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Serum Granulysin in Differentiation of Stevens-Johnson Syndrome/toxic Epidermal Necrolysis and Erythema Multiforme

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Abstract

BACKGROUND: Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are acute, life-threatening drug reactions, which lead to massive epidermal necrolysis. Granulysin plays an important role as a key mediator for keratinocyte apoptosis in these conditions. Erythema multiforme (EM) may have skin manifestation similar to SJS/TEN.

AIMS: The aim of the study was to compare serum granulysin levels in patients with SJS/TEN and EM as well as to investigate a possible association between serum granulysin levels and the severity of SJS/TEN.

METHODS: In total, 48 patients with SJS/TEN, 43 patients with EM, and 20 health controls (HCs) were enrolled. We measured serum granulysin levels using enzyme-linked immunosorbent assay.

RESULTS: The average level of serum granulysin in the SJS/TEN patients was 23.0 ng/ml (range 1.2–144.6 ng/ml), significantly higher than that of EM group (20.1 ng/ml; range 8.5–121 ng/ml, $p < 0.05$) and HCs group (20.8 ng/ml; range 10.1–46.7 ng/ml, $p < 0.05$). Of 48 SJS/TEN patients, the 25 samples collected <6 days after onset showed higher level of serum granulysin (27.7 ng/ml; range 2.5–144.6 ng/ml) than those collected ≥6 days after onset (17.9 ng/ml; range 1.2–59 ng/ml; $p > 0.05$). No significant correlation was found between serum granulysin levels and the body surface area affected and the modified-SCORTEN. At the day of re-epithelialization, serum granulysin levels were not different compared with those at the day of hospitalization.

CONCLUSIONS: Serum granulysin levels are significantly higher in SJS/TEN group than in EM group. After the onset, serum granulysin levels in patients with SJS/TEN are not a good biomarker to evaluate the severity of the diseases.

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 [1], [2]. The most common causative drugs of SJS/TEN are carbamazepine, allopurinol, abacavir, phenytoin, and lamotrigine [1], [3], [4], [5]. The period between taking a drug and onset of symptoms ranges from a few days to 2 months [1], [2], [6]. SJS and TEN are categorized based on the percentage of epidermal detachment area: (i) SJS: <10%, (ii) TEN: >30%, and (iii) overlapping SJS/TEN: 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 [4], [5], [7], [8], [9]. There is a strong association between *HLA-B*15:02* and carbamazepine-induced SJS/TEN [10], [11], [12], *HLA-B*58:01* and allopurinol-induced SJS/TEN [13], [14], [15], and *HLA-B*57:01* and abacavir-induced SJS/TEN [15], [16], [17]. CD8+ cytotoxic T cells (CTLs) and natural killer (NK) cells play an important role in the pathogenesis of SJS/TEN [1], [4], [5], [8], [9]. The immune response may be triggered by binding an antigenic drug to a specific *HLA* on a keratinocyte [4], [5]. Specific T cell receptors recognize the drug-*HLA* complex and upon the activation, CD8+CTLs and NK cells produce cytokines, chemokines, and cytotoxic proteins, particularly granulysin, that cause extensive keratinocytes apoptosis [4], [5]. Chung reported that granulysin concentrations in the blister fluid of SJS/TEN patients were 2–4 times higher than perforin, granzyme B, or soluble Fas ligand concentrations [18]. Granulysin in the blister fluid was 15-kDa secretory form, and injection of it into mouse skin resulted in features mimicking SJS/TEN [5], [18], [19]. Abe showed that serum granulysin levels in patients with SJS/TEN were elevated before skin detachment or mucosal lesions developed [20]. The rapid immunochromatographic test has been developed for serum granulysin to diagnose early SJS/TEN [21].

Erythema multiforme (EM) may have skin manifestation similar to SJS/TEN [2], [22], [23], 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 [24]. However, this test is not clinically rapid. On the other hand, serum granulysin levels may be elevated among SJS/TEN patients [20], [25]. To the best of our knowledge, there is no study compared serum granulysin levels in SJS/TEN with those in EM. We conducted this study to investigate serum granulysin levels in patients with SJS/TEN and EM as well as possible associations between serum granulysin levels and the severity of SJS/TEN.

