MINISTRY OF EDUCATION AND TRAINING MINISTRY OF HEALTH

HANOI MEDICAL UNIVERSITY

TRAN THI HUYEN

IDENTIFY SOME GENS, MOLECULES RELATING TO SJS/TEN IN VIETNAMESE PEOPLE

Major : Internal Medicine (Dermatology)
Code : 9720107

SUMMARY OF MEDICAL DOCTORAL THESIS

HANOI - 2021

THE STUDY WAS COMPLETED AT

HANOI MEDICAL UNIVERSITY

Supervisors:

1. **Assoc. Prof. Pham Thi Lan, MD, PhD**
2. **Prof. Riichiro Abe, MD, PhD**

Opponent 1: Assoc. Prof. Dang Van Em

Opponent 2: Assoc. Prof. Ly Tuan Khai

Opponent 3: Assoc. Prof. Tran Dang Quyet

The thesis has been defended at University-level Thesis Evaluation Council held in Hanoi Medical University

Time: 14:00, Date: May 11th 2021

This thesis may be found at:

- National Library
- Library of Hanoi Medical University

LIST OF PUBLISHED PAPERS RELATING TO THE THESIS

1. **Tran Thi Huyen**, Riichiro Abe, Pham Thi Lan et al (2020). The link between HLA-B alleles and causative drugs in Vietnamese patients with Stevens-Johnson syndrome/toxic epidermal necrolysis. *Open Access Maced J Med Sci*, **8(B)**, 395-400.
2. **Tran Thi Huyen**, Riichiro Abe, Pham Thi Lan et al (2020). Serum granulysin in differentiation of Stevens-Johnson syndrome/toxic epidermal necrolysis and erythema multiforme. *Open Access Maced J Med Sci*, **8(B)**, 381-388.
3. **Tran Thi Huyen**, Pham Dinh Hoa, Pham Thi Lan (2020). High levels of serum interferon-gamma in patients with Stevens-Johnson syndrome/toxic epidermal necrolysis. *Journal of Medical Research*, **127 E6(3)**, 67-72.
4. **Tran Thi Huyen**, Nguyen Thi Hoa, Pham Thi Lan et al (2020). Serum levels of interleukin-6 and interleukin-8 in patients with Stevens-Johnson syndrome/toxic epidermal necrolysis and erythema multiforme. *Vietnamese Journal of Dermatology and Venereology*, **30**, 53-62.
5. **Tran Thi Huyen**, Nguyen Hoang Phuong, Pham Thi Lan et al (2020). Carbamazepine, allopurinol and traditional medicine-induced Stevens-Johnson syndrome and toxic epidermal necrolysis: clinical features and *HLA-B* alleles. *Journal of Medical Research*, **128(4)**, 85-94.
6. **Tran Thi Huyen**, Nguyen Thi Hoa, Pham Thi Lan et al. (2020). Study on the causes of Stevens-Johnson syndrome and toxic epidermal necrolysis at National Hospital of Dermatology and Venereology. *Vietnamese Journal of Dermatology and Venereology*, **30**, 53-62.

INTRODUCTION

1. Justification of study issues

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are severe cutaneous adverse drug reactions (SCARs). Though their incidence is of 2 per million per year, they are life-threatening with a mortality rate of 5-30%. The common culprit drugs are allopurinol, carbamazepine, phenytoin, phenobarbital, lamotrigine, abacavir and others. The time between the date of taking medicine and the onset of symptoms ranges from some days to two months.

The pathogenesis of SJS/TEN is not fully understood, but there are some immunological and genetic factors which are believed to be involved There is a strong association between *HLA-B*15:02* and carbamazepine-induced SJS/TEN, *HLA-B*58:01* and allopurinol-induced SJS/TEN, *HLA-B*57:01* and abacavir-induced SJS/TEN. CD8+ cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells play an important role in the pathogenesis of SJS/TEN. The immune response may be triggered by binding an antigenic drug to a specific *HLA* on a keratinocyte. Specific T cell receptors recognize the drug-*HLA* complex and upon the activation, CD8+CTLs and NK cells produce cytokines, chemokines and cytotoxic proteins that cause extensive keratinocyte apoptosis. Apoptotic molecules, including alpha tumor necrosis factor (TNF-α), interferon gamma (IFN-γ), inducible nitric oxide (NO), are the bridge between the drug-induced immune response and the damage of keratinocyte. Factors such as ligand ligand (FasL) soluble, perforin and granzyme B are all emphasized in the apoptosis of keratinocyte but the most recent research supports the important role of granulysin. Chung reported that granulysin concentrations in the blister fluid of SJS/TEN patients were two to four times higher than perforin, granzyme B or soluble Fas ligand concentrations. Granulysin in the blister fluid was 15-kDa secretory form, and injection of it into mouse skin resulted in features mimicking SJS/TEN.

In Vietnam, there have been studies that prove the relation between *HLA-B*58:01* and allopurinol-induced SCARs, between *HLA-B*15:*02 and carbamazeoine-induced SCARs. In many cases of SJS/TEN, the causative drugs are unknown. A lot of Vietnamese SJS/TEN patients use traditional medicine. In fact, these drugs cause SJS/TEN in one person but cause erythema multiforme (EM) in another. This may depend on the individual *HLA* allele. In addition, assessing the key role of certain cytokines may help prognosis of disease and promise new therapies. Researches in Vietnam were mainly related to clinical and paraclinical characteristics, less research investigated genes, molecules and cytokines that play an important role in the pathogenesis of SJS/TEN. Therefore, we conducted the thesis "Identify some gens, molecules relating to SJS/TEN in Vietnamese people" with the following objectives:

1. Identify some gens (HLA-B alleles) relating to SJS/TEN in Vietnamese people.

2. Investigate serum levels of granulysin and 13 cytokines in SJS/TEN.

2. New contributions of the thesis

*HLA-B*15:02* was the most common *HLA-B* allele in Vietnamese patients (Kinh ethnicity) with SJS/TEN. *HLA-B*51:02* allele may play an important role in the pathogenesis of the traditional medicine-induced SJS/TEN. There may possibly have a link between *HLA-B* alleles and causative drugs of SJS/TEN. The *HLA-B* genotypes may be useful for suggesting the causative drugs in some cases and preventing SJS/TEN.

