CLINICAL CHARACTERISTICS AND SERUM CONCENTRATIONS OF SOME T-HELPER 2-DERIVED CYTOKINES IN PATIENTS WITH GENERALIZED ERYTHEMA MULTIFORME

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ABSTRACT

Objectives: To describe the clinical characteristics of patients with erythema multiforme (EM) presenting with generalized skin lesions and to investigate the serum concentrations of certain T-helper 2 (Th2) cytokines in these patients, exploring their correlation with specific clinical features.

Subjects and methods: This cross-sectional descriptive study included 33 patients diagnosed with EM featuring generalized skin lesions at the National Hospital of Dermatology and Venereology from April 2017 to August 2019. Additionally, 32 healthy controls (HCs) participated as the control group. Clinical examinations, medical history interviews, and serum sample collections were conducted for all subjects. The fluorescence covalent microbead immunosorbent assay technique was used to detect multiple cytokines simultaneously: IL-4, IL-5, and IL-13. The Mann-Whitney U test compared the serum cytokine levels between the two groups, with statistical significance set as $p < 0.05$. Pearson's test was applied to evaluate the correlation between two quantitative variables.

Results: The mean age of EM patients with generalized lesions was 42.2 years; 30.3% were male, and 69.7% were female. Twenty-two patients (66.7%) had an illness duration of less than one week a history of medication use before illness onset was reported by 60.6% of patients. Only 15.2% of patients had mucosal lesions. In the EM group, serum levels of IL-4 and IL-5 were 1.55 ± 4.35 pg/ml and 7.12 ± 18.91 pg/ml, respectively, which were lower than those in the HCs group (9.15 ± 10.17 pg/ml and 21.38 ± 20.85 pg/ml, respectively), $p < 0.001$. Serum IL-13 levels were identical in both groups (1.48 pg/ml). No correlation was found between serum IL-4 levels and age ($r = 0.08$; $p > 0.05$), nor between serum IL-5 levels and age ($r = -0.055$; $p > 0.05$).

Conclusions: Th2 cytokine levels in the EM group with generalized skin lesions were lower compared to the HCs group, with no correlation between these cytokine levels and age. Th2 is likely not an important factor in the pathogenesis of generalized EM.

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1. INTRODUCTION

Erythema multiforme (EM) is a mucocutaneous syndrome first described by Von Hebra in 1860[1]. Characteristic skin lesions include typical and/or atypical target lesions. The disease is classified into EM minus (without mucosal erosions, only affecting skin and lips) and EM majus (with mucosal erosions). Commonly affected mucosal sites include the eyes, mouth, nose, and genital area. The exact cause of the disease is unknown; however, EM can be drug-induced or associated with infections. Among these, herpes simplex virus (HSV) is considered the most commonly related pathogen, causing a wide range of clinical presentations, including typical target lesions on extremities, mucosal involvement, and even giant, generalized targets on the hands, feet, trunk, and face[1-4]. EM caused by *Mycoplasma pneumoniae* primarily involves severe oral mucosal lesions, with minimal or no skin involvement[1,5-7]. Drug-related EM often presents with generalized skin lesions[1], although in some cases, it may be limited to mucocutaneous involvement[8,9].

The pathogenesis of EM remains unclear. Most studies have concentrated on EM associated with HSV[1]. HSV has not been isolated from EM skin lesions; however, HSV-DNA has been identified through polymerase chain reaction (PCR) methods[2,3]. Keratinocytes in the epidermis carry viral DNA fragments, including the Pol gene of the virus. These DNAs localize in the basal and spinous cells in the layers adjacent to the basement membrane. The virus's *Pol* protein is synthesized. HSV-specific lymphocytes, including cytotoxic cells, are drawn to the site. Initially, there are specific immune responses to the virus, followed by non-specific responses amplified by autoactive T cells. The cytokines these cells produce trigger a delayed hypersensitivity reaction, as observed histopathologically in EM lesions[1]. T helper 1 (Th1) cells are crucial in this process, with interferon-gamma from these cells playing several roles in EM pathogenesis: enhancing antigen presentation by keratinocytes, increasing cytokine release, promoting keratinocyte lysis by macrophages and cytotoxic T cells, and activating macrophages[10,11]. T helper 2 (Th2) cells are likely less involved in the pathogenesis of HSV-induced EM.