Materials and Methods

Participants

SJS/TEN patients

In total, 48 patients with SJS/TEN were enrolled from January 2018 to October 2019 at two hospitals in Hanoi, Vietnam (National Hospital of Dermato-venereology and Bach Mai Hospital). The SJS/TEN patients had their vital signs and systemic symptoms and the percentage of body surface area affected (skin detachment) examined. SJS and TEN were classified in accordance with Bastuji-Garin *et al*. [2]. All patients were aged 18 or older. The onset was defined as the day mucocutaneous or ocular lesions were first eroded or ulcerated [20]; the re-epithelialization was defined as complete healing of the skin without any erosion [26]. The severity of SJS/TEN patients was also assessed by the modified-SCORTEN (SCORe of TEN) scale that was composed of six well-defined criteria, including age >40 years, malignancy, tachycardia>120 beats/min, percentage of epidermal detachment >10%, serum urea >10 mmol/L, and serum glucose >14 mmol/L. This scale was based on SCORTEN scale of Bastuji-Garin *et al.* consisting of seven criteria [27].

EM patients and health controls (HCs)

There were 43 patients with EM recruited in this study. 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. Twenty HCs recruited were healthy medical staff in the National Hospital of Dermatology and Venereology, Ha Noi, Vietnam.

Measuring serum granulysin levels

In the SJS/TEN group, we took blood samples at 2-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 10–20 min, then centrifuged in 20 min at a speed of 2000–000 r.p.m, finally serum was taken and stored at −20°C until proceeding the granulysin measurement.

Using enzyme-linked immunosorbent assay (ELISA) (Human Granulysin ELISA Kit, MELSIN, China), we quantified granulysin in all serum samples. The kit used ELISA-double antibody sandwich principle to assess granulysin levels. The microELISA strip plate provided in this kit has been coated by purified granulysin antibody to make solid-phase antibody, in sequence granulysin is added to wells, combined with granulysin antibody labeled by horseradish peroxidase, which then becomes an antibody-antigen-enzyme-antibody complex. After washing completely to remove the uncombined enzyme, chromogen solution A and chromogen solution B were added, changing the color of the liquid to blue which in turn turns into yellow due to the effect of acid. The color change was measured spectrophotometrically at a wavelength of 450 nm. The concentration of granulysin in the samples was then determined by comparing the O.D. of the samples to the standard curve. The minimum detectable dose of granulysin is typically <0.15 ng/ml (following manufacturer's instruction).

Statistical analysis and ethical clearance

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

The study was approved by the Ethical Review Committee on Research Involving Human Subjects, Hanoi Medical University (Number 04NCS17, dated February 08, 2018). Written consent was obtained from all participants.

Results

There were 19 SJS patients (39.5%) and 29 TEN patients (60.5%) participating in our study. Their characteristics are shown in Table 1. The mean age was 49.3, range 19–77 years (47.9% males; 52.1% females). The most common causative drugs were traditional medicine (29.1%), carbamazepine (12.5%), and allopurinol (12.5%). The time between the onset and the day of hospitalization was 5.9 ± 2.7 days (range 2–18 days). The mean body surface area affected was 43.7%. The mean m-SCORTEN score was 1.6. The mean time of re-epithelialization was 15.9 days (range 9–31 days). All SJS/TEN patients got re-epithelialization and total resolution, no one with in-hospital mortality.

The characteristics of EM patients are shown in Table 2. The mean age was 41.4, range 19–76 (30.2% males; 69.8% females). The medication was responsible for 41.9% of patients with EM.