Serum granulysin levels were significantly higher in SJS/TEN patients than in EM patients. After the onset of SJS/TEN, serum granulysin levels were not associated with the severity of the diseases. In SJS/TEN patients, serum levels of GM-CSF, TNF-α, IFN-γ, IL-6 and IL-12 were significantly higher than those in EM

patients. At the day of re-epithelialization, serum levels of GM-CSF, TNF-α, IFN-γ, IL-1β, IL-5, IL-6 and IL-12 were significantly lower than those at the day of hospitalization. Serum level of IFN-γ may be a good biomarker to differentiate SJS/TEN from EM as well as to evaluate the progress and the severity of SJS/TEN.

3. The structure of the thesis

The thesis consists of 142 pages, in which the introduction section is 02 pages, the literature review section is 38 pages, the methodology section is 25 pages, the result section is 39 pages, the discussion section is 35 pages, the conclusion section is 02 page and the recommendation section is 01 page. The results section has 15 tables and 23 figures. The thesis has 163 references (9 Vietnamese documents and 154 English documents).

CHAPTER 1. LITERATURE REVIEW

1.1. The association between *HLA* (MHC class I) and SCARs

Numerous studies have indicated an association between MHC class I and SCARs. In Han-Chinese patients with SJS/TEN, there was a strong association between aromatic antiepileptic agents such as carbamazepine, phenytoin, oxcarbazapine, lamotrigine and *HLA-B*15:02*, between allopurinol and *HLA-B*58:01*. The association between *HLA-B*15:02* and carbamazepine-induced SJS/TEN was also observed in Thai, Malaysian, and South Indian patients, but not in Japanese, Korean or European. In Caucasians, there was an association between *HLA-B*57:01* and abacavir-induced SJS/TEN, between *HLA-A*31:01* and carbamazepine-induced SJS/TEN. The research in Japan showed that *HLA-B*15:11* was a risk factor for carbamazepine-induced SJS/TEN, and there was an association between *HLA-A*02:06* and acetaminophen-induced SJS/TEN.

1.2. The role of granulysin in SJS/TEN

Granulysin is a molecule found in the cytotoxic granules (along with granzyme B and perforin) of TCD8+, NK cells, NK/T cells, it acts as tumor killer and bactericide. In the presence of drug interactions with specific *HLA* and T cell receptor (TCR) of TCD8+, granulysin is released from the granules of TCD8+, inducing apoptosis of keratinocytes. Granulysin appears to cross the target cell membrane, causing ionic imbalance, damaging the mitochondria, releasing oxidants and caspase cascades, causing apoptosis of keratinocytes.

In the study of granulysin in TEN, Chung *et al* compared the expression of the bullous fluid cell gene with the peripheral blood mononuclear cells, the results showed that the granulysin gene expression of the bullous cells increased 10-20 times, granzyme B increased 8 times, perforin increased 3 times, soluble FasL increased 2 times. The concentration of granulysin in the blister fluid was 2-4 times higher than that of perforin, granzyme B and soluble FasL, the concentration of granulysin correlated with the severity of the disease. Erythema multiforme may have skin manifestation similar to SJS/TEN but they can be distinguished immunopathologically. In cases of SJS/TEN, the inflammatory infiltrates expressing granulysin, granzyme B and perforin accumulated predominantly in the lower epidermal and subepidermal bulla, in contrast, they were relatively sparse in EM. Abe showed that serum granulysin levels in patients with SJS/TEN were elevated before skin detachment or mucosal lesions developed. The rapid immunochromatographic test has been developed for serum granulysin to diagnose early SJS/TEN. The above studies prove that granulysin may play an important role in the pathogenesis of SJS/TEN, it is also a marker for early diagnosis and prognosis of disease severity.

1.3. Cytokines associated with SJS/TEN

Caproni *et al* showed that skin biopsy samples of SJS/TEN expressed all cytokines and chemokins (TNF-α, IFN-γ, IL-2, IL-5, IL-13, CCR3, CXCR3 and CXCR4) stronger than those in EM skin samples. All skin samples of SJS/TEN and EM expressed these cytokines stronger than those in healthy controls. Comparing Th1 or Th2 related cytokines/chemokins shows that the Th1 response is dominant in EM, the imbalance between Th1 and Th2 is not significant in SJS/TEN.

Wang *et al* showed that serum concentrations of CCL27 and TNF-α increased significantly in SJS/TEN patients during the acute phase compared to the resolution phase and healthy controls. CCL27 concentration positively correlated with the TNF-α concentration in the acute phase. The CCL27 concentration in the bullous fluid was higher than that in serum during the acute phase. TNF-α concentration in bullous fluid was higher than that in serum at the acute and resolution stages. CCL27 and TNF-α have an important role in the initiating of the pathogenesis of SJS/TEN. CCL27 works in the early stages of the disease, via circulation, while TNF-α works throughout the course of the disease, in skin lesions.

Su *et al* suggested that the IL-15 concentration correlated with the severity and mortality of SJS/TEN. When added, exogenous IL-15 stimulated bullous cells in SJS/TEN to secrete granulysin in 3 samples, and if IL-15 is neutralized, it reduces drug-induced cell stimulation in a TEN patient. Other factors, including IL-6, IL-8, and TNF-α correlated with severity of SJS/TEN (clinical progression, skin lesion area) but not as prominent as IL-15. This finding raises the possibility of using IL-15 concentrations for disease prognosis.

