Prevalence of Antibody to Toxic Shock Syndrome Toxin-1 in Burn Patients

Ji-Young Park, M.D.¹, Jae-Seok Kim, M.D.¹, and Heungjeong Woo, M.D.²
Departments of Laboratory Medicine¹ and Internal Medicine², Hallym University College of Medicine, Chuncheon, Korea

Background: Burn wounds lack normal barriers that protect against pathogenic bacteria, and burn patients are easily colonized and infected by *Staphylococcus aureus*. Toxic shock syndrome (TSS) is a rare but fatal disease caused by *S. aureus*. A lack of detectable antibodies to TSS toxin-1 (TSST-1) in serum indicates susceptibility to TSS.

Methods: A total of 207 patients (169 men and 38 women; median age, 42.5 yr) admitted to a burn center in Korea were enrolled in this study. The serum antibody titer to TSST-1 was measured by sandwich ELISA. *S. aureus* isolates from the patients’ nasal swab culture were tested for TSST-1 toxin production by PCR-based detection of the TSST-1 toxin gene.

Results: One hundred seventy-four (84.1%) patients showed positive results for antibody against TSST-1. All patients aged ≥61 yr (n=28) and <26 months (n=7) were positive for the anti-TSST-1 antibody. *S. aureus* was isolated from 70 patients (33.8%), and 58.6% of the isolates were methicillin resistant. Seventeen patients were colonized with TSST-1-producing *S. aureus*. The antibody positivity in these 17 carriers was 88.2%, and the positivity in the non-carriers was 83.7%.

Conclusions: Most burn patients had antibody to TSST-1, and nasal colonization with TSST-1-producing *S. aureus* was associated with positive titers of anti-TSST-1 antibody. Additionally, patients with negative titers of anti-TSST-1 antibody might be susceptible to TSS.

Key Words: Burns, Toxic shock syndrome, Toxic shock syndrome toxin-1, *Staphylococcus aureus*, Antibodies, Prevalence

INTRODUCTION

Burn patients are susceptible to invasive infections, and complications from infection are a leading cause of burn-related morbidity and mortality [1, 2]. *Staphylococcus aureus* is the most frequent cause of infection in burn patients [3, 4]. Some *S. aureus* strains produce a variety of exotoxins such as toxic shock syndrome toxin-1 (TSST-1), staphylococcal enterotoxins, and exfoliative toxin [5], which increase the morbidity and mortality via systemic pathways that can induce shock and cause host immune disruption [2, 6].

Toxic shock syndrome (TSS) is an acute febrile illness caused by *S. aureus* and is characterized by fever, rashes, desquamation, hypotension, and multi-organ involvement [6, 7]. There are several toxins associated with staphylococcal TSS, but the major cause is TSST-1 [7, 8]. Reduced levels of serum antibody to TSST-1 are correlated with TSS development [9]. Many reports have shown that the prevalence of this antibody increases with age, and a majority of the adult population has already developed antibodies to TSST-1 [10-12]. Among patients with menstrual TSS, low or negative concentrations of such antibodies have been reported in 90.5% of patients, and more than 50% of these patients failed to seroconvert within 2 months of acquiring the infection [9]. TSS caused by *S. aureus* has rarely been re-
ported; to our knowledge, thus far, only one case of TSS caused by methicillin-resistant *S. aureus* (MRSA) harboring TSST-1 gene has been reported in a burn patient from Korea [13]. In addition, the presence of the anti-TSST-1 antibody has not yet been characterized in the Korean population.

In this study, we evaluated the prevalence of the anti-TSST-1 antibody and nasal colonization of TSST-1-producing *S. aureus* among patients admitted to a burn center.

**METHODS**

1. **Subjects**
   A total of 207 patients (169 men and 38 women; median age, 42.5 yr [range, 10 months to 87 yr]) admitted to the burn center of Hangang Sacred Heart Hospital, Seoul, Korea, from April through November 2009 were enrolled in the study. None of the patients had TSS before or during the hospital stay.

   Serum and nasal swab samples were collected within 7 days of admission. The patients’ sera were stored at -70°C for analysis by ELISA, and nasal swabs were streaked onto mannitol salt agar plates for *S. aureus* screening.

   The study protocol, informed consent, and other associated documents were reviewed and approved by the Institutional Review Board of Hangang Sacred Heart Hospital.

