynamic therapy program for some patients, seriously affecting the therapeutic effect in the patients and limiting the application of aminolevulinic acid photodynamic therapy in the treatment of condyloma acuminatum.

The photodynamic effect is a photochemical reaction that includes photosensitive molecules, light sources with photosensitized wavelengths, and tissue oxygen [4]. Tissue hypoxia and singlet oxygen generation are the causes of oxidative damage and cell death due to the photodynamic effect, while its singlet oxygen downstream

products can attack the photosensitizer to make it ineffective (i.e. photobleaching effect). Foreign studies have found that the therapeutic efficacy of aminolevulinic acid photodynamic therapy is mainly related to the total energy of phototherapy [5] and not directly related to the intensity of light [6], and photodynamic such as controlling the total energy of light to reach a certain total amount of treatment can guarantee the therapeutic effect. Some other studies have shown that the pain caused by photodynamic therapy is related to light intensity, and reducing the intensity of phototherapy can significantly reduce the degree of pain in the patients receiving photodynamic therapy [7]. Through this finding, domestic and foreign researchers found that the aminolevulinic acid photodynamic two-step irradiation method can effectively relieve patients' pain in photodynamic therapy [8]. In this study, on the basis of the theory of the two-step photodynamic method of aminolevulinic acid, it was found that the complete response rate of the experimental group was 66.7% (20/30), the partial response rate was 33.3% (10/30), and the null response rate was 0. The complete response rate of the control group was 70% (21/30), the partial response rate was 30% (9/30), and the null response rate was 0. The difference in complete response rate between the experimental and control groups was not statistically significant ($\chi^2 = 0.527$, $P = 0.706$). This suggests that there is no significant difference in the efficacy of the aminolevulinic acid photodynamic two-step irradiation method compared to conventional photodynamic therapy. We maintained the total phototherapy energy at 100 J/cm^2 and reduced the initial phototherapy intensity to 50 mW/cm^2, which not only effectively relieved patients' pain during treatment at the initial stage of photodynamic therapy, but also significantly relieved pain after increasing the phototherapy intensity in the latter course of treatment and 30 minutes after the end of treatment. It is believed that the slow photobleaching effect of the photosensitizer can reduce the production of singlet oxygen in the tissues and the oxidative damage, thus alleviating the local pain, without significantly aggravating the pain after increasing the intensity of phototherapy in the latter course of the treatment. The specific mechanism of this phenomenon is still to be further investigated.

5. Conclusion

In summary, compared with conventional photodynamic therapy, the aminolevulinic acid photodynamic two-step irradiation method can significantly alleviate the patient's pain during and after treatment, and did not have a negative impact on the effect of photodynamic therapy. In the post-treatment observation, it was found that the photodynamic two-step irradiation treatment did not increase the risk of producing erosion, exudation, blisters, and other adverse reactions. Therefore, the aminolevulinic acid photodynamic two-step irradiation method can improve patient compliance and reduce patient pain, which is worth promoting in clinical treatment.

Disclosure statement

The authors declare no conflict of interest.

References

[1] Morton CA, Szeimies RM, Sidoroff A, et al., 2013, European Guidelines for Topical Photodynamic Therapy Part 2: Emerging Indications—Field Cancerization, Photorejuvenation and Inflammatory/Infective Dermatoses. J Eur Acad Dermatol Venereol, 27(6): 672–679.

[2] Yu H, Ma H, 2021, Application of Pain Management in Patients with Condyloma Acuminatum Treated by Aminolevulinic Acid Photodynamic Therapy. International Journal of Medicine and Health, 27(15): 2396–2399.

http://doi.org/10.3760/cma.j.issn.1007-1245.2021.15.047

[3] Wang XL, Wang HW, Wang HS, et al., 2004, Topical 5-Aminolaevulinic Acid-Photodynamic Therapy for the Treatment of Urethral Condylomata Acuminata. Br J Dermatol, 151(4): 880–885.

[4] Photodynamic Therapy Research Center of Dermatology and Venereology Branch of Chinese Medical Association, 2015, Expert Consensus on the Clinical Application of Aminolevulinic Acid Photodynamic Therapy. Chinese Journal of Dermatology, 48(10): 675–678.