CHAPTER 2. METHODOLOGY

2.1. Study subjects

The study was conducted on 83 SJS/TEN patients at National Hospital of Dermatology and Venereology and Bach Mai Hospital in Hanoi, Vietnam from January 2018 to October 2019. Diagnosis of SJS, TEN or overlap SJS/TEN was based on Bastuji-Garin's classification, which was examined by at least two dermatologists and/or allergologists: 1) SJS is defined as epidermal detachment less than 10% body surface area (BSA) plus widespread purpuric macules or flat atypical targets; 2) overlap SJS/TEN is defined as detachment of 10-30% BSA plus widespread purpuric macules or flat atypical targets; 3) TEN is defined as epidermal detachment more than 30% BSA.

Inclusion criteria

Age 18 and over; patient or patient's legal representative consenting to participate in the study, signed consent inform. With the objective 1: selecting Vietnamese SJS/TEN patients (Kinh ethnicity). With the objective 2: having at least 2 good serum samples at two different times: at the day of hospitalization and at the day of re-epithelialization.

Exclusion criteria

With the objective 1: not having quality total blood samples for typing *HLA-B*. With the objective 2: patients with sepsis (blood procalcitonin >2 ng/ml and/or positive blood cultures); having an immunodeficiency disease; not having enough serum samples at the day of hospitalization and at the day of re-epithelialization.

2.2. Methodology

2.2.1. Study design

This was a cross-sectional study.

2.2.2. Sampling method

The participants were selected to the study by the convenient chronological sampling method.

2.2.3. Sample size

For objective 1: Apply formula to estimate a rate for a population, n=60.

For objective 2: Apply formula to estimate the mean serum granulysin concentration in SJS/TEN patients, calculated as n=48. The control groups consisted of 43 patients with EM and 20 healthy controls (HCs).

2.2.4. Study procedure

Medical history

Investigating medical history according to the available questionnaire. Identify causative drugs based on the following important criteria: the time between taking medicine and the onset; the history of allergy to the same drug or other drug in the same class; the risk of causing SJS/TEN of the drug according to previous studies in Vietnam and in the world. Additionally, we have a reference to the ALDEN (Algorithm for Drug Causality) table. If the patient took more than one drug, the drug with a higher score was identified as the culprit drug.

Clinical examination

Evaluation of severity of SJS/TEN patients according to 6-criterion-SCORTEN: age >40, with associated malignancy, heart rate >120 beats/minute, skin detachment area >10% BSA, blood urea concentration >10 mmol/L, blood glucose concentration>14 mmol/L. Each criterion was calculated 1 point.

Taking, storing blood and serum samples

- Each patient with SJS/TEN has blood taken at least two time points: 1) at the day of hospitalization (03 ml of whole blood for typing *HLA-B*; 04 ml of serum for measuring granulysin and 13 cytokines); 2) at the day of re-epithelialization (04 ml of serum for measuring granulysin and 13 cytokines).

- Each EM patient has blood taken at the day of hospitalization: 04 ml of serum for measuring granulysin and 13 cytokines.

- Each healthy control has one blood sample taken: 04 ml of serum for measuring granulysin and 13 cytokines.

All blood and serum samples were stored at -80°C until tests were performed.

2.2.5. Techniques performed in the study

2.2.5.1. Typing HLA-B

It was performed at National Institute of Hematology and Blood Transfusion in Hanoi, Vietnam. Polymerase chain reaction-sequence specific oligonucleotide (PCR-SSO) assay and LuminexTM Multiplex Technology were used to analyze *HLA-B* alleles. To summarize, PCR products were hybridized against a panel of oligonucleotide probes coated on polystyrene microspheres that have sequences which complement stretches of polymorphic sequence within the target *HLA-B* alleles. By using a colorimetric reaction and fluorescence detection technology we were able to see the amplicon probe complex.

2.2.5.2. Measuring serum granulysin levels

It was conducted at the Department of Immunology, Military Medical Academy by using ELISA (Human Granulysin ELISA Kit, MELSIN, China). The kit used ELISA-double antibody sandwich principle to assess granulysin levels.

2.2.5.3. Measuring serum levels of 13 cytokines

It was conducted at the Department of Immunology, Military Medical Academy. We used the fluorescence covalent microbead immunosorbent assay (FCMIA) (ProcartaPlex Immunoassay Panels kit, Thermo Fisher Scientific, USA) for simultaneously detecting 13 cytokines: GM-CSF (granulocyte-macrophage-colony-stimulating factor), IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-17A and TNF-α.

2.3. Statistical analysis

Data entry and analysis were conducted by using SPSS software version 16.0 (IBM, Armonk, NY, USA). The Mann-Whitney U test and Wilcoxon test was used to compare quantitative variables. The χ^2 test was used to compare qualitative variables. Differences were considered to be statistically significant at $p<0.05$.

2.4. Ethical clearance

The study was approved by the Ethical Review Committee on Research Involving Human Subjects, Hanoi Medical University (Number 04NCS17, dated 8th February 2018). Written consent was obtained from all participants.

CHAPTER 3. RESULTS

3.1. Identify some gens (HLA-B alleles) relating to SJS/TEN in Vietnamese people

3.1.1. Demographic characteristics and causative drugs in 83 SJS/TEN patients

Table 3.1. Demographic characteristics of patients

	SJS (n=38)	TEN (n=45)	SJS/TEN (n=83)
Age, years	47.9 ± 16.9	51.4 ± 14.9	49.8 ± 15.9
Group of age, n (%)			
<30	6 (15.8)	5 (11.1)	11 (13.3)
30-39	8 (21.1)	5 (11.1)	13 (15.7)
40-50	6 (15.8)	7 (15.6)	13 (15.7)
>50	18 (47.3)	28 (62.2)	46 (55.3)
Gender, n (%)			
Male	20 (52.6)	22 (48.9)	42 (50.6)
Female	18 (47.4)	23 (51.1)	41 (49.4)

The table 3.1 shows that the average age of the SJS/TEN patients was 49.8 (range 17-77). The sex distribution was equal (male: 50.6%, female: 49.4%). The most common group of age was over 50 (55.3%).