2. **Measurement of anti-TSST-1 antibody**
   Serum antibody titers to TSST-1 were measured by sandwich ELISA, according to the method of Parsonnet et al. [11] with minor modifications. In brief, serum samples were serially diluted from 1:2 to 1:4,096 with phosphate-buffered saline and poured into wells of a microtiter plate precoated with TSST-1 (Sigma-Aldrich, St. Louis, MO, USA). Each plate was treated with goat anti-human IgG-horseradish peroxidase (MP Biomedicals, Solin, OH, USA) and subsequently with the substrate 3,3',5,5'-tetramethylbenzidine. The enzyme reaction was terminated by addition of 100 μL of 2M H₂SO₄ solution when the positive control wells almost reached an optical density of 1.0 at 405 nm. Commercially available human immunoglobulin G (I.V.-Globulin S inj.; Green Cross, Cheongju, Korea), diluted to 1:1,024 was arbitrarily used as a positive control, and a serum aliquot from a healthy volunteer was used as a titer control (1:16 dilution) in each ELISA for ensuring quality control. Samples with titers ≥1:16 were considered positive and those with titers ≤1:2 were considered negative. Titers of 1:4 and 1:8 were considered intermediate.

3. **Identification of TSST-1-producing *S. aureus* isolated from the nasal cavity**
   We selected 2 or 3 suspected colonies from the mannitol salt agar plates for identification of *S. aureus*. Tests for identification of and susceptibility to *S. aureus* isolates were performed by using Microscan (Siemens, West Sacramento, CA, USA). PCR was performed to detect the TSST-1 gene [14].

4. **Statistical analysis**
   A Chi-square test was used to compare the prevalence of the anti-TSST-1 antibody or TSST-1-producing strain. SPSS statistics 19 doctor’s pack (SPSS Inc., Chicago, IL, USA) was used for statistical analysis, and *P* values <0.05 were considered significant.

**RESULTS**

1. **Serum antibody to TSST-1**
   Among the 207 patients, 174 (84.1%) had positive titers of antibody to TSST-1 (≥1:16) and 18 (8.7%) had negative titers (≤1:2). All patients aged ≥61 yr (n=28) and <26 months of age (n=7) had positive titers of anti-TSST-1 antibody. No difference in the antibody prevalence was observed between men and women (84.0% and 84.2%, respectively) (Table 1).

2. **S. aureus colonization and anti-TSST-1 antibody**
   Of the 207 patients, 70 (33.8%) were colonized with *S. aureus*.

| Characteristics | N of patients | N (%) positive for antibody | N (%) intermediate for antibody | N (%) negative for antibody |
|-----------------|---------------|-----------------------------|-------------------------------|-----------------------------|
| Age (yr)        |               |                             |                               |                             |
| ≤6*             | 7             | 7 (100.0)                   | 0 (0.0)                       | 0 (0.0)                     |
| 7-18†           | 2             | 1 (50.0)                    | 1 (50.0)                      | 0 (0.0)                     |
| 19-30           | 23            | 20 (87.0)                   | 2 (8.7)                       | 1 (4.3)                     |
| 31-40           | 46            | 37 (80.4)                   | 4 (8.7)                       | 5 (10.9)                    |
| 41-50           | 63            | 48 (76.2)                   | 7 (11.1)                      | 8 (12.7)                    |
| 51-60           | 38            | 33 (86.8)                   | 1 (2.6)                       | 4 (10.5)                    |
| ≥61             | 28            | 28 (100.0)                  | 0 (0.0)                       | 0 (0.0)                     |
| Sex             |               |                             |                               |                             |
| Male            | 169           | 142 (84.0)                  | 12 (7.1)                      | 15 (8.9)                    |
| Female          | 38            | 32 (84.2)                   | 3 (7.9)                       | 3 (7.9)                     |
| Total           | 207           | 174 (84.1)                  | 15 (7.2)                      | 18 (8.7)                    |

*The age range is 10-26 months (median, 12 months); †Seven and 11 yr old.
and among them, 41 (58.6%) were MRSA carriers. Seventeen patients (8.2%; 24.3% of S. aureus carriers) were colonized with TSST-1-producing S. aureus; 11 isolates (64.7%) were identified as MRSA. Fifteen TSST-1-producing S. aureus carriers (88.2%) had positive titers for the anti-TSST-1 antibody (Table 2). Among the TSST-1-producing S. aureus carriers (n=17), all patients with methicillin-susceptible S. aureus (MSSA) colonization (n=6) were positive for the anti-TSST-1 antibody, and 5 of them had high titers of anti-TSST-1 antibody (≥1:512), while the MRSA carriers (n=11) had a lower positive titer for the anti-TSST-1 antibody (median titer, 1:1,536 vs. 1:32). Among the TSST-1-producing MRSA carriers (n=11), 2 patients were negative for the antibody, and only one patient had a high antibody titer (1:4,096).