[5] Novak B, Heesen L, Schary N, et al., 2018, The Influence of Different Illumination Parameters on Protoporphyrin IX Induced Cell Death in Squamous Cell Carcinoma Cells. Photodiagnosis Photodyn Ther, 2018(21): 385–392. http://doi.org/10.1016/j.pdpdt.2018.02.007

[6] Chaves YN, Torezan LA, Niwa AB, et al., 2012, Pain in Photodynamic Therapy: Mechanisms of Action and Management Strategies. An Bras Dermatol, 87(4): 521–526. http://doi.org/10.1590/s0365-05962012000400001

[7] Apalla Z, Sotiriou E, Panagiotidou D, et al., 2011, Effect of Different Fluences on Pain and Clinical Outcome in Patients with Actinic Keratoses Treated with Photodynamic Therapy. Photodermatol Photoimmunol Photomed, 27(4): 181–185. http://doi.org/10.1111/j.1600-0781.2011.00595.x

[8] Chen J, Zhou F, Chen S, et al., 2020, Effect of Two-Step Irradiance Schedule on Pain Control During and Clinical Efficacy of Aminolevulinic Acid-Based Photodynamic Therapy for Moderate-to-Severe Acne. Chinese Journal of Dermatology, 53(11): 900–904. http://doi.org/10.35541/cjd.20200088

Dermatological Health, 2024, Volume 2, Issue 1
http://ojs.bbwpublisher.com/index.php/DH

Analysis of the Efficacy of Intense Pulsed Light Combined with Doxycycline in the Treatment of Rosacea

Zhengfang Sun*, Xinhui Wang, Dongxia Fu

Tangchang County People's Hospital, Longnan 748500, Gansu Province, China

Corresponding author: Zhengfang Sun, sun6226361@sina.com

Abstract: *Objective:* To investigate the clinical efficacy of intense pulsed light combined with doxycycline in the treatment of rosacea. *Methods:* A sample of 60 patients with rosacea admitted to our hospital from January 2021 to December 2023 were selected and grouped into the observation group (n = 30) and the control group (n = 30) by using the randomized numerical table sampling method. The patients in the control group were treated with doxycycline, and the patients in the observation group were treated with intense pulsed light combined with doxycycline. The clinical effective rate and recurrence rate, clinical symptom score, skin barrier function indexes, and the incidence of adverse reactions in the two groups were compared. *Results:* The clinical effective rate of the observation group was higher than that of the control group, and the recurrence rate was lower than that of the control group ($P < 0.05$); the clinical symptom score of the observation group was lower than that of the control group after treatment ($P < 0.05$); the water content of the stratum corneum of the observation group was higher than that of the control group after treatment, and the amount of transepidermal water loss was lower than that of the control group ($P < 0.05$); the incidence of adverse reactions of the two groups did not have any significant difference in comparison ($P > 0.05$). *Conclusion:* The treatment effect of intense pulsed light combined with doxycycline in rosacea patients is remarkable. It can alleviate clinical symptoms and improve the skin barrier function, with a lower recurrence rate after treatment and higher therapeutic safety, which is suitable for popularization and use in healthcare institutions.

Keywords: Intense pulsed light; Doxycycline; Rosacea

Online publication: March 29, 2024

1. Introduction

Rosacea is a common chronic inflammatory skin disease, the causes of which include damage to the skin barrier function, inflammatory response, genetics, etc. The lesions can affect the facial blood vessels, follicles, and sebaceous glands around the unit, and the affected site is mainly in the middle of the face, and it mainly manifests as skin erythema, papules, pustules, capillary dilatation, etc. The lesions worsen with the prolongation of the disease, which can seriously affect the aesthetic of the facial area [1,2]. Clinical treatment

of rosacea includes drug therapy, physical therapy, etc. Doxycycline is a tetracycline antibacterial drug, which can kill follicular helminth mites and other pathogenic bacteria, thereby alleviating inflammatory reactions and infections, and reducing local symptoms [3]. Intense pulsed light is a physical therapy that can damage dilated capillaries and repair the damaged skin barrier [4]. In this study, a sample of 60 patients with rosacea was selected to investigate the clinical efficacy of intense pulsed light combined with doxycycline treatment.