Table 3.2. Causatives drugs of 83 SJS/TEN patients

Drugs	SJS		TEN		SJS/TEN	
	n	**%**	**n**	**%**	**n**	**%**
Allopurinol	12	31.7*	4	8.9*	16	19.3
Carbamazepine	10	26.3	6	13.4	16	19.3
Traditional medicine	2	5.2**	15	33.4**	17	20.5
Antibiotics[ε]	3	7.9	2	4.4	5	6
Diclofenac	0	0	1	2.2	1	1.2
Phenylbutazone	1	2.6	0	0	1	1.2
Piracetam	0	0	1	2.2	1	1.2
Piroxicam	0	0	1	2.2	1	1.2
Thalidomide	0	0	1	2.2	1	1.2
Lamotrigine	0	0	1	2.2	1	1.2
Unknown	10	26.3	13	28.9	23	27.7
Total	38	100	45	100	83	100

*[ε]amoxicilline, cefalexine, zodocine (metronidazole and spiramycine), metronidazole; * p<0.05; **p<0.05 (comparing SJS group with TEN group)*

The table 3.2 shows that in SJS/TEN, traditional medicine was the most common causative drugs, followed by allopurinol and carbamazepine. There were 23 patients with unknown culprit drugs. The frequence of traditional medicine in the TEN group was higher than that in the SJS group, the rate of allopurinol in the SJS group was higher than that in the TEN group, the difference was statistically significant with $p<0.05$ (test $\chi 2$). The other drugs did not differ between the two groups.

3.1.2. Identify some gens (HLA-B alleles) relating to SJS/TEN in Vietnamese people

Table 3.3. HLA-B genotypes (n=60)

Patient No.	Diagnosis	Causative medicine	Allele 1	Allele 2
1	SJS	Carbamazepine	15:02	15:25
2	SJS	Carbamazepine	15:02	40:01
3	SJS	Carbamazepine	15:02	51:01
4	SJS	Carbamazepine	15:02	56:04
5	SJS	Carbamazepine	15:02	15:02
6	SJS	Carbamazepine	15:02	46:01
7	SJS	Carbamazepine	15:02	46:01
8	TEN	Carbamazepine	15:02	44:03
9	TEN	Carbamazepine	15:02	40:01
10	TEN	Carbamazepine	15:02	55:02
11	TEN	Carbamazepine	15:02	54:01
12	TEN	Carbamazepine	15:02	13:01
13	TEN	Carbamazepine	15:02	13:01
14	SJS	Unknown	15:02	51:02
15	SJS	Unknown	15:02	13:01
16	SJS	Unknown	15:02	73:01
17	SJS	Unknown	15:02	57:01
18	SJS	Unknown	15:02	40:01
19	TEN	Unknown	15:02	35:05
20	TEN	Unknown	15:02	15:02
21	TEN	Traditional medicine	15:02	51:02
22	TEN	Traditional medicine	15:02	54:01
23	SJS	Allopurinol	15:02	58:01
24	SJS	Zodocine	15:02	15:02
25	TEN	Piroxicam	15:02	15:02
26	SJS	Allopurinol	58:01	13:02
27	SJS	Allopurinol	58:01	57:01
28	SJS	Allopurinol	58:01	40:06
29	SJS	Allopurinol	58:01	13:01
30	SJS	Allopurinol	58:01	46:01
31	SJS	Allopurinol	58:01	51:01

Patient No.	Diagnosis	Causative medicine	Allele 1	Allele 2
32	SJS	Allopurinol	58:01	38:02
33	SJS	Allopurinol	58:01	40:01
34	TEN	Allopurinol	58:01	40:01
35	TEN	Allopurinol	58:01	46:01
36	TEN	Allopurinol	58:01	56:04
37	TEN	Allopurinol	58:01	40:01
38	TEN	Unknown	58:01	07:02
39	SJS	Unknown	58:01	35:05
40	SJS	Unknown	07:05	46:01
41	SJS	Unknown	38:02	46:01
42	TEN	Unknown	15:25	46:01
43	TEN	Unknown	13:01	51:02
44	TEN	Unknown	56:04	51:02
45	SJS	Unknown	13:01	38:02
46	SJS	Unknown	13:01	07:02
47	SJS	Unknown	07:05	51:01
48	TEN	Unknown	07:05	51:01
49	SJS	Unknown	56:02	38:02
50	TEN	Unknown	07:05	73:01
51	TEN	Traditional medicine	51:02	46:01
52	TEN	Traditional medicine	15:01	51:02
53	TEN	Traditional medicine	15:21	51:02
54	TEN	Traditional medicine	37:01	51:02
55	TEN	Traditional medicine	15:25	51:02
56	TEN	Traditional medicine	54:01	54:01
57	TEN	Diclofenac	13:01	51:01
58	SJS	Phenylbutazone	35:05	38:02
59	TEN	Thalidomide	15:21	57:01
60	TEN	Lamotrigine	15:21	46:01

**Zodocine includes metronidazole and spiramycin; **treatment of multiple myeloma*

The table 3.3 shows that the most common *HLA-B* allele was *HLA-B*15:02* (25/60 patients, 41.7%), followed by *HLA-B*58:01*

(15/60 patients, 25%) and *HLA-B*46:01* (9/60 patients, 15%). Other less common alleles were *HLA-B*51:01* (eight patients,13.2%); *HLA-B*13:01* (eight patients, 13.2%); *HLA-B*07:05* (four patients, 6.6%).