Table 1: Characteristics of patients with SJS/TEN

Characteristics	SJS (n=19)	TEN (n=29)	SJS/TEN (n=48)
Age, years	44.9±15.3	52.2±14.3	49.3±15.0
(Range)	(19-72)	(25-77)	(19-77)
Sex, n (%)			
Male	11 (57.9)	12 (41.4)	23 (47.9)
Female	8 (42.1)	17 (58.6)	25 (52.1)
Causative drugs, n (%)			
Carbamazepine	5 (26.2)	1 (3.4)	6 (12.5)
Allopurinol	4 (21.1)	2 (6.9)	6 (12.5)
Traditional medicine	2 (10.5)	12 (41.5)	14 (29.1)
Antibiotics	1 (5.3)	2 (6.9)	3 (6.2)
NSAIDs	1 (5.3)	3 (10.3)	4 (8.4)
Thalidomide	0 (0)	1 (3.4)	1 (2.1)
Unknown	6 (31.6)	8 (27.6)	14 (29.2)
The time between onset and hospitalization, days	5.5 ± 1.6	6.1 ± 3.2	5.9 ± 2.7
Taking corticosteroids before hospitalization, n (%)			
Yes	7 (36.8)	13 (44.8)	20 (41.7)
No	8 (42.1)	13 (44.8)	21 (43.7)
Unknown	4 (21.1)	3 (10.4)	7 (14.6)
Body surface area affected, %	7±7.4	67.8±21.7	43.7±34.7
m-SCORTEN score	1±0.9	2±0.7	1.6±0.9
Fever, n (%)	8 (42.1)	19 (65.5)	27 (56.2)
High levels of liver enzymes, n (%)	11 (57.9)	19 (65.5)	30 (62.5)
Malfunction of kidney	6 (31.6)	14 (48.3)	20 (41.7)
Mucous membrane lesions	18 (94.7)	21 (72.4)	39 (81.2)
Pneumonia	0 (0)	8 (27.6)	8 (16.7)
Treatment given during hospitalization, n (%)			
Systemic corticoid	16 (84.2)	19 (65.5)	35 (72.9)
Ciclosporin A	3 (15.8)	8 (27.6)	11 (22.9)
Only care support	0 (0)	2 (6.9)	2 (4.2)
The time of re-epithelialization, days	13.8±3.7	17.2±4.6	15.9±4.6
(Range)	(9–23)	(10–31)	(9–31)

SJS: Stevens-Johnson syndrome, TEN: Toxic epidermal necrolysis, NSAIDs: Nonsteroid anti-inflammatory drugs, SCORTEN: SCORe of toxic epidermal necrolysis

Table 2: Characteristics of patients with EM and HCs

Characteristics	EM (n=43)	HCs (n=20)
Age, years	41.4±17.3	28.4±3.5
(range)	(19–76)	(25–37)
Sex, n (%)		
Male	13 (30.2)	10 (50)
Female	30 (69.8)	10 (50)
Causes, n (%)		NA
Medication	18 (41.9)	
Traditional medicine	8 (44.4)	
Antibiotics	5 (27.8)	
Allopurinol	3 (16.6)	
Herbal food	2 (11.2)	
Unknown	25 (58.1)	
Cutaneous lesions, n (%)		NA
Typical targets	7 (16.3)	
Atypical targets	30 (69.7)	
Both typical and atypical targets	6 (14)	
Mucous membrane lesion, n (%)	9 (20.9)	NA
Fever, n (%)	10 (23.3)	NA

EM: Erythema multiforme, HCs: Healthy controls, NA: Not applicable.

Serum granulysin levels of patients with SJS/TEN (23.0 ng/ml; range 1.2–144.6 ng/ml) were significantly higher than those of patients with EM (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 1). There was no significant difference with regard to serum granulysin levels among SJS and TEN patients (21.7 ng/ml versus 23.9 ng/ml; $p > 0.05$) (Figure 2a). In the SJS/TEN group, serum granulysin levels were higher in the 25 samples collected <6 days of the onset (27.7 ng/ml; range 2.5–144.6 ng/ml) than in the 23 samples collected ≥6 days after the onset (17.9 ng/ml; range 1.2–59 ng/ml) but it was not significantly different ($p > 0.05$) (Figure 2b). Serum granulysin levels were not significantly different between male and female patients with SJS/TEN (Figure 2c). In patients with SJS/TEN, there was a negative correlation between serum granulysin levels and the ages with r = (−0.25), but it was not statistically significant (p = 0.081) (Figure 3a). There was no correlation between serum granulysin levels and the body surface area affected (r = 0.12; p = 0.417) (Figure 3b); between serum granulysin levels and modified-SCORTEN with r = (−0.16), p = 0.29 (Figure 3c). Serum granulysin levels were not affected by some clinical/paraclinical such as fever, malfunction of kidney, pneumonia, high level of liver enzymes, and mucous membrane lesion (Figure 4). We had a TEN patient whose progress of disease is shown in Figure 5.

