

ORIGINAL ARTICLE

REVEALING THE MOLECULAR-GENETIC AND CLINICAL PREDICTORS OF GLUCOCORTICOID RESISTANCE IN PATIENTS WITH HAND ECZEMA

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ABSTRACT

The aim: To reveal the possible predictors of the glucocorticoid resistance in patients with hand eczema (HE) based on the demographic, clinical, and molecular-genetic data.

Materials and methods: 143 patients with HE were included in the study. Demographic, clinical, biochemical (blood content of IgE, IL-17A, IL-2, 25(OH)D), and genetic (rs41423247 genotypes) data were obtained from all patients.

Results: After 2 weeks of treatment by glucocorticoids, all subjects were divided into "responder" and "non-responder" groups according to change of the Hand Eczema Severity Index (HECSI). Statistical analysis was done using SPSS (version 22.0.). Binary logistic regression was used to identify predictors of glucocorticoid resistance. P-value 0.05). The results of the multivariate regression showed that Bcl-1 G-allele (OR =3.83; P = 0.033), and severe eczema (OR = 2.52; P = 0.023) are linked with an elevated risk of glucocorticoid resistance in patients with hand eczema

Conclusions: Insensitivity to glucocorticoids in HE patients is associated with NR3C1 gene Bcl-1 polymorphism, eczema severity and blood level of IL-17, IL-2, 25(OH)D. The final adjustment showed that minor C-allele of the Bcl-1 polymorphism and severe eczema are the strongest predictors of the glucocorticoid resistance

KEY WORDS: eczema, interleukin, glucocorticoid, gene polymorphism, 25(OH)D

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INTRODUCTION

Hand eczema (HE) is one of the most common chronic inflammatory skin diseases, which leads to disability and economic burden [1]. HE ranks the 2nd place among the causes of temporary disability, in both the case number and the course duration [2]. In addition, the prevalence of hand eczema has increased during the COVID-19 pandemic [3-5].

Currently, there is no single approach to treating patients with HE in different countries. Immunosuppressants, immunomodulators, vitamins, physical factors are most often among the list of preferred therapeutic agents [6]. In recent years, a number of fundamentally new low molecular weight drugs have also been developed that can selectively block certain cytokines and intracellular signaling proteins [7]. However, most of these drugs have not yet completed the testing phase. Moreover, all of them are expensive and not available in various countries.

Thus, in the absence of a clear management protocol and unconditionally effective treatment options of eczema, the basis for HE patient treatment is corticosteroids (both topical and systemic) [8]. However, its effectiveness may decrease over the treatment course [9]. At the same time, some patients do not respond to treatment with glucocorticoids at all.

To date, there is a significant amount of work devoted to revealing the molecular mechanisms of glucocorticoid resistance [10-12]. It is known that sensitivity to glucocorticosteroids largely depends on the structure and function of the glucocorticoid receptor [11, 13]. It has been shown that some cytokines and vitamins can affect the production of various glucocorticoid receptor subunits, modulating the cell response to glucocorticoids [14]. It has also been shown that the sensitivity of glucocorticoid receptor depends on the structure of its own gene (*NR3C1*) [15]. The association between some SNPs of the *NR3C1* gene and the emergence of glucocorticoid resistance in some patients has been proved [16, 17]. However, the final disclosure of pathogenetic mechanisms and the detection of highly specific predictors of glucocorticoid resistance are still far away.

THE AIM

The aim of the study was to reveal the possible effective predictors of glucocorticoid resistance in patients with HE based on the demographic, clinical, and molecular-genetic data.

MATERIALS AND METHODS

Totaly 143 patients with HE (42 % female and 58% male, mean age – 42.2 ± 11.1 years) were included in the study. All patients were treated in the outpatient setting at the Medical Center «Eledia» (Ukraine, Sumy) – the clinical

Table I. Characteristics of the patients with hand eczema stratified by the sensitivity to glucocorticoids

