HIGH LEVELS OF SERUM INTERFERON-GAMMA IN PATIENTS WITH STEVENS-JOHNSON SYNDROME AND TOXIC EPIDERMAL NECROLYSIS

Tran Thi Huyen✉, Pham Dinh Hoa, Pham Thi Lan

Hanoi Medical University, Hanoi, Vietnam
National Hospital of Dermatology and Venereology, Hanoi, Viet Nam

Stevens - Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are acute, life - threatening drug reactions. Cytotoxic proteins and cytokines may play an important role in the pathogenesis of SJS/TEN. This study was conducted to measure serum interferon - gamma (IFN - γ) levels in patients with SJS/TEN as well as to investigate possible associations between serum IFN - γ levels and the progress of SJS/TEN. In total, 48 SJS/TEN patients, 43 erythema multiforme (EM) patients and 32 healthy controls (HCs) were enrolled. We measured serum IFN - γ levels by using FCMIA, serum granulysin levels by using ELISA. The average level of serum IFN - γ in SJS/TEN patients was 32.1 pg/ml, significantly higher than that of HCs (0.3 pg/ml; $p < 0.05$), and that of EM (6.1 pg/ml, $p < 0.05$). At the time of re - epithelialization, serum IFN - γ level of patients with SJS/TEN was 0.4 pg/ml, significantly lower as compared with those at the day of being admitted to the hospital (32.1 pg/ml, $p < 0.001$). There was a weak correlation between IFN - γ and granulysin levels in the serum of SJS/TEN patients. Serum IFN - γ level may be a good biomarker to differentiate SJS/TEN from EM as well as to evaluate the progress of SJS/TEN.

Keywords: Steven-Johnson syndrome, toxic epidermal necrolysis, granulysin, interferon-gamma, erythema multiforme

I. INTRODUCTION

Stevens - Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are severe cutaneous adverse drug reactions (SCARs) characterized by extensive epidermal necrolysis, blisters and skin sloughing. The most common causative drugs of SJS/TEN are carbamazepine, allopurinol, abacavir, phenytoin and lamotrigine.[1] The period between taking a drug and onset of symptoms ranges from a few days to two months. Stevens - Johnson syndrome and TEN are categorized based on the percentage of epidermal detachment area: i) SJS: less than 10%, ii) TEN: greater than 30%, iii) and SJS/TEN overlap: 10 - 30%.[2]

The pathogenesis of SJS/TEN is not fully understood, but there are some immunological and genetic factors which are believed to be involved.[3] 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.[4] CD8+ cytotoxic T cells (CTLs) and natural killer (NK) cells play a principal role in the pathogenesis of SJS/TEN.[5] 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 disseminated

Corresponding author: Tran Thi Huyen,
Hanoi Medical University,
Email: drhuyentran@gmail.com
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keratinocyte death,[3] among them, granulysin is a key mediator6. Serum interleukin (IL) - 13 levels are increased in patients with SJS/TEN but not in those with EM.[7] Increased levels of IL - 15 were associated with in - hospital mortality in SJS/TEN.[1] There were high concentrations of IFN - γ, soluble TNF - α (tumor necrosis factor - alpha) and soluble Fas - ligand in the blister fluid of TEN patients compared with burn fluid.[8]

Erythema multiforme (EM) may have skin manifestation similar to SJS/TEN with typical or atypical target lesions. Serum IL - 17 levels may have prognostic and diagnostic value in patients with EM or SJS/TEN reactions, and can provide a valuable approach in management.[9] However, serum IFN - γ was not elevated in EM compared with HCs.[10] In Vietnam, to our knowledge, there are no published data on serum IFN - γ levels in SJS/TEN or EM. We conducted this study to measure serum IFN - γ levels in patients with SJS/TEN, EM and in HCs group as well as to investigate possible associations between serum IFN - γ levels and the progress of SJS/TEN, the correlation between IFN - γ and granulysin concentrations in the serum of patients with SJS/TEN.

II. METHODS

1. Participants

In total, 48 patients with SJS/TEN were enrolled at the National Hospital of Dermatology and Venereology (NHDV) and Bach Mai Hospital, Hanoi, Vietnam, from January 2018 to October 2019. The SJS/TEN patients with their vital signs, systemic symptoms and the percentage of body surface area affected (skin detachment) were examined. SJS and TEN were classified in accordance with Bastuji - Garin.[2] All patients were aged 18 or older. The onset in patients with SJS/TEN was defined as the day mucocutaneous or ocular lesions were first eroded or ulcerated. Re - epithelialization is defined as complete healing of the skin without any erosion.

There were 43 patients with EM recruited in this study (aged 41.4 ± 17.3, range 19 - 76 years, male 30.3%; female 69.7%). They had the presence of typical or atypical cutaneous target lesions, with or without mucous membrane lesions. The causes of EM were either drugs or unknown.