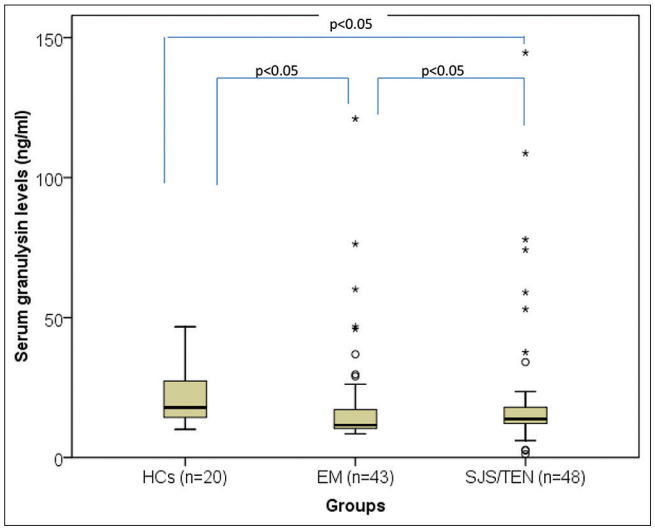

Figure 1: *Serum granulysin levels of healthy controls, erythema multiforme group, and Stevens-Johnson syndrome/toxic epidermal necrolysis p > 0.05 group*

Discussion

This study showed that serum granulysin levels in SJS/TEN patients were statistically higher than those in EM patients. There was no significant difference with regard to serum granulysin levels in SJS and TEN patients. Serum granulysin levels in patients hospitalized <6 days of the onset were higher than after ≥6 days; however, it was not statistically significant.

Granulysin is a cytolytic molecule presenting in human CTL and NK cell granules [28] and is lytic against a variety of tumor cell targets and microbes [28], [29]. In combination with purified perforin, recombinant granulysin breaks up 90% of intracellular *Mycobacterium tuberculosis*, inducing lesions on the mycobacterial cell surface [29]. Previous studies as regards SJS/TEN support the concept that SJS/TEN are SCARs initiated by CTLs [5], [8], [18], [30]. It has been observed that in patients with SJS/TEN, CTLs, and NK cells infiltrate the skin lesions, which point to cutaneous recruitment of antigen-primed CTLs in the pathogenesis of SJS/TEN [8], [18], [31]. Chung demonstrated that SJS/TEN blister cells were, in most part, formed by CD8+CTLs, CD56+NK, and NK cells,