In clinical practice, many patients are diagnosed with EM due to the presence of typical and/or atypical targets with a generalized skin distribution. These patients do not have a history of HSV infection before the onset of EM and do not exhibit severe oral mucosal lesions as seen in EM caused by *Mycoplasma pneumoniae*. The pathogenesis of these EM cases may differ from that of HSV-induced EM. In many instances, the cause cannot be determined, suggesting the possibility of spontaneous EM. This raises the question of whether Th2 cells, which are minimally involved in HSV-related EM, might play a role in the pathogenesis of these cases. This study aims to describe the clinical characteristics of EM patients with generalized skin lesions and to investigate the serum levels of specific Th2 cytokines in this condition and their correlation with particular clinical features.

2. RESEARCH SUBJECTS AND METHODS

2.1. Research subjects

There were 33 EM patients with generalized skin lesions and 32 healthy controls (HCs) participating in the study. Samples were collected conveniently without calculating the sample size. The diagnostic criteria for EM included having typical target lesions (three rings: a central blister,

an infiltrated pale ring, and an outer erythematous halo elevated above normal skin) and/or atypical target lesions (two rings above normal skin). Mucosal involvement could be present or absent. The cause could be drug-related or unknown. Skin lesions were generalized, affecting the hands, feet, trunk, and face. EM diagnosis was based on independent clinical assessment by at least two dermatologists.

Inclusion criteria for EM patients were: age 18 or older; having typical and/or atypical target lesions with generalized distribution without epidermal detachment. Exclusion criteria included: EM patients with typical target lesions localized to the extremities (face, hands, feet, knees, elbows, around natural orifices) since these cases are often associated with herpes virus or other microbial agents; patients with other ongoing infections or systemic/internal diseases. This study included 33 EM patients who met the inclusion criteria.

For the healthy control group, we selected 32 individuals with no history of drug allergies, bacterial infections, allergic conditions (such as rhinitis, urticaria, spring conjunctivitis, or bronchial asthma), or other internal or surgical conditions.

2.2. Research methods

Research design

This prospective cross-sectional descriptive study was conducted from April 2017 to August 2019 at the National Hospital of Dermatology and Venereology, where EM patients received inpatient treatment, and at the Department of Immunology, Vietnam Military Medical University, where serum quantification tests for IL-4, IL-5, and IL-13 were performed.

Research procedure steps

Subjects were selected for the study using a convenience sampling method based on chronological order. They were assessed for medical history, underwent clinical examination, and had basic laboratory tests, all of which were recorded in the research medical records.

Serum storage and cytokine quantification: After obtaining consent from the patients or their legal representatives, each EM patient had 4 ml of blood drawn to separate serum upon admission. Similarly, each healthy control had 4 ml of blood drawn for serum separation. Blood samples were kept at room temperature for 10 - 20 minutes, then centrifuged for 20 minutes at 2000 - 3000 rpm to separate the serum, which was then stored at -80°C until serum cytokine quantification. The fluorescence covalent microbead immunosorbent assay technique was used to detect multiple cytokines simultaneously: interleukin (IL)-4, IL-5, and IL-13.

Data processing

Data were processed using SPSS 20.0 software. Variables are presented as mean, standard deviation, median, minimum value, maximum value, percentage, and frequency. The Shapiro-Wilk test (for sample sizes below 50) was used to assess the normal distribution of a quantitative variable before comparing quantitative variables. Statistical tests used to compare two means included the t-test for normally distributed variables and non-parametric tests (Wilcoxon and Mann-Whitney U) for non-normally distributed variables. Pearson's test was applied to evaluate the correlation between two quantitative variables. Statistical significance was defined as $p < 0.05$.

2.3. Ethics in research

The investigators ensured that the study procedures adhered to the Declaration of Helsinki on ethics in research. The study was approved by the Research Ethics Committee of Hanoi Medical University under decision number 04NCS17/HĐĐĐ-ĐHYHN, dated February 8, 2018.