2. General information and methods

2.1. General information

A sample of 60 patients with rosacea admitted to our hospital from January 2021 to December 2023 were selected and grouped into the observation group (n = 30) and the control group (n = 30) by using the randomized numerical table sampling method. There were 9 males and 21 females in the observation group, with an age range of 25–44 (34.58 ± 3.59) years and a disease duration of 5–16 (10.48 ± 2.06) months. In the control group, there were 10 males and 20 females, the age range was 27–41 (34.65 ± 3.52) years old and the disease duration was 3–17 (10.52 ± 2.09) months. The difference in the results of comparing the general information of patients in the two groups was not statistically significant ($P > 0.05$).

Inclusion criteria: (1) Meet the diagnostic criteria of papulopustular rosacea in the "Chinese Rosacea Diagnosis and Treatment Expert Consensus"; (2) No other skin diseases; (3) Sign the informed consent form. Exclusion criteria: (1) Keloid; (2) Recently received rosacea therapeutic intervention; (3) Combined with skin breakdown or facial infectious skin diseases.

2.2. Methods

Patients in the control group were treated with oral doxycycline, which was taken orally twice a day, with a single dose of 100 mg, for a total duration of 8 weeks.

Based on the control group's treatment program, patients in the observation group also received intense pulsed light (IPL) therapy, selecting patients to complete one treatment at the end of the 2nd week of oral doxycycline, at the end of the 5th week, and at the end of the 8th week. The treatment was completed using our IPL therapy device, selecting the acne treatment mode, choosing an energy range of 12–18 J, and performing a spot test before the first treatment to select the appropriate energy parameters. Before treatment, the patient's face was cleaned, no anesthesia was required, and both the physician and the patient wore goggles. An appropriate amount of gel was evenly applied to the treatment area, and the treatment was completed according to the site of the rosacea. If the treatment area produced light to medium red spots, it was considered to be the endpoint of the treatment, and if the patient did not reach the endpoint of the treatment, then the treatment was repeated. After the completion of the treatment, the patient's face was cleaned in a timely manner, and a collagen mask was applied coldly for 30 minutes. The physician informed the patient that the face could not be washed until 24 hours after the treatment and that cosmetics were prohibited for 48 hours. The patient was advised to pay attention to sunscreen in his daily life and avoid using skin-care products containing allergenic antiseptics, fragrances, and alcohols, as well as cleansing devices, and exfoliating scrubs. A too-hot diet was also avoided, patients were to abstain from smoking and alcohol, and avoid going to the salon to treat the disease.

2.3. Evaluation criteria

(1) The clinical efficiency of the two groups was evaluated after 8 weeks of treatment, if the symptoms of erythema, papule, and pustule disappear, and the skin lesions are completely repaired, then it is cured;

if the symptoms of erythema, papule, and pustule disappear, and the skin lesions improve, then it is effective; if it does not meet the criteria of cured and effective, it is ineffective; and the sum of the percentage of the patients who are cured and effective is the clinical efficiency. After the treatment, the two groups of patients were followed up for 3 months, and the recurrence rate was counted.

(2) Before treatment and after 8 weeks of treatment, the erythema, papule, pustule, and capillary dilatation symptom scores of the two groups were evaluated with reference to the criteria of the Chinese Rosacea Expert Consensus, and the scores were 0–4, with higher scores indicating more severe symptoms.

(3) The skin barrier function indexes of the two groups were evaluated before treatment and after 8 weeks of treatment. The water content of the stratum corneum was measured by using a skin moisture tester, and the amount of transdermal water loss was measured by using a Vapometer.

(4) The incidence of adverse reactions in the two groups was recorded.

2.4. Statistical methods

SPSS23.0 software was used to analyze the research data, the measurement data [mean ± standard deviation (SD)] used *t*-test, and the count data % used χ^2 test. $P < 0.05$ was considered to have statistically significant differences.

3. Results

3.1. Clinical effective rate and recurrence rate of the two groups

The clinical effective rate of the observation group was higher than that of the control group, and the recurrence rate was lower than that of the control group ($P < 0.05$), as shown in **Table 1**.

Table 1. Clinical effective rate and recurrence rate of the two groups [n (%)]

Groups	Cured	Effective	Ineffective	Total effectiveness	Recurrence rate
Observation group (n = 30)	21	8	1	29 (96.70)	2 (6.70)
Control group (n = 30)	15	7	8	22 (73.30)	8 (26.70)
χ^2	-	-	-	6.405	4.320
P	-	-	-	0.011	0.037

3.2. Clinical symptom scores of the two groups

The clinical symptom score of the observation group was lower than that of the control group after treatment ($P < 0.05$), as presented in **Table 2**.