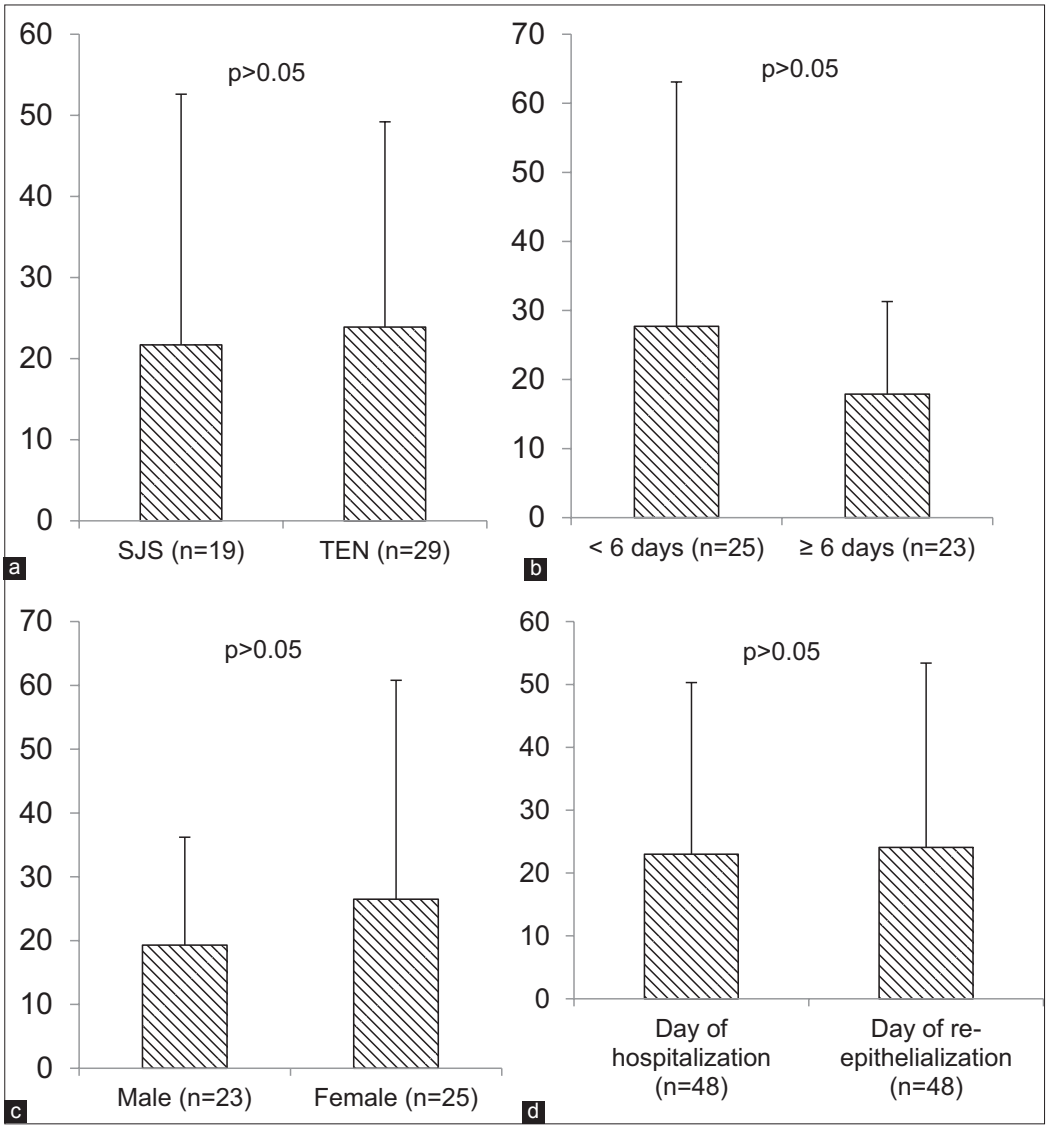

Figure 2: *Comparison of serum granulysin levels (ng/ml) in SJS/TEN: Between SJS and TEN (a); the 25 samples collected <6 days of the onset and the 23 samples collected ≥6 days after the onset (b); male and female patients (c); At the day of hospitalization and re-epithelialization (d). SJS: Stevens-Johnson syndrome; TEN: toxic epidermal necrolysis*

and these effector cells showed cytotoxicity against target cells [18]. These findings are favorable to the key role of granulysin, a product of CTLs and NK cells, in the pathogenesis of SJS/TEN.

EM is a cutaneous reaction characterized by typical or atypical target lesions that mimic cutaneous manifestations of SJS/TEN in the early phase [2], [22], [23]. The causes of EM may be a viral infection or drug reaction [22], [23], [32]. Serum granulysin can be also elevated in viral infected conditions [33] 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 [33], [34], [35], sepsis [36], cancers [34], [37], age [33], and immunological condition [35], hence, it was a large range among our SJS/TEN patients. However, systemic corticosteroid treatment before hospitalization did not affect serum granulysin levels in patients with SJS/TEN.

In the present study, there was no association between serum granulysin levels and some clinical/paraclinical manifestations of 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 reactions [20]. However, the mean level of serum granulysin in this study was consistent with that in Abe's *et al*. study (24.8 ng/ml) [20]. It could be due to the difference in the size of samples in each study.