3. RESULTS

3.1. General characteristics of study participants

Table 1. General characteristics of study participants (N = 33)

Characteristics	n	%
Gender, n (%)		
Female	23	69.7
Male	10	30.3
Duration of illness		
< 1 week	22	66.7
≥ 1 week	11	33.3
History of medication use		
Yes (allopurinol, antibiotics, other drugs)	20	60.6
No	13	39.4
History of hsv infection before disease		
No	33	100
Itching		
Yes	32	97
No	1	3
Mucosal lesions		
Yes	5	15.2
No	28	84.8
Fever		
Yes	10	30.3
No	23	69.7
Number of skin lesions		
20 - 50 papules	3	9.1
> 50 papules	30	90.9
Leukocytosis		
Yes	18	54.5
No	15	45.5

A total of 33 patients with generalized EM participated in the study. The mean age was 42.2 ± 17.5 years, ranging from 19 to 76 years, with a predominance of females. Most patients (66.7%) had a disease duration of less than one week, with the remainder having a duration of one week or more. A history of medication use was reported by 60.6% of the patients, while 39.4% had no history of medication use before disease onset. None of the patients had a history of HSV infection or reactivation. Most patients experienced itching (97%) and had generalized lesions; 90.9% had more than 50 papules, presenting as typical or atypical target lesions. Fever was present in 10% of the patients. Mucosal involvement (one or more sites) was observed in 15.2% of the patients. Elevated peripheral blood leukocyte counts were found in 54.5% of the patients (Table 1). Additionally, 32 healthy controls (HCs) participated in the study, with a mean age of 28.5 ± 4.7 years (ranging from 22 to 46 years), and an equal male-to-female ratio (50%).

3.2. Th2 cytokine levels in EM and HCs groups

Table 2. Th2 cytokine levels in EM and HCs groups (N = 33)

Cytokine (pg/ml)	EM group	HCs group	p*
IL-4			
Mean	1.55 ± 4.35	9.15 ± 10.17	< 0.001
Range	0.79 - 25.78	0.79 - 42.65	
Median	0.79	5.81	
IL-5			
Mean	7.12 ± 18.91	21.38 ± 20.85	< 0.001
Range	0.49 - 92.94	0.49 - 52.22	
Median	0.49	24.10	
IL-13			
Mean	1.48 ± 0	1.48 ± 0	1
Range	1.48 - 1.48	1.48 - 1.48	
Median	1.48	1.48	

** Mann-Whitney U test.*

In the EM group, serum concentrations of IL-4 and IL-5 were 1.55 ± 4.35 pg/ml and 7.12 ± 18.91 pg/ml, respectively, which were lower than those in the HCs group (9.15 ± 10.17 pg/ml and 21.38 ± 20.85 pg/ml, respectively). The differences were statistically significant with $p < 0.001$. The serum level of IL-13 was the same in both groups (1.48 pg/ml). The ranges of serum levels for IL-4 and IL-5 were quite wide.

3.3. Th2 cytokine levels in EM group by gender

Table 3. Th2 cytokine levels in EM group by gender (N = 33)

Cytokin (pg/ml)	Female (n = 23)	Male (n = 10)	p*
IL-4			
Mean	1.87 ± 5.21	0.79 ± 0	> 0.05
Range	0.79 - 25.78	0.79-0.79	
Median	0.79	0.79	
IL-5			
Mean	7.95 ± 21.75	5.21 ± 10.47	> 0.05
Range	0.49 - 92.94	0.49 - 30.93	
Median	0.49	0.49	
IL-13			
Mean	1.48 ± 0	1.48 ± 0	> 0.05
Range	1.48 - 1.48	1.48 - 1.48	
Median	1.48	1.48	

** Mann-Whitney U test.*

In 23 female EM patients, serum concentrations of IL-4, IL-5, and IL-13 were 1.87 ± 5.21 pg/ml, 7.95 ± 21.75 pg/ml, and 1.48 pg/ml, respectively. These levels did not differ from those in 10 male EM patients (0.79 pg/ml, 5.21 ± 10.47 pg/ml, and 1.48 pg/ml, respectively), with p > 0.05 (Table 3).