Table 2. Clinical symptom scores of the two groups (mean ± SD)

Groups	Erythema		Papules		Pustules		Capillary dilatation	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Observation group (n = 30)	3.08 ± 0.45	0.82 ± 0.14	3.12 ± 0.48	0.77 ± 0.12	3.05 ± 0.64	0.83 ± 0.15	2.96 ± 0.51	0.79 ± 0.12
Control group (n = 30)	3.11 ± 0.43	1.79 ± 0.36	3.09 ± 0.53	1.25 ± 0.41	3.09 ± 0.57	1.67 ± 0.44	2.88 ± 0.54	1.49 ± 0.38
t	0.264	13.755	0.230	6.154	0.256	9.897	0.590	9.621
P	0.793	0.000	0.819	0.000	0.799	0.000	0.558	0.000

3.3. Skin barrier function indexes of the two groups

The water content of the stratum corneum of the observation group was higher than that of the control group after treatment, and the amount of transepidermal water loss was lower than that of the control group ($P < 0.05$), as demonstrated in **Table 3**.

Table 3. Skin barrier function indexes of the two groups (mean ± SD, g/h/cm^2)

Groups	Water content of the stratum corneum		Transepidermal water loss	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Observation group (n = 30)	46.81 ± 4.58	60.12 ± 5.38	27.94 ± 4.33	18.12 ± 2.06
Control group (n = 30)	46.77 ± 4.64	53.76 ± 3.19	27.89 ± 4.29	22.15 ± 3.82
t	0.034	5.569	0.045	5.086
P	0.973	0.000	0.964	0.000

3.4. Incidence of adverse reactions in the two groups

No serious adverse reactions occurred during the treatment of the two groups of patients. In the observation group, one case had obvious pain during intense pulsed light treatment, and the symptoms disappeared without therapeutic intervention. Two cases in the control group experienced stomach pain during doxycycline administration, and the symptoms disappeared after stopping the drug, and there was no significant difference between the results of the two groups ($P > 0.05$).

4. Discussion

Rosacea is a common chronic inflammatory lesion of skin tissue and its pathogenesis is related to inflammatory response. The main clinical symptoms of patients are erythema, papules, pustules, capillary dilatation, etc., which can affect facial aesthetics, and therefore it is necessary to take an early and effective therapeutic intervention to control the progress of the disease [5,6].

The results of this study confirmed that the clinical effective rate of patients in the observation group was higher than that of the control group, and the recurrence rate was lower than that of the control group, suggesting that the treatment of rosacea patients with intense pulsed light combined with doxycycline was effective with low recurrence rate. Analyzing the specific reasons, the pathogenesis of rosacea correlates with infection and inflammatory response, so it requires anti-inflammatory and anti-infective drugs. Doxycycline is the preferred oral treatment drug for rosacea recommended in the relevant guidelines, which is classified as a tetracycline antibiotic, and its components can bind to the A region of the 30S subunit of the bacterial ribosome, inhibit the bacterial protein synthesis and peptide chain growth, and kill follicle mites, thereby alleviating the local infections and inflammatory reactions [7]. Pharmacological treatment alone has a slow onset of action and is ineffective in some severely ill patients. Intense pulsed light is a physical treatment in which the use of intense photon irradiation of the lesion area during treatment can induce a chemical reaction in the collagen fibers in the dermis, thus achieving the role of cosmetic spot removal [8]. At the same time, intense pulsed light therapy can gather energy in a smaller area, and then directly act on lesions such as erythema, papules, and pustules, gradually restoring the barrier function of the skin tissue [9]. Intense pulsed light combined with doxycycline in the treatment of rosacea can realize the synergistic effect between the two regimens, synchronously complete the intervention of anti-infection and local lesions, and can promote the reconstruction of the skin barrier, significantly reducing the recurrence rate, and its application value is significantly better than the single oral

doxycycline treatment [10].

The results of this study showed that the clinical symptom scores of the observation group were lower than those of the control group after treatment, suggesting that intense pulsed light combined with doxycycline treatment can reduce multiple symptoms. Infection and local inflammatory reactions are important factors in the pathogenesis of rosacea, while oral doxycycline can kill pathogenic microorganisms, long-term use of medication easily leads to drug resistance and the treatment efficacy in some patients with severe conditions is poor [11]. In intense pulsed light treatment process, photothermal effect and heat conduction can destroy the dilated capillaries and can act on the sebaceous glands, significantly decreasing the total amount of sebum secretion and reducing the symptoms of pustules. The thermal effect produced during the light can kill local pathogens, exert anti-inflammatory effects, and repair the skin barrier function, which can reduce the symptoms of pustules and erythema [12,13].