The antibody positivity in the group of patients colonized with TSST-1-producing S. aureus was 88.2% (15/17) and that in the patients without TSST-1-producing S. aureus was 83.7% (159/190) (P > 0.05). Additionally, patients with antibody titers ≥1:2,048 were frequently found to be colonized with the TSST-1-producing strain (Fig. 1).

### DISCUSSION

TSS is a life-threatening condition, wherein superantigen-mediated activation of T cells results in overproduction of cytokines, resulting in systemic inflammation and shock [6, 15]. Although TSS appears to be a rare disease, the severity of superantigen-mediated disease is underestimated [7].

Burn wounds lack normal barriers that protect against pathogenic bacteria including S. aureus, and in many burn patients

| Table 2. Methicillin resistance of Staphylococcus aureus isolated from nasal carriers and the presence of serum anti-toxic shock syndrome toxin-1 (TSST-1) antibody in TSST-1-producing and nonproducing strains (n=70) |
|---------------------------------|-----------------|-----------------|-----------------|
| **Methicillin resistance**      | **TSST-1 producing strains (n=17)** | **TSST-1 nonproducing strains (n=53)** | **Total (n=70)** |
| Resistant                       | 11 (64.7%)      | 30 (56.6%)      | 41 (58.6%)      |
| Susceptible                     | 6 (35.3%)       | 23 (43.4%)      | 29 (41.4%)      |
| **Presence of anti-TSST-1 antibody** | **Total (n=70)** |
| Positive                        | 15 (88.2%)      | 46 (86.8%)      | 61 (87.1%)      |
| Intermediate                    | 0 (0.0%)        | 5 (9.4%)        | 5 (7.1%)        |
| Negative                        | 2 (11.8%)       | 2 (3.8%)        | 4 (5.7%)        |

Nasal colonization with TSST-1-producing S. aureus was found in 8.2% of patients in the current study. The incidence was higher than that of healthy women living in North America (6%) or Japan (3%) [11, 12]. Peck et al. [21] reported 52.6% prevalence rate of TSST-1-producing strains in S. aureus isolates from nasal swabs of children attending an outpatient clinic in a Korean tertiary-care hospital; this rate is higher than ours (24.3%). In addition, they found no difference in the prevalence of TSST-1-producing strains between MRSA and MSSA strains (50.0% vs. 53.3%). In our study, the TSST-1-producing isolates accounted for 26.8% (11/41) of MRSA strains and 20.7% (6/29) of MSSA strains (P > 0.05). On the contrary, in healthy Japanese women, the carriage rate of TSST-1-producing isolates was 100% (2/2) for MRSA strains and 6.5% (10/155) for MSSA strains [12].

Nasal carriers of TSST-1-producing S. aureus tended to have a high rate of antibody positivity, which is known to be associ-
ated with a lower risk of TSS. In the TSST-1-positive group, MRSA carriers had lower antibody levels. Because MRSA is less prevalent in the community, many MRSA carriers might be colonized with these strains after admission to the hospital. A TSST-1-producing MRSA carrier with the highest antibody titer (1:4,096) was referred to us from another hospital, and his total duration of hospital stay was 15 days, which is a sufficient period to generate antibodies to hospital-acquired MRSA.

In our study, an antibody titer ≥1:2,048 was more frequently observed in patients with colonization of the TSST-1-producing strain than in those without the toxigenic strain (23.5% vs. 2.1%). This finding is compatible with those of previous studies on menstruating women colonized with TSST-1-producing Staphylococcus aureus [11, 12].

In summary, most patients (84.1%) admitted to a burn center in Korea had serum antibody to TSST-1. Elderly patients aged ≥61 yr and children aged <26 months had 100% prevalence of the antibody to TSST-1, while a few patients (~10%) aged 31-60 yr showed a negative antibody titer to TSST-1. Most of the burn patients with TSST-1-producing S. aureus had a positive titer of anti-TSST-1 antibody, and some patients had a negative titer of anti-TSST-1 antibody; such patients might be at risk for developing TSS.

Authors’ Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

Acknowledgments

This study was supported by the Korea Healthcare Technology R&D Project, Ministry for Health and Welfare, Republic of Korea (grant A084589).