Of the 25 patients possessing *HLA-B*15:02* allele, the culprit drugs were carbamazepine among 13 patients (52%), traditional medicine two patients (8%); allopurinol one patient (4%) (who had *HLA-B* phenotype was *15:02* and *58:01*); piroxicam one patient (4%); zidocine (metronidazole and spiramycin) one patient (4%), and unknown drugs seven patients (28%). Of the 15 patients with *HLA-B*58:01*, there were 13 patients with allopurinol-induced SJS/TEN and two patients with unknown causative drugs. Among eight patients with traditional medicine-induced SJS/TEN, there were six *HLA-B*51:02* allele carriers (75%).

3.2. Investigate serum levels of granulysin and 13 cytokines in SJS/TEN

Levels of serum granulysin and 13 cytokines were measured in 48 patients with SJS/TEN, 43 patients with EM and 20 HCs.

3.2.1. Serum granulysin levels

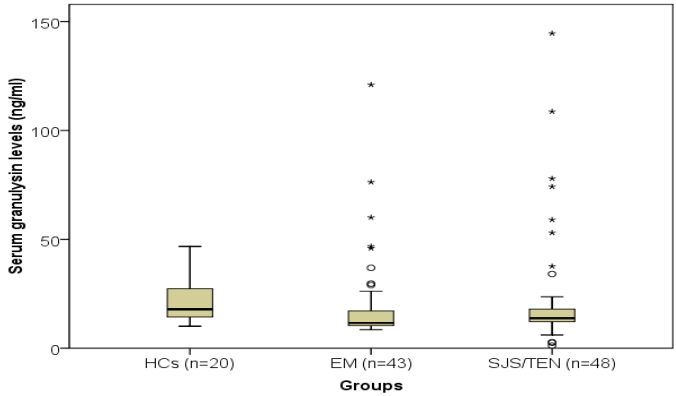


Figure 3.1. Serum granulysin levels of the three groups

The figure 3.1. shows that serum granulysin levels of SJS/TEN patients (23.0 ng/ml, range 1.2-144.6 ng/ml) were significantly higher than those of EM patients (20.1 ng/ml, range 8.5-121 ng/ml, $p<0.05$) and those of HCs (20.8 ng/ml, range 10.1-46.7 ng/ml, $p<0.05$)

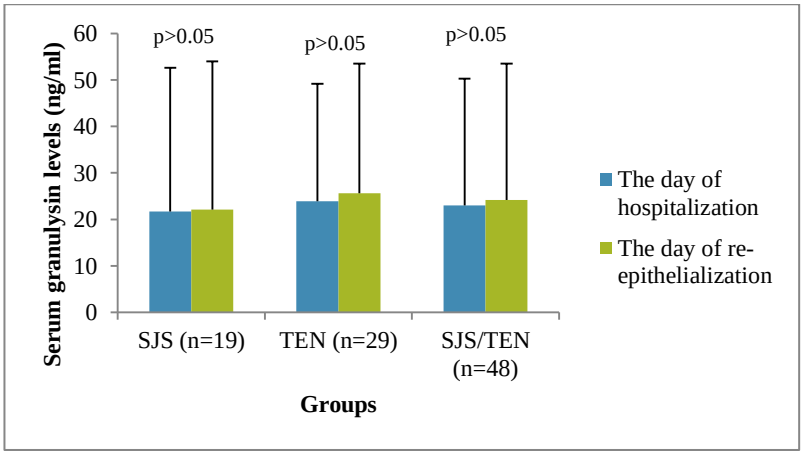


Figure 3.2. Serum granulysin levels at two time points

The figure 3.2 shows that in patients with SJS/TEN, serum granulysin concentrations at the day of hospitalization were not different from those at the day of re-epithelialization.

3.2.2. Serum levels of 13 cytokines

Table 3.4. Serum mean levels of 13 cytokines (pg/ml) in 3 groups

Cytokines	**SJS/TEN (n=48)**	**EM (n=43)**	**HCs (n=20)**	p1; p2
GM-CSF	10.6 ± 19.2	6.3 ± 14.6	10.4 ± 15.6	**<0.05**; >0.05
IFN-γ	32.1 ± 91.6	6.1 ± 13.4	0.4 ± 0.9	**<0.05; <0.01**
IL-1β	26.4 ± 81.7	33 ± 179	98.6 ± 130.6	>0.05; <**0.001**
IL-2	7.6 ± 17	4.8 ± 12	4.2 ± 4,5	>0.05; >0.05
IL-4	3 ± 7.5	1.6 ± 4.1	2.5 ± 3	>0.05; <**0.05**
IL-5	4.5 ± 9.8	6.9 ± 18.5	8.2 ± 14.5	>0.05; >0.05
IL-6	455.0 ± 1696.9	302.6 ± 1324	675.3 ± 1206	**<0.001;** >0.05
IL-8	364.6 ± 647.9	239.4 ± 546.8	916.1 ± 577.9	>0.05; <**0.001**
IL-10	8.4 ± 14.4	9.8 ± 15.4	4.4 ± 4.6	>0.05; >0.05
IL-12	1.9 ± 2.6	1 ± 2.5	2.6 ± 2.7	**<0.001;** >0.05
IL-13	1.6 ± 0.6	1.5	1.5	>0.05; >0.05
IL-17A	0.6 ± 1.1	0.4 ± 0.8	0.3 ± 0.1	>0.05; >0.05
TNF-α	32.6 ± 125	7.6 ± 26.9	27.7 ± 37.9	>0.05; <**0.05**

p1: comparing SJS/TEN group with EM group; p2: comparing SJS/TEN group with HCs group (Mann-Whitney U test)