3.4. Th2 cytokine levels in EM group by duration of illness

Table 4. Th2 cytokine levels in EM group by duration of illness

Cytokine (pg/ml)	Duration of illness		p*
	< 1 week (n = 22)	≥ 1 week (n = 11)	
IL-4			
Mean	1.93 ± 5.33	0.79 ± 0	> 0.05
Range	0.79 - 25.78	0.79 - 0.79	
Median	0.79	0.79	
IL-5			
Mean	10.44 ± 22.59	0.49 ± 0	> 0.05
Range	0.49 - 92.94	0.49 - 0.49	
Median	0.49	0.49	

Cytokine (pg/ml)	Duration of illness		p*
	< 1 week (n = 22)	≥ 1 week (n = 11)	
IL-13			
Mean	1.48 ± 0	1.48 ± 0	> 0.05
Range	1.48 - 1.48	1.48 - 1.48	
Median	1.48	1.48	

** Mann-Whitney U test.*

For the 22 EM patients with a disease duration of less than one week, serum levels of IL-4, IL-5, and IL-13 were 1.93 ± 5.33 pg/ml, 10.44 ± 22.59 pg/ml, and 1.48 pg/ml, respectively, showing no difference compared to the group of 11 EM patients with a disease duration of one week or more, with p > 0.05 (Table 4).

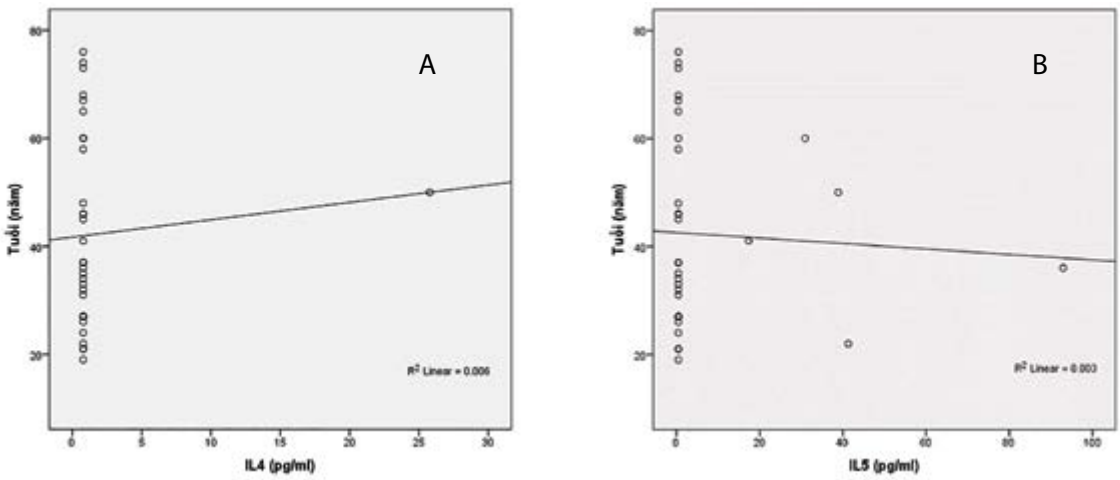

Figure 1. Correlation between serum levels of IL-4 and IL-5 and age in the EM group

In the generalized EM group, there was no correlation between serum IL-4 levels and age, r = 0.08; p > 0.05 (Figure A). Likewise, no correlation was observed between serum IL-5 levels and age, r = (-0.055); p > 0.05 (Figure B).

4. DISCUSSION

The study included 33 patients with generalized EM. The mean age was 42.2 years, ranging from 19 to 76 years, with a majority being female. According to the literature, EM typically has a higher incidence in males. However, our study showed a higher female prevalence, likely due to the small and non-random sample size not accurately representing the EM patient population. Most patients had a disease duration of less than one week, while the remainder had a duration of one week or more. Almost all patients experienced itching (mostly mild) and had generalized lesions, with 90.9% having more than 50 papules, either typical or atypical target lesions. Traditionally, EM target lesions are distributed on extremities