Parameter	Responders n = 92	Non-responders n = 51	P
Age, years	43.1 ± 11.6	40.7 ± 9.9	0.200
Female (%)	35 (38.0)	25 (49.0)	0.203
Male (%)	57 (62.0)	26 (51.0)	
BMI, kg/m^2	25.7 ± 3.9	25.4 ± 4.0	0.681
BMI ≥ 25 kg/m^2 (%)	55 (59.8)	31 (60.8)	0.907
Smokers (%)	30 (32.6)	21 (41.2)	0.306
Exposure (%)	20 (21.7)	12 (23.5)	0.806
IgE, iu/ml	101.4 ± 33.9	109.9 ± 31.1	0.138
IL-17, pg/ml	80.2 ± 28.3	93.2 ± 27.5	0.009
IL-2, pg/ml	26.6 ± 8.9	29.8 ± 8.9	0.045
25(OH)D, ng/ml	32.4 ± 12.2	28.7 ± 10.4	0.070
Bcl-1 C/C (%)	32 (34.8)	7 (13.7)	0.025
Bcl-1 C/G (%)	46 (50.0)	33 (64.7)	
Bcl-1 G/G (%)	14 (15.2)	11 (21.6)	
Mild eczema (%)	36 (39.1)	11 (21.6)	0.009
Moderate eczema (%)	36 (39.1)	17 (33.3)	
Severe eczema (%)	20 (21.8)	23 (45.1)	
HECSI baseline score	26.4 ± 21.1	35.3 ± 21.7	0.018
HECSI 2-week score	11.1 ± 8.7	25.8 ± 17.4	< 0.001

Notes: BMI – body mass index; HECSI – Hand Eczema Severity Index.

Table II. The regression analysis of glucocorticoid resistance predictors

Predictor	Univariate Analysis			Multivariate Analysis		
	OR	95 % CI	P	OR	95 % CI	P
Gender	0.64	0.32-1.28	0.204			
Overweight	1.04	0.52-2.10	0.907			
Smoking	1.45	0.71-2.94	0.307			
Exposure	1.11	0.49-2.50	0.806			
IL-17	2.61	1.28-5.29	0.008	1.92	0.85-4.35	0.117
IL-2	2.30	1.14-4.64	0.019	1.87	0.87-4.01	0.110
25(OH)D	2.29	1.13-4.62	0.021	1.89	0.84-4.22	0.122
Bcl-1 C/G + G/G	3.35	1.36-8.29	0.009	3.83	1.19-13.10	0.033
Bcl-1 C/G	1.83	0.91-3.71	0.092	0.73	0.27-1.99	0.545
Bcl-1 G/G	1.53	0.64-3.68	0.340			
Severe eczema	2.96	1.41-6.21	0.004	2.52	1.14-5.56	0.023

Note: OR – odds ratio, 95 % CI – 95 % confidence interval.

base of the dermatovenerology department of the Medical Institute of Sumy State University (government license № 597170). The diagnosis of «Hand eczema» was established in accordance with the International Guidelines for the diagnosis, prevention, and treatment of hand eczema [18]. The complaints, medical history, and clinical criteria (localization, erythema, serous wells, infiltration, vesicles, cracks, peeling) were used. Eczema severity was measured using Hand Eczema Severity Index described by – HECSI (1-16 = mild, 17-37 = moderate, > 37 = severe) [19, 20].

Study Design. Demographic (age, gender, smoking, exposure (potentially harmful occupation, eg welder, chemical worker), and clinical data (body mass index (BMI), baseline HECSI) were determined in all patients before treatment. Also, the plasma content of IgE, IL-17A, IL-2, 25(OH)D were measured in all patients. Moreover, all subjects were genotyped by Bcl-1 polymorphism of