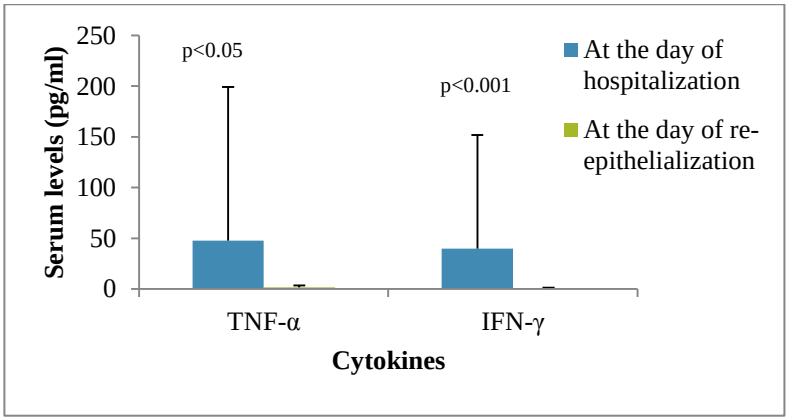
The table 3.4 shows that in patients with SJS/TEN, serum concentrations of GM-CSF, IFN-γ, IL-6 and IL-12 were higher than

those in the EM group; serum concentrations of IFN-γ, IL-4 and TNF-α in the SJS/TEN group were higher than those in the HCs group. Serum concentrations of IL-1β and IL-8 in the HCs group were higher than those in the SJS/TEN group. The difference is statistically significant with $p<0.05$.

Table 3.5. Comparison of serum mean concentration of cytokines (pg/ml) at two time points in 48 SJS/TEN patients

Cytokines	**At the day of hospitalization (n=48)**	**At the day of re-epithelialization (n=48)**	**p** (test Wilcoxon)
GM-CSF	10.6 ± 19.2	3.6 ± 3.7	**<0.05**
IFN-γ	32.1 ± 91.6	0.4 ± 0.9	**<0.001**
IL-1β	26.4 ± 81.7	1.9 ± 5,6	**<0.01**
IL-2	7.6 ± 17	3 ± 4.7	>0.05
IL-4	3 ± 7.5	1.1 ± 2.1	>0.05
IL-5	4.5 ± 9.8	1 ± 2.8	**<0.05**
IL-6	455.0 ± 1696.9	78 ± 341.6	**<0.001**
IL-8	364.6 ± 647.9	211.7 ± 542.1	>0.05
IL-10	8.4 ± 14.4	4.9 ± 7.4	>0.05
IL-12	1.9 ± 2.6	0.8 ± 0.8	**<0.01**
IL-13	1.6 ± 0.6	1.5 ± 0	>0.05
IL-17A	0.6 ± 1.1	1.2 ± 7.9	>0.05
TNF-α	32.6 ± 125	2.7 ± 7.9	**<0.01**

The table 3.5 shows that serum concentrations of GM-CSF, IFN-γ, IL-1β, IL-5, IL-6, IL-12 and TNF-α at the day of re-epithelialization were significantly lower than those at the day of hospitalization ($p<0.05$). Concentrations of other cytokines did not change between the two time points.



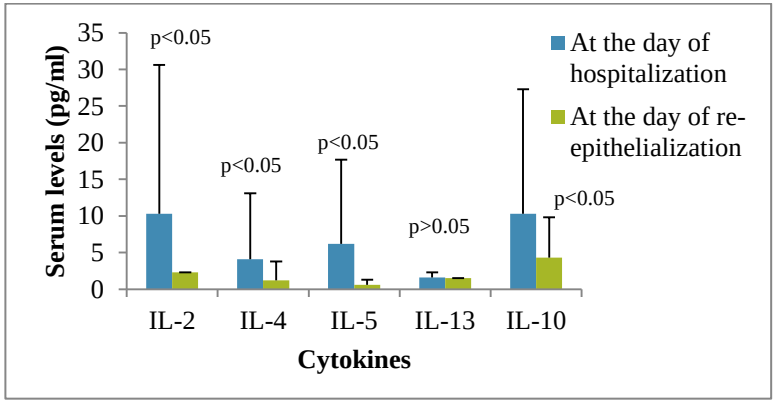


Figure 3.3. Alteration of some cytokines before and after systemic corticosteroid therapy in SJS/TEN patients (n=32)

The figure 3.3 shows that serum concentrations of TNF-α, IFN-γ, IL-2, IL-4, IL-5 and IL-10 after treatment decreased significantly compared to before treatment ($p<0.05$). The IL-13 concentration did not differ between the two time points ($p>0.05$). The most significant change is with serum IFN-γ levels ($p<0.001$).

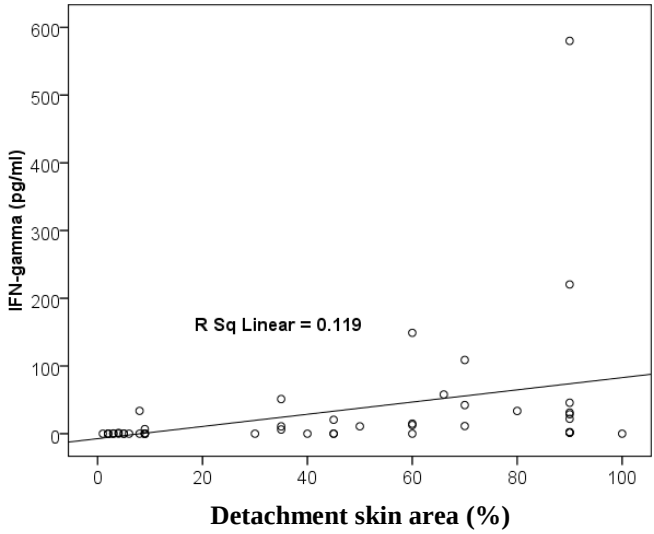


Figure 3.4. Correlation of detachment skin area with IFN-γ levels

Among 13 cytokines and granulysin, only serum IFN-γ concentration correlated with the area of skin detachment in SJS/TEN patients with r=0.345, p<0.05 (as shown in Figure 3.4). However, in the TEN group, there was no correlation between the area of skin detachment and IFN-γ serum concentration (r=0.241, p>0.05).