(lips, ears, shoulders, elbows, knees, hands, feet) and are often associated with HSV. In this study, patients had generalized lesions with numerous lesions, and most had no history of HSV infection or reactivation before the disease onset. The incidence of mucosal involvement (one or more sites) was 15.2%, with mild lesions, unlike the severe oral lesions in *Mycoplasma pneumoniae*-induced EM. A limitation of this study is the absence of PCR testing for HSV-DNA at skin lesions and serological tests for HSV and *Mycoplasma pneumoniae* (both IgM and IgG), making it challenging to determine the etiology in these patients. A history of medication use before disease onset was reported by 60.6% of the patients. Since drug-induced EM, if present, is a type IV hypersensitivity reaction, we examined the medications used by patients within 1 - 8 weeks before disease onset. Due to patient recall errors and the lack of precise tests for diagnosing drug allergies, this variable may not be entirely accurate. However, the literature suggests that in some cases of drug allergies, there is an interaction between the drug, microbial infection, and the immune system, triggering the disease's pathogenesis, as seen in DRESS syndrome and infectious mononucleosis. High-risk medications identified in this study include allopurinol, antibiotics, and traditional medicines. These drugs can also cause Stevens-Johnson syndrome, but in that syndrome, systemic symptoms and mucosal lesions are more severe, with skin lesions presenting as atypical target-like necrotic patches, blisters, and erosions.

Interleukin-4 (IL-4) and Interleukin-13 (IL-13) are cytokines produced by activated Th2 cells. While they share approximately 30% structural similarity, they exhibit different biological activities. The specific receptor for IL-4, which does not bind to IL-13, is found in T cells and natural killer cells. This receptor comprises IL-4Rα (CD124) and γc, transmitting signals via Janus kinase (JAK)1 and JAK3. The second receptor complex can bind either IL-4 or IL-13 and is found in keratinocytes, endothelial cells, and other non-hematopoietic cells. It consists of IL-13α1 and IL-4Rα and transmits signals through JAK1 and JAK2. These receptors have low expression in resting cells but increase when activated by specific signals[12].

The biological effects of IL-4 vary depending on the receptor and cell type but primarily involve stimulating the growth and differentiation of Th2 cells while inhibiting Th1 cells[13]. When naïve T cells are exposed to IL-4, they mature and differentiate into Th2 cells, which then produce more IL-4, enhancing local stimulation and prolonging the Th2 response. Therefore, early expression of IL-4 in the immune response can initiate a cascade favoring Th2 development, leading to a predominant Th2 response. Naïve T cells can produce low levels of IL-4 upon activation, and IL-4 is also produced by natural killer cells[12].

In this study, serum levels of IL-4 and IL-5 in the EM group were lower compared to the HCs group, while IL-13 levels did not differ between the two groups. According to Quaglino et al., EM patients do not exhibit increased IL-13 levels[14]. The role of Th2 is more prominent in other skin conditions such as atopic dermatitis, graft-versus-host disease, lepromatous leprosy, and disseminated leishmaniasis. Dupilumab, a biologic agent effective in treating adult atopic dermatitis[12], works by inhibiting the α subunit of the IL-4 receptor,

thereby blocking both IL-4 and IL-13, structurally similar[12]. In acute asthma in children, serum concentrations of Th2 cytokines are increased, whereas TNF-α levels are reduced (compared to healthy children), indicating a heightened Th2-mediated inflammatory response and a reduced Th1 response[15]. To explain the lack of increased IL-4 and IL-5 levels in the EM group compared to the HCs group, we suggest that in EM, including generalized EM not related to HSV, Th1 plays a more dominant role than Th2. Th2 is insufficient to suppress Th1, resulting in unchanged or even decreased serum concentrations of Th2 cytokines due to Th1 predominance. Our study's limitations include the absence of skin biopsies, the lack of cytokine expression evaluation in biopsy samples, and incomplete statistical data on the healthy control group due to retrospective serum sample collection. According to Caproni, immunohistochemical observations indicate that Th1 response predominates over Th2 in EM[16]. Additionally, the cytokine network in the body is highly complex and interactive, with serum levels influenced by multiple factors. Quantitative testing of serum cytokine levels may be subject to various sources of error.

5. CONCLUSIONS

In the group of EM patients with generalized skin lesions, the levels of Th2 cytokines were lower compared to the HCs group, and there was no correlation between these cytokine levels and age. Th2 may not have a significant role in the pathogenesis of generalized EM.

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