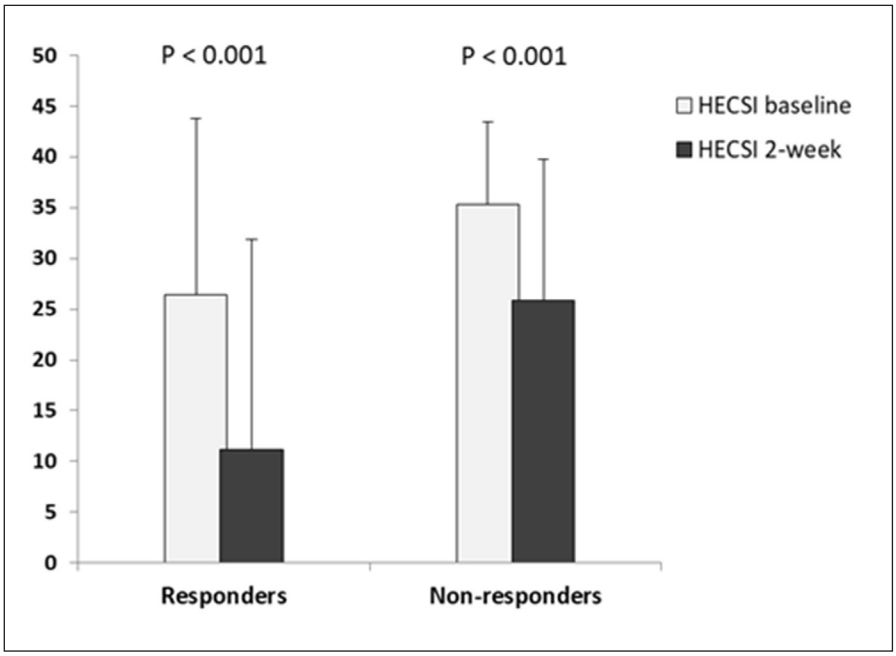

Fig. 1. The baseline HECSI and 2-week HECSI in responders and non-responders groups. The P values are obtained from dependent t-test for paired samples

the *NR3C1* gene. All patients were treated with corticosteroids for 2 weeks (mild and moderate eczema – topical corticosteroids (0.1 % Mometasone Furoate 2 times a day), severe eczema – topical corticosteroids (0.1 % Mometasone Furoate 2 times a day) + systemic corticosteroids (Dexamethasone: 3 days – 8 mg per day, 2 days 4 mg per day)). After 2 weeks of treatment, the HECSI score was assessed (HECSI 2-week score). If the HECSI was reduced by more than 50 % (HECSI≥50), the patient was considered as «responder». If the HECSI was reduced by less than 50 % or increased (HECSI<50), the patient was considered as «non-responder». After that, a retrospective comparative analysis of demographic, clinical, and molecular-genetic data between the two formed groups was performed. Finally, the attempts to determine the effective predictors of glucocorticoid resistance were made.

The study was performed in accordance with the principles of the Helsinki Declaration of the World Medical Association, Order of the Ministry of Health of Ukraine № 690 (23.09.2009), and approved by the Bioethics Commission of Sumy State University. Written informed consent was received from each patient before the experiment.

The level of IL-17A in blood serum was measured using Human IL-17A ELISA Kit (Enzyme-linked Immunosorbent Assay for quantitative detection of human IL-17A, Invitrogen, Carlsbad, CA, USA) according to manufacturer's protocol. All samples were evaluated in duplicate. Absorbance was measured at 450 nm. The detection of IL-2 serum concentration was based on Sandwich-ELISA (Enzyme-linked Immunosorbent Assay) method using Human IL-2 (Interleukin 2) Kit (Elabscience, Wuhan, China) according to manufacturer's instructions. The duplicate evaluation of samples was done. The optical density was measured at a wavelength of 450 nm. Blood level of vitamin D3 was measured using 25-OH-Vitamin D direct ELISA Kit (Enzyme immunoassay for the quantitative direct determination of 25-OH-Vitamin D in human serum and plasma, IBL International GMBH, Hamburg, Germany). The absorbance of each well was measured at 450 nm.

The Bcl-1 polymorphism (rs41423247) of the glucocorticoid receptor gene (NR3C1) was genotyping using the PCR-RFLP method according to Fleury et al. [21]. Whole venous blood was used for genetic analysis (blood was collected in vacutainers with EDTA and stored at -20°C).

Statistical analysis was performed using the program SPSS 22.0. Quantitative variables were tested for normal distribution by the Shapiro-Wilk method. Continuous data in graphs and tables are expressed as mean ± standard deviation. Comparisons of the averages between responders and non-responders were performed using the independent t-test for unpaired samples. The mean values of baseline HECSI and 2-weeks HECSI were compared by dependent t-test for paired samples. A comparison of the frequencies of factors distribution between two subgroups was performed using the Pearson test. Binary logistic regression was used to reveal possible predictors of glucocorticoid resistance under the univariate analysis. Multivariate logistic regression was used to identify the most effective predictors of glucocorticoid resistance. P-value < 0.05 was considered significant.

RESULTS

Corticosteroid therapy was prescribed to all 143 patients with HE. Two weeks after treatment, the patients were divided into two subgroups depending on the change in the HECSI score. The characteristics of two subgroups are presented in Table I.

The differences in age, male/female ratio, BMI, smokers, people with a harmful profession, and the IgE plasma content were not revealed (P > 0.05). Wherein, the plasma level of IL-17A and IL-2 in non-responders was significantly

higher compared to responders (P = 0.009, and P = 0.045 respectively). On the other hand, 25(OH)D blood content in non-responders was lower, but this difference was not confirmed statistically (P = 0.070). In addition, the number of patients with severe eczema (P = 0.009), the baseline (P = 0.022), and 2-week (P < 0.001) HECSI scores in non-responders were significantly higher than in responders. The results of the NR3C1 gene Bcl-1-polymorphism genotyping showed that the ratio of C/C, C/G, and G/G genotypes significantly differs between the subgroups of responders and non-responders (P = 0.025).