By using the sandwich ELISA method, a study in Japan shows the mean level of serum granulysin among

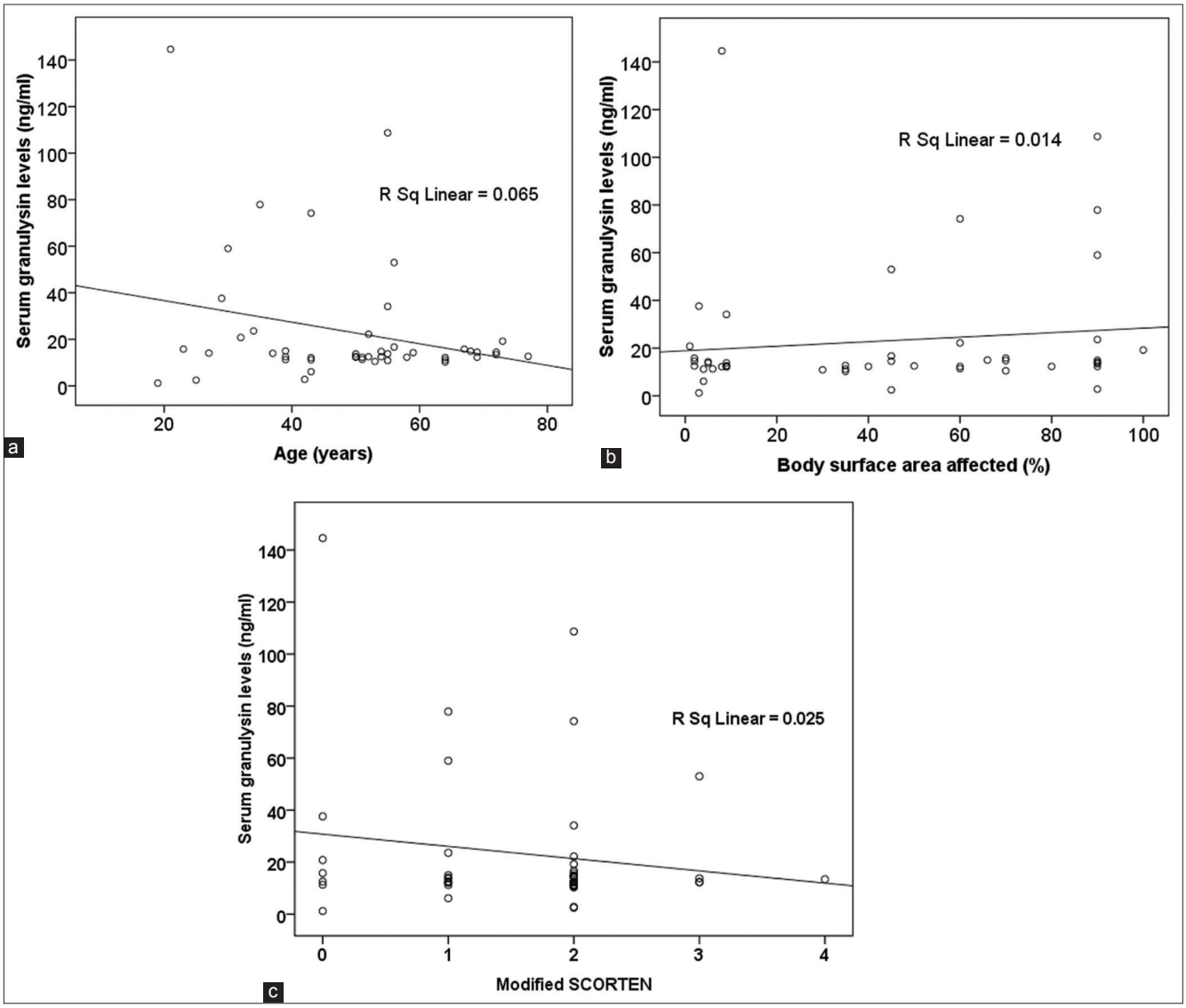

Figure 3: *Correlation between serum granulysin levels in SJS/TEN patients and the ages (a), the body surface area affected (b), the modified-SCORTEN (c). SJS: Stevens-Johnson syndrome; TEN: Toxic epidermal necrolysis; SCORTEN: SCORe of toxic epidermal necrolysis*