CHAPTER 4. DICUSSION

4.1. Identify some gens (HLA-B alleles) relating to SJS/TEN in Vietnamese people

4.1.1. Demographic characteristics and causative drugs in 83 SJS/TEN patients

Our research results showed that the average age of SJS/TEN patients was 49.8, the most common group of age was over 50 years old. These findings were similar to those of previous studies. Luong et al revealed that drug-induced SJS/TEN could happen in all age, the

mean age was 47.3 (range 11-79). The most ordinary age of SJS/TEN patients was 40-50 (21,7%). Su et al showed that the mean age of 77 Taiwanese patients with SJS/TEN was 52.8, that of 52 European patients was 49.1. However, the age of patients with SJS/TEN may be younger on certain epidemic conditions. For example, in sub-Saharan region, the mean age of patients with SJS/TEN was 32.3 years. Among them, there was a high rate of positive HIV, the most common culprit drugs were anti-tuberculous agents, sulfonamide and nevirapine.

Male patients was 50,6%, similar to female patients. This result was consistent with those of other studies. In general, the distribution of sex in patients with SJS/TEN is quite equal. Exceptionally, the study of Saka revealed that in SJS/TEN, male patient was 36.2, female patient was 63.8%. This can be explained by the difference with regard to the place of study, the original diseases of taking medicine among SJS/TEN patients. Saka conducted her study at Togo, Africa where the incidence of HIV is high, it affects more on women than men. Among 115 SJS/TEN patients who underwent HIV test, the positive result was 59.1%.

This study demonstrated that the most popular causative drug SJS/TEN was traditional medicine. Other classic drugs such as allopurinol and carbamazepine were quite frequent but lower than other previous studies. A study at Bach Mai hospital in 2014 showed that there were 33 drugs that caused SJS/TEN, in which allopurinol was 21.7%; traditional medicine was 21.7%; followed by carbamazepine (20%). In Vietnam, the use of traditional medicine and other drugs without prescription is still prevalent. Consequently, it is more difficult to identify the offending drugs as well as to identify ingredients in traditional medicine. In this study, we identified the causative drugs based on ALDEN, did not perform

allergic tests such as lymphocyte transformation test and patch test. In fact, identifying culprit drugs is still a difficult problem. *In vitro* tests have low sensitivity and specificity because some drugs, when entering the body, are metabolized through the liver, to become metabolites that play a role as hapten. Researchs in Europe showed that ALDEN may be a good tool in clinical practice.

4.1.2. Identify some gens (HLA-B alleles) relating to SJS/TEN in Vietnamese people

Among 60 SJS/TEN patients, *HLA-B*15:02* was the most common allele (25/60 patients; 41.7%), which is higher than that in general Kinh population. This finding might be due to the fact that all participants in our study were SJS/TEN patients, common culprit drug was carbamazepine (13/60 patients, 21.7%). Of the 25 *HLA-B*15:02* carriers in this study, there were 13 carbamazepine-induced SJS/TEN patients (52%). Studies in Asian countries have demonstrated the strong association between carbamazepine-induced SJS/TEN and *HLA-B*15:02*. A previous study in Vietnam shows the most common *HLA-B* allele among general Kinh population is *HLA-B*15:02*. While, a study in Korean population shows that *HLA-B*44:03* is the most frequent type in *HLA-B* genes; allele frequency of *HLA-B*15:02* is 0.3%. Among Thai population, the most common *HLA-B* alleles observed is *HLA-B*46:01* (11.5%), while, the *HLA-B*15:02* allele frequency is 8.2%. These show the diversity of *HLA-B* polymorphisms in different ethnicities. The high frequency of *HLA-B* pharmacogenomic markers in population emphasizes the importance of such screening to predict and avoid SCARs.

In the present study, there were 15 out of 60 patients carry *HLA-B*58:01* allele (25%). Among them, there were 13 patients with allopurinol-induced SJS/TEN and two patients with unknown drug-

induced SJS/TEN. The strong association between *HLA-B*58:01* and allopurinol-induced SJS/TEN has been shown in Asian as well as in Caucasians, while the association between *HLA-B*15:02* and carbamazepine has been only observed in Asian ancestries. We found 15% of SJS/TEN patients (nine patients) having *HLA-B*46:01* allele, which is similar to the findings from previous studies among Vietnamese, Korean and Thai. A study in Vietnam shows that the prevalence of *HLA-B*46:01* in the carbamazepine-induced SCARs group was significantly lower than that in the carbamazepine-tolerant epilepsy patient group. This allele may be considered as a protective factor against the development of carbamazepine-induced SCARs in Vietnamese. But in the present study, *HLA-B*46:01* allele did not reveal the association with carbamazepine-induced SJS/TEN. Therefore, the role of *HLA-B*46:01* in SCARs needs to be investigated further.

We also observed an allopurinol-induced SJS patient possessing *HLA-B*15:02.* In fact, this patient's *HLA-B* genotype was *15:02* and *58:01*. He was at high risk of being allergic to both carbamazepine and allopurinol. In addition, there were two traditional-induced SJS/TEN patients and seven unknown drug-induced SJS/TEN patients all carrying *HLA-B*15:02;* two patients with unknown causative drugs carrying *HLA-B*58:01*. Typing of *HLA-B* alleles in these patients could be significant to avoid the high risk of drug-induced SCARs. Interestingly, among eight patients with traditional medicine-induced SJS/TEN, there were six patients with *HLA-B*51:02* allele (75%). In Vietnam, the use medicines without prescription is rather common, even mixing western medicines in some traditional medicines intentionally done by traditional healers is not rare. Consequently, it is more difficult to identify the offending

drugs. Therefore the possible association between *HLA-B*51:02* allele and traditional medicine-induced SJS/TEN needs to be investigated further, and, studies about *HLA-B* genotypes in SCARS are crucial and may provide evidences for advising certain patients avoid using certain medicines.