The results of comparison between baseline HECSI and 2-week HECSI in responders and non-responders groups are presented in Figure 2. After two weeks of treatment, it was found that HECSI has decreased by 63% in the responders group (P < 0.001), and by 8% – in the non-responders group (P = 0.010).

The final step of our study was the regression analysis of the obtained data in order to identify possible predictors of glucocorticoid insensitivity (Table II). The results of the univariate analysis showed that such factors as serum IL-17A more than 85 pg/mol, serum IL-2 more than 28 pg/mol, blood vitamin 25(OH)D less than 30 ng/mol, severe eczema and the C/G- and GG-genotypes of *NR3C1* gene Bcl-1 polymorphism significantly increases the risk of glucocorticoid resistance (P > 0.05).

After that, all factors with a P value of less than 0.1 were used for multivariate logistic regression. The results of the final model showed that Bcl-1 C/G- and GG-genotypes (OR =3.83; P = 0.033), as well as severe eczema (OR = 2.52; P = 0.023), are the significant predictors of the glucocorticoid resistance onset.

DISCUSSION

Today, glucocorticoids are the treatment basis for patients with HE [8]. However, not all patients have well response to corticosteroid treatment, while some are completely insensitive to these drugs [9].

It is known that the sensitivity of cells to glucocorticoids depends mainly on the structure and activity of the glucocorticoid receptor [11, 13]. In our work, we have analyzed the association between Bcl-1-polymorphism of the glucocorticoid receptor gene (*NR3C1*) and GR. Bcl-1-polymorphism is a C to G substitution in 646 position of the 2 intron of the *NR3C1* gene [21]. Some studies have shown that the minor G-allele is associated with increased sensitivity to glucocorticoids [17, 22], while others have shown that the G-allele is associated with decreased production of the glucocorticoid receptor alpha-subunit and increased methylation of the *NR3C1* gene promoter [16, 23]. The results of our study revealed that GR more often occurs in minor G-allele carriers. It was also established that the C/G- and G/G-genotypes are the strong independent predictors of GR in patients with HE.

It has been shown that alpha-subunit of the glucocorticoid receptor is required for the binding of glucocorticoids, while the presence of a beta-subunit (instead of alpha-subunit) leads to a loss of glucocorticoid sensitivity [11]. Experiments by Vazquez-Tello et al. demonstrated that IL-17 and IL-23 increase the expression of beta-subunit, while IL-2 leads to the inhibition of alpha-subunit production [24]. Thus, these interleukins inhibit the interaction between glucocorticoid receptor and glucocorticoids through different mechanisms.

Our results showed that the blood content of IL-17A and IL-2 in patients with GR is significantly higher compared to hormone-sensitive subjects. Also, the univariate regression demonstrated that elevated blood levels of these cytokines are the predictors of the GR onset in patients with HE.

It is also known that vitamin D can modulate the immune response and influence the production of various cytokines [25]. Nanzer et al. revealed that vitamin D3 inhibits the IL-17A and IL-22 expression in cell culture from asthmatic patients [26]. Interesting to note that synthesis of mentioned cytokines in this experiment was not affected by glucocorticoids.

In our study, it was found that patients with GR have significantly lower blood content of vitamin D3 than eczema patients with preserved response to glucocorticoids. Also, vitamin D3 deficiency has been identified as a predictor of GR development.

There are several important limitations in our work that should be mentioned. Our study was not prospective and placebo-controlled. The cytokines, IgE, and 25(OH)D were not evaluated after the treatment. Only one clinically significant *NR3C1* gene polymorphism was investigated. Moreover, the study was conducted on a relatively small patient sample. All these circumstances allow us to draw only preliminary conclusions and push us to conduct further research.

CONCLUSIONS

Thus, the obtained results revealed that insensitivity to glucocorticoids in patients with hand eczema is related to *NR3C1* gene Bcl-1 polymorphism, eczema severity and blood level of IL-17A, IL-2, 25(OH)D. The results of the final adjustment showed that minor G-allele of the *NR3C1* gene Bcl-1 polymorphism and severe eczema are the strongest predictors of the glucocorticoid resistance.

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