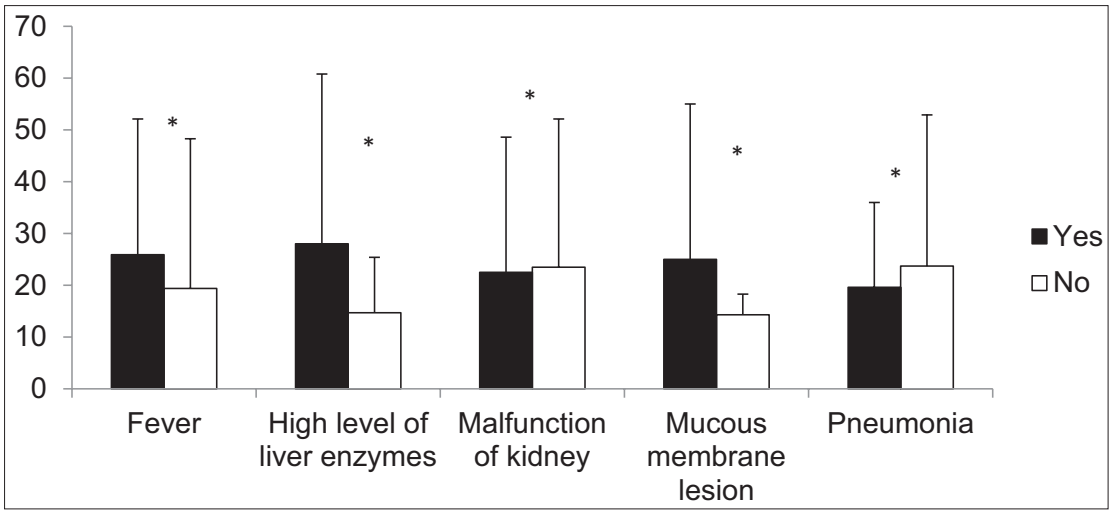

Figure 4: *Serum granulysin levels (ng/ml) and some clinical/paraclinical in patients with SJS/TEN; *p > 0.05. SJS: Stevens-Johnson syndrome; TEN: Toxic epidermal necrolysis*

healthy individuals is 3.7 ng/ml, and it also demonstrates a positive correlation between serum granulysin levels and age, but no significant difference in serum granulysin levels between genders [33]. Furthermore,