This study confirmed that the water content of the stratum corneum of the observation group was higher than that of the control group after treatment, and the amount of transepidermal water loss was lower than that of the control group, suggesting that intense pulsed light combined with doxycycline treatment can improve the skin barrier function. The skin barrier function of rosacea patients is impaired and external stimuli invade the dermis, leading to aggravation of the condition. Doxycycline is an antimicrobial drug that can kill pathogens, but has limited effect on the improvement of skin barrier function. Intense pulsed light therapy can produce selective photothermal effects, destroy dilated capillaries, and remodel collagen, regulate collagen fiber disorders, restore the elasticity of skin tissues, and repair the skin barrier function [14].

In this study, there was no significant difference in the incidence of adverse reactions between the two groups. Compared with other tetracycline antibiotics, doxycycline has a higher medication safety and a slight effect on liver and kidney function. During intense pulsed light treatment, physicians can adjust the energy parameters according to the patient's condition, and the energy is focused on the lesion site, which can avoid damaging healthy skin tissues and have high therapeutic safety [15].

5. Conclusion

In conclusion, it can be seen that the treatment effect of intense pulsed light combined with doxycycline in rosacea patients is remarkable, which can alleviate the clinical symptoms and improve the skin barrier function, with a lower recurrence rate after treatment and higher therapeutic safety, which is suitable for popularization and use in healthcare institutions.

Disclosure statement

The authors declare no conflict of interest.

References

[1] Huo YP, Zhang ZY, Li DJ, et al., 2023, Clinical Effect Observation of Doxycycline Combined with Red and Blue Light Irradiation in the Treatment of Rosacea. China Health Standard Management, 14(8): 193–198.

[2] Liu Y, Wang X, Liu C, et al., 2023, Clinical Efficacy Observation of Doxycycline Hydrochloride Sequential Isotretinoin in the Treatment of Papulopustular Rosacea. Chinese Journal of Integrative Dermatology and Venereology, 22(6): 539–542.

[3] Dou X, Sheng P, Liu H, 2022, The Efficacy of Doxycycline Combined with 30% Supramolecular Salicylic Acid and

Fire Needle in the Treatment of Papulopustular Rosacea. China Medical Aesthetics, 12(4): 24–30.

[4] Chen H, Li W, Ma L, 2023, Clinical Analysis of Gold Microneedle Radiofrequency Combined with Doxycycline and Metronidazole Gel in the Treatment of Papulopustular Rosacea. Systemic Medicine, 8(21): 55–58.

[5] Bu Q, Xu S, Zhang F, et al., 2021, Observation on the Efficacy of Supramolecular Salicylic Acid Combined with Doxycycline in the Treatment of Papulopustular Rosacea. Journal of Hubei Medical College, 40(6): 620–624.

[6] Xie L, Peng H, Li L, et al., 2021, Clinical Efficacy of Hydroxychloroquine Sulfate and Doxycycline Combined with 0.1% Tacrolimus Ointment in the Treatment of Rosacea. Journal of Gannan Medical College, 41(1): 65–67, 77.

[7] Qian JL, Liu JL, He JY, et al., 2023, Comparison of the Effects of Two Types of Intense Pulsed Light in the Treatment of Erythematous Capillary-Dilated Rosacea. China Aesthetic Medicine, 32(7): 110–113.

[8] Yang S, Wan H, He G, 2023, Intense Pulsed Light Combined with Botulinum Toxin Type A in the Treatment of Rosacea. China Aesthetic Medicine, 32(5): 108–111.

[9] Lai M, Yang M, Qi N, et al., 2024, Clinical Efficacy Study of Detoxification and Penetration Formula Combined with Dual-Wavelength Intense Pulsed Light in the Treatment of Rosacea with Heatiness of Lung and Stomach. Zhejiang Journal of Integrative Medicine, 34(1): 55–57.

[10] Han J, Zi S, Liu A, 2023, Efficacy and Safety Analysis of Compound Acid-Assisted Intense Pulsed Light Therapy for Rosacea. China Aesthetic Medicine, 32(7): 118–121.