4.2. Investigate serum levels of granulysin and 13 cytokines in SJS/TEN

4.2.1. Serum granulysin concentration

This study showed that serum granulysin levels in SJS/TEN patients were statistically higher than those in EM patients. Erythema multiforme is a cutaneous reaction characterized by typical or atypical target lesions that mimic cutaneous manifestations of SJS/TEN in the early phase. The causes of EM may be viral infection or drug reaction. Serum granulysin can be also elevated in viral infected conditions such as virus-associated EM. Nevertheless, our results may imply the use of serum granulysin as a biomarker for distinguishing between EM and SJS/TEN. In fact, serum granulysin levels can be affected by some factors such as infection, sepsis, cancers, age, immunological condition, hence, it was a large range among our SJS/TEN patients. However, systemic corticosteroid treatment before hospitalization did not affect serum granulysin level in patients with SJS/TEN.

At the day of re-epithelialization, serum granulysin levels were not significantly different compared with those at the day of being hospitalized. These findings could be explained by the fact that nearly all the 48 patients with SJS/TEN in this study had their serum granulysin measured after the onset (mean 5.9 days) when serum granulysin levels could be decreased. Abe *et al* showed that in SJS/TEN, serum granulysin levels were elevated before the onset

(when skin detachment or mucosal lesion develop) and higher than those of ordinary drug-induced skin reaction. But the mean level of serum granulysin in this study were consistent with that in Abe's study (24.8 ng/ml). It could be due to the difference in the size of samples in each study.

4.2.2. Serum levels of some cytokines in SJS/TEN

In our study, the TNF-α concentration in SJS/TEN patients was 32.6 pg/ml, higher than that in the EM group (7.6 pg/ml) and HCs (27.7 pg/ml). After the treatment, when patients got re-epithelialization, the TNF-α concentration was only 2.7 pg/ml, much lower than at admission, the difference was statistically significant with $p<0.01$. The serum TNF-α concentration in this study was lower than that in Su's study but higher than that in Wang's study. Research on the role of TNF-α could open up a new therapeutic direction by using biotherapy in SJS/TEN.

The serum IFN-γ concentration of SJS/TEN patients in this study was higher than that of the EM and HCs groups. At the day of re-epithelialization, the serum IFN-γ concentration of SJS/TEN patients was only 0.4 pg/ml, much lower than that at the day of hospitalization ($p<0.001$). Thus, in SJS/TEN, IFN-γ is produced a lot by Th1 cells and NK cells. The previous research also showed that in bullous fluid of SJS/TEN, there is a very high concentration of IFN-γ, secreted by mononuclear cells in the bullous fluid. This can be a good serum marker to assess the progression and severity of SJS/TEN as well as to differentiate SJS/TEN from EM.

In addition, IFN-γ is also a biomarker for evaluating the efficacy of treatment in SJS/TEN. In the Hirahara's study, serum levels of IFN-γ and TNF-α were measured in 5 SJS/TEN patients treated with pulse-dose methylprednisolone. On the fourth day of treatment, the

mean concentration of these cytokines decreased compared to that of before treatment, but only changed significantly with IFN-γ. On day 19, the decrease was significant for both IFN-γ and TNF-α. In our study, 32 SJS/TEN patients treated with systemic corticosteroid were measured serum level of cytokines. After treatment, cytokine concentrations decreased compared to before treatment but the most significant was with IFN-γ ($p<0.001$). Systemic corticosteroid work to reduce proinflammatory cytokines, helping patients with SJS/TEN avoid death. Serum IFN-γ concentrations were much higher in SJS/TEN patients with fever than in patients without fever (50.2 pg/ml vs 7.7 pg/ml, $p<0.01$).

Th2-derived cytokines (IL-4, IL-5 and IL-13) did not differ between the SJS/TEN and EM groups, between at the day of hospitalization and at the day of re-epithelialization. Our results were different from those of the previous research. According to Quaglino et al, the SJS/TEN patients had an increase in serum IL-13 levels while the EM patients did not. In our study, the concentrations of Th1-derived cytokines (TNF-α, IFN-γ) in the SJS/TEN group were higher than those in the EM group, especially at the day of hospiatalization. The concentrations of Th2-derived cytokines (IL-4, IL-5 and IL-13) in the SJS/TEN group were not higher than those in the EM group. They had no significant change according to the progress of SJS/TEN. This proves that, in SJS/TEN, Th1 may play a more important role than Th2. Our results were also consistent with the previous research. The role of Th2 is more important in other skin diseases such as atopic dermatitis, host-versus-graft disease and leishmaniasis.

CONCLUSIONS

- *HLA-B*15:02* was the most common *HLA-B* allele in Vietnamese patients (Kinh ethnicity) with Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN). *HLA-B*51:02* allele may play an important role in the pathogenesis of the traditional medicine-induced SJS/TEN. There may possibly have a link between *HLA-B* alleles and causative drugs of SJS/TEN. The *HLA-B* genotypes may be useful for suggesting the causative drugs in some cases and preventing SJS/TEN.

- Serum granulysin levels were significantly higher in SJS/TEN patients than in EM patients. After the onset of SJS/TEN, serum granulysin was not associated with the severity of the diseases.

- In SJS/TEN patients, serum levels of GM-CSF, TNF-α, IFN-γ, IL-6 and IL-12 were significantly higher than those in EM patients. At the day of re-epithelialization, serum levels of GM-CSF, TNF-α, IFN-γ, IL-1β, IL-5, IL-6 and IL-12 were significantly lower than those at the day of hospitalization. Serum level of IFN-γ may be a good biomarker to differentiate SJS/TEN from EM as well as to evaluate the progress and the severity of SJS/TEN.

IMPLICATIONS

1. It is advisable to identify *HLA-B* allele before indicating certain drugs with a high risk of causing SJS/TEN. For example *HLA-B*15:02* for carbamazepine, *HLA-B*58:01* for allopurinol.

2. Based on significant changes in concentration of some cytokines (TNF-α, IFN-γ), the new biological therapy should be applied in the treatment of SJS/TEN, the new technique should be used in the diagnosis of causative drugs in SJS/TEN.