Healthy controls recruited were healthy medical staffs in the NHDV (aged 28.5 ± 4.7, range 22 - 46 years, male 50%; female 50%).

2. Methods

Measuring serum IFN - γ and granulysin levels

In the SJS/TEN group, blood samples were drawn at two time points: 1) at the day of hospitalization and 2) at the day of re - epithelialization. In the EM group, blood samples were taken at the day of hospitalization. All blood samples were left to coagulate at room temperature for 10 - 20 minutes, then centrifuged for 20 minutes at a speed of 2000 - 3000 r.p.m. Finally, serum was taken and stored at - 20^0C until it was time for granulysin measurement.

We measured serum IFN - γ levels by using the fluorescence covalent microbead immunosorbent assay (FCMIA) (ProcartaPlex Immunoassay Panels kit, Thermo Fisher Scientific, USA). By using ELISA (Human Granulysin ELISA Kit, MELSIN, China) we quantified the granulysin level in all serum samples.

3. Statistical analysis

Data entry and analysis were conducted by using SPSS software version 16.0 (IBM, Armonk, NY, USA). The Mann - Whitney U and the Wilcoxon tests were used to compare quantitative variables. The Pearson test was used to investigate the correlation between two

variables. Differences were considered to be statistically significant at $p < 0.05$.

4. Ethical clearance

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

III. RESULTS

1. Demographic and clinical characteristics of SJS/TEN patients

There were 48 patients with SJS/TEN (19 SJS patients, 39.5%; 29 TEN patients, 60.5%) participating in the study. Characteristics of patients with SJS/TEN are shown in Table 1. The mean age of patients was 49.3 ± 15.0, range 19 - 77 years (47.9% males; 52.1% females). The most common causative drugs of SJS/TEN were traditional medicine (29.1%), carbamazepine (12.5%) and allopurinol (12.5%). There were 14 patients (29.2%) with unknown culprit drugs. Twenty patients (41.7%) were treated with systemic corticosteroid before being hospitalized. The time between the onset and the day of hospitalization was 5.9 ± 2.7 days (range 2 - 18 days). The main treatment was systemic corticosteroid (72.9%). Ciclosporin A was indicated in 11 patients (22.9%). There were two patients (4.2%) treated with supportive care only. The mean time of re - epithelialization was 15.9 days (range 9 - 31 days).

2. Serum IFN - γ levels in SJS/TEN patients

The mean level of serum IFN - γ among SJS/TEN patients was 32.1 pg/ml (range 0 - 579.9 pg/ml). It was significantly higher than that of patients with EM (6.1 pg/ml; range 0 - 70.6 pg/ml; $p < 0.05$) and that of HCs (0.3 pg/ml; range 0.07 - 3.0 pg/ml; $p < 0.05$), as shown in Figure 1. There was a significant difference with regard to serum IFN - γ levels among SJS and TEN patients (2.6 pg/ml versus 52.1 pg/ml; $p < 0.001$), as shown in Figure 2. In the SJS/TEN group, serum IFN - γ levels were higher in the 25 samples collected within 5 days of the onset (49.1 pg/ml; range 0.1 - 580 pg/ml) than in the 23 samples collected 5 days after the onset (12.9 pg/ml; range 0.1 - 108.9 pg/ml) but it was not significantly different ($p > 0.05$). In 20 patients treated with systemic corticosteroids before being hospitalized, serum IFN - γ levels were 10.5 pg/ml (range 0.1 - 108.9 pg/ml); it was not significantly lower than that of the 21 patients without systemic corticosteroid before hospitalization (45 pg/ml, $p = 0.52$). In 27 SJS/TEN patients with fever, serum IFN - γ levels were 50.2 pg/ml (range 0.07 - 579.9 pg/ml), which was significantly higher than those in patients without fever (7.7 pg/ml, range 0.07 - 57.9 pg/ml, $p < 0.05$).

At the time of re - epithelialization, serum IFN - γ levels of patients with SJS/TEN were 0.4 ± 0.9 pg/ml (range 0.1 - 4 pg/ml), significantly lower compared with those at the day of being admitted to hospital (32.1 pg/ml; range 0.1 - 579.9 pg/ml; $p < 0.001$), as shown in Figure 3.

Serum granulysin levels in SJS/TEN patients were 23.0 ng/ml (range 1.2 - 144.6 ng/ml). There was a weak correlation between serum IFN - γ levels and serum granulysin levels ($r = 0.368$, $p = 0.011$), as shown in Figure 4.

IV. DISCUSSION

In this study, we demonstrated that serum IFN - γ levels were significantly higher in patients with SJS/TEN compared with HCs; they were also higher in patients with TEN compared with SJS patients and in SJS/TEN patients with fever compared with SJS/TEN patients without fever.