[11] Yan Y, 2023, Effect of Dual-Wavelength Intense Pulsed Light Combined with 0.1% Tacrolimus Ointment on Erythema and Capillary Dilatation Scores in the Treatment of Rosacea. Contemporary Medicine Series, 21(17): 67–69.

[12] Hao RH, Zhang YQ, Ren JW, 2023, Clinical Effect of Intense Pulsed Light Combined with Rehabilitation New Liquid and Metronidazole Gel in the Treatment of Rosacea. Clinical Medicine Research and Practice, 8(7): 94–96.

[13] Duan X, Zhang Z, Zhou Z, et al., 2023, Observations on the Efficacy of Intense Pulsed Light Combined with 0.1% Tacrolimus Ointment in the Treatment of Erythematous Capillary-Dilated Rosacea. China Aesthetic Medicine, 32(1): 92–95.

[14] Zhu X, Zhang E, Fu M, 2023, Intense Pulsed Light Combined with Platelet-Rich Plasma Injection in the Treatment of Rosacea. China Aesthetic Medicine, 32(10): 102–105.

[15] Shi H, Yang K, Zhang Y, 2023, Clinical Efficacy of Intense Pulsed Light Combined with Azelaic Acid Gel in the Treatment of Type II Rosacea. Journal of Naval Medicine, 44(5): 527–529.

Integrated Services Platform of International Scientific Cooperation

Innoscience Research (Malaysia), which is global market oriented, was founded in 2016.Innoscience Research focuses on services based on scientific research. By cooperating with universities and scientific institutes all over the world, it performs medical researches to benefit human beings and promotes the interdisciplinary and international exchanges among researchers.

Innoscience Research covers biology, chemistry, physics and many other disciplines. It mainly focuses on the improvement of human health. It aims to promote the cooperation, exploration and exchange among researchers from different countries. By establishing platforms, Innoscience integrates the demands from different fields to realize the combination of clinical research and basic research and to accelerate and deepen the international scientific cooperation.

Cooperation Mode

Clinical Workers

In-service Doctors

Foreign Researchers

China Collaborators

Oversea Collaborators

Hospital

University

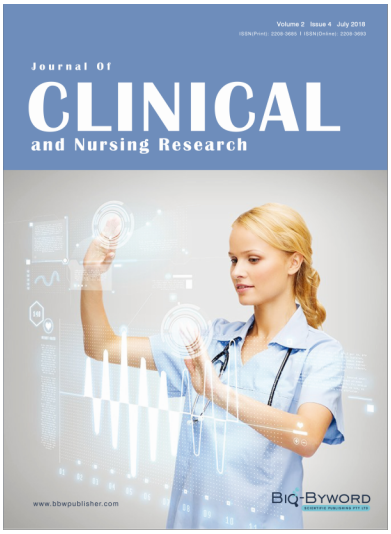
Scientific institutions

Our Journals

The *Journal of Architectural Research and Development* is an international peer-reviewed and open access journal which is devoted to establish a bridge between theory and practice in the fields of architectural and design research, urban planning and built environment research.

Topics covered but not limited to:

- Architectural design
- Architectural technology, including new technologies and energy saving technologies
- Architectural practice
- Urban planning
- Impacts of architecture on environment



Journal of Clinical and Nursing Research (JCNR) is an international, peer reviewed and open access journal that seeks to promote the development and exchange of knowledge which is directly relevant to all clinical and nursing research and practice. Articles which explore the meaning, prevention, treatment, outcome and impact of a high standard clinical and nursing practice and discipline are encouraged to be submitted as original article, review, case report, short communication and letters.

Topics covered by not limited to:

- Development of clinical and nursing research, evaluation, evidence-based practice and scientific enquiry
- Patients and family experiences of health care
- Clinical and nursing research to enhance patient safety and reduce harm to patients
- Ethics
- Clinical and Nursing history
- Medicine

Journal of Electronic Research and Application is an international, peer-reviewed and open access journal which publishes original articles, reviews, short communications, case studies and letters in the field of electronic research and application.

Topics covered but not limited to:

- Automation
- Circuit Analysis and Application
- Electric and Electronic Measurement Systems
- Electrical Engineering
- Electronic Materials
- Electronics and Communications Engineering
- Power Systems and Power Electronics
- Signal Processing
- Telecommunications Engineering
- Wireless and Mobile Communication