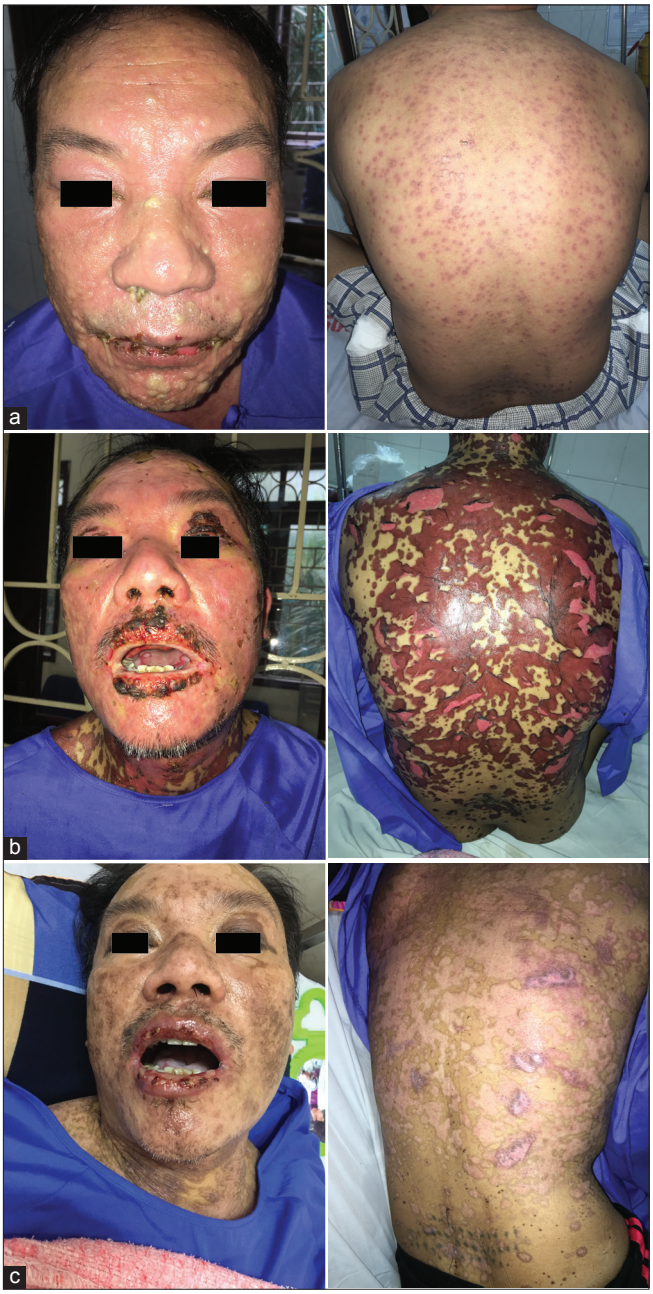

Figure 5: A 52-year-old male patient with allopurinol-induced toxic epidermal necrolysis and serum granulysin level results. (a) At the day of hospitalization: Serum granulysin level was 22.2 ng/ml, (b) ten days after hospitalization, extensive necrolysis of skin, (c) at the day of re-epithelialization: Serum granulysin level was 17.1 ng/ml

a study among health staffs from tuberculosis hospitals in Hanoi, Vietnam, also shows lower levels of serum granulysin as compared with those of the present study [35]. This may be due to the difference in study populations, age, and immunological conditions. On the other hand, HCs in our study were medical staff, who possibly have higher chances of being exposed to bacterial or viral infections in hospital environments.

Cho *et al.* showed that the serum level of granulysin of patients with generalized bullous fixed drug eruption (GBFDE) was significantly lower than that of patients with SJS/TEN overlap or TEN [25]. In GBFDE, granulysin is mainly produced by NK cells, whilst in SJS/TEN both NK cells and CTLs play an essential role. The presence of granulysin in lesional skin and in serum is a principal factor to distinguish GBFDE from SJS/TEN [25]. Furthermore, serum granulysin levels are not elevated exclusively in SJS/TEN; other conditions have also shown to be responsible for high serum granulysin levels, such as graft-versus-host disease [33], [38], drug reaction with eosinophilia and systemic symptoms, or drug-induced hypersensitivity syndrome [39]. Another study in Taiwan shows that high plasma granulysin levels were associated with renal impairment and mortality rates of patients with allopurinol-induced SJS/TEN [14]. Deceased patients showed higher concentrations of granulysin for extended periods of time when compared to the survivors [14]. Su *et al.* demonstrated that the levels of interleukin-15 and granulysin were significantly correlated with the disease extension in SJS/TEN [36]. These studies implied that serum granulysin levels may be used as a biomarker for not only the diagnosis but also the prognosis of SCARs. However, in our study, after the onset of SJS/TEN, serum granulysin level is not useful for evaluating the severity of SJS/TEN.

This study had some limitations. First, we collected serum samples several days after the onset of SJS/TEN when serum granulysin levels might have been decreased a lot. Second, HCs group were healthy health staffs whose serum granulysin levels might be higher than those of the general population. Third, we only measured granulysin levels in sera and were not able to quantify them in blister fluids and identify the presence of granulysin on skin tissue. Fourth, we did not identify the cells secreting granulysin. In fact, there are other cytotoxic proteins and molecules such as perforin, granzyme B [5], [18], [40], and annexin A1 [41] relating to the pathogenesis of SJS/TEN but we could not investigate all of them. Finally, we used modified-SCORTEN as serum bicarbonate test was not available in the study settings. However, the mean m-SCORTEN in this study was consistent with the mean SCORTEN of a previous study in Vietnam [11].

Conclusion

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

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