IFN - γ is a pro - inflammatory cytokine that is mainly produced by activated NK cells, Th1 and CD8+ cytotoxic T cells11. Its production is

tightly regulated and stimulated by macrophage - derived cytokines, especially TNF - α, IL - 2 and IL - 18.[11] Cellular effects of IFN - gamma include up - regulation of pathogen recognition, the antiviral state, inhibition of cellular proliferation and effects on apoptosis, activation of microbicidal effector functions, immunomodulation, and leukocyte trafficking. IFN - γ is normally not detectable in the plasma of healthy humans, but its levels can be elevated in patients with sepsis or systemic inflammatory response syndrome,[11] in which body temperature may be higher than 38^0C or lower than 36^0C.[11] In SJS/TEN, IFN - γ has been reported to play an important role by initiating the cytotoxic activities, which is a shared mechanism connecting the involvement of TNF - α and FasL (Fas ligand/CD95L/CD178). The apoptotic effects of IFN - γ can also be explained by its transcriptional regulation of a variety of genes that are vital for apoptosis, such as TNF - a receptor, Fas/FasL, caspase - 1, - 4, and - 8. Finally, IFN - γ contributes to the antigen processing and presentation and thus stimulate the cell - mediated immunity by upregulation of MHC molecules, further supporting a pathogenic role in SJS/TEN.[5]

Caproni et al shows that IFN - γ may contribute to the pathogenesis of both EM and SJS/TEN.[12] IL - 2, IL - 5 and IL - 13 may contribute to the cutaneous immuno - inflammation in these diseases. Chemokine receptors may be involved strongly in the recruitment of inflammatory cells in skin lesions. There was a sharp polarization towards a Th1 pattern in EM, while the SJS/TEN lesions showed a mixed Th1/Th2 pattern.[12] In our study, serum IFN - γ levels in patients with SJS/TEN was significantly higher than those in patients with EM. In patients with EM, they were higher than those in HCs. These findings revealed that in the pathogenesis of SJS/TEN and EM, a Th1 pattern plays an important role. However, our results were not consistent with a previous study. Akkurt et al showed that serum IFN - γ levels in 32 patients with EM were not significantly different compared to HCs.[10] Nevertheless, Nassif et al revealed that IFN - γ was the cytokine with the highest concentration in blister fluid of TEN, and always present in TEN when not found in burns.[8] In the present study, we did not investigate IFN - γ levels in blister fluid.

In a recent study, Su SC et al reported that some cytokine or cytotoxic proteins such as IL - 151, IL - 179 and granulysin correlated with the severity of the mortality of SJS/TEN1. In this study, we could not investigate the correlation between serum IFN - γ levels and SCORE of TEN (SCORTEN) because the serum bicarbonate test was not available in the study settings. However, we found that serum IFN - γ levels in patients with TEN were significantly higher than those in patients with SJS. So we concluded that IFN - γ may be associated with the severity of SJS/TEN.

Furthermore, IFN - γ is considered to be a good biomarker to evaluate the response of treatment in SJS/TEN. Serum IFN - γ and TNF - α levels were significantly decreased after 4 days of pulse methylprednisolone therapy. This therapy reduced levels of proinflammatory cytokines, hence the SJS/TEN patients could avoid mortality.[13] In our study, at the day of re - epithelialization, serum IFN - γ levels in SJS/TEN patients sharply decreased compared with those at the day of hospitalization. Some SJS/TEN patients had taken systemic corticosteroids before the day of hospitalization. We should exclude them from the study but such a study is not realistic. In fact, we analyzed the effect of taking corticosteroid before hospitalization on IFN - γ levels and found that there was not a significant difference. The decreased IFN - γ levels reported on the day of re - epithelialization

may be due to both the treatment (corticosteroid, ciclosporin A) and the spontaneous progression of SJS/TEN.

Granulysin is a cytolytic molecule presenting in human CTL and NK cell granules and is lytic against a variety of tumor cell targets and microbes. In combination with purified perforin, recombinant granulysin breaks up 90% of intracellular Mycobacterium tuberculosis, inducing lesions on the mycobacterial cell surface.[14] In the pathogenesis of SJS/TEN, upon the activation, granulysin and IFN - γ are produced simultaneously by NK cells and CD8+ cytotoxic T cells.[5] This can explain for the correlation between these two biomarkers in this study, although it was not strong. Granulysin is a key mediator for the extensive death of keratinocytes,[6] while, the activation of keratinocytes by IFN - γ is an essential step in making them sensitive to lysis by cutaneous T lymphocytes.[15]

V. CONCLUSION

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. There was a mild correlation between IFN - γ and granulysin levels in serum of SJS/TEN patients.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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