REFERENCES

1. Weber J and Macmanus A; Nursing Committee of the International Society for Burn Injuries. Infection control in burn patients. Burns 2004; 30:A16-24.
2. Shupp JW, Ortiz RT, Moffatt LT, Jo DY, Randad PR, Njimoluh KL, et al. Treatment with an oxazolidinone antibiotic inhibits toxic shock syndrome toxin-1 production in MRSA-infected burn wounds. J Burn Care Res 2013;34:267-73.
3. Lee HG, Jang J, Choi JE, Chung DC, Han JW, Woo H, et al. Bloodstream infections in patients in the burn intensive care unit. Infect Chemother 2013;45:194-201.
4. Murray CK, Holmes RL, Ellis MW, Mende K, Wolf SE, McDougal LK, et al. Twenty-five year epidemiology of invasive methicillin-resistant Staphylococcus aureus in a burn center. J Burn Care Res 2013;34:253-66.
Staphylococcus aureus (MRSA) isolates recovered at a burn center. Burns 2009;35:1112-7.

5. Marples RR and Wienke AA. Enterotoxins and toxic-shock syndrome toxin-1 in non enteric staphylococcal disease. Epidemiol Infect 1993;110:477-88.

6. McCormick JK, Yanwood JM, Schlievert PM. Toxic shock syndrome and bacterial superantigens: an update. Annu Rev Microbiol 2001;55:77-104.

7. DeVries AS, Lesher L, Schlievert PM, Rogers T, Villaume LG, Danila R, et al. Staphylococcal toxic shock syndrome 2000-2006: epidemiology, clinical features, and molecular characteristics. PLoS One 2011;6:e22997.

8. Andrews MM, Parent EM, Barry M, Parsonnet J. Recurrent nonmenstrual toxic shock syndrome: clinical manifestations, diagnosis, and treatment. Clin Infect Dis 2001;32:1470-9.

9. Lappin E and Ferguson AJ. Gram-positive toxic shock syndrome. Lancet Infect Dis 2009;9:281-90.

10. Quan L, Morita R, Kawakami S. Toxic shock syndrome toxin-1 (TSST-1) antibody levels in Japanese children. Burns 2010;36:716-21.

11. Parsonnet J, Hansmann MA, Delaney ML, Modern PA, Dubois AM, Wieland-Alter W, et al. Prevalence of toxic shock syndrome toxin 1-producing Staphylococcus aureus and the presence of antibodies to this superantigen in menstruating women. J Clin Microbiol 2005;43:4628-34.

12. Parsonnet J, Goering RV, Hansmann MA, Davis CC, et al. Prevalence of toxic shock syndrome toxin 1 (TSST-1)-producing strains of Staphylococcus aureus and antibody to TSST-1 among healthy Japanese women. J Clin Microbiol 2008;46:2731-8.

13. Choi JH, Choi JH, Kim DI, Kim JS, Choi EH. A case of toxic shock syndrome caused by methicillin-resistant Staphylococcus aureus (MRSA) following a burn injury. Korean J Pediatr Infect Dis 2009;16:205-9.

14. Mehrotra M, Wang G, Johnson WM. Multiplex PCR for detection of genes for Staphylococcus aureus enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1, and methicillin resistance. J Clin Microbiol 2000;38:1032-5.

15. Xu SX and McCormick JK. Staphylococcal superantigens in colonization and disease. Front Cell Infect Microbiol 2012;2:52.

16. Kooistra-Smid M, Nieuwenhuis M, van Belkum A, Verbrugh H. The role of nasal carriage in Staphylococcus aureus burn wound colonization. FEMS Immunol Med Microbiol 2009;57:1-13.

17. Bonventre PF, Linnemann C, Weckbach LS, Staneck JL, Buncher CR, Vgdorh E, et al. Antibody responses to toxic-shock-syndrome (TSS) toxin by patients with TSS and by healthy staphylococcal carriers. J Infect Dis 1984;150:662-6.

18. Dinges MM, Orwin PM, Schlievert PM. Exotoxins of Staphylococcus aureus. Clin Microbiol Rev 2000;13:16-34.

19. Parsonnet J, Hansmann MA, Delaney ML, Modern PA, Dubois AM, Wieland-Alter W, et al. Prevalence of toxic shock syndrome toxin 1-producing Staphylococcus aureus and the presence of antibodies to this superantigen in menstruating women. J Clin Microbiol 2005;43:4628-34.

20. Park BG and Lee MK. Nasal carriage of methicillin-resistant Staphylococcus aureus among healthcare workers and community students in 1997 and 2006. Korean J Nosocomial Infect Control 2007;12:85-90.

21. Peck KR, Baeck JY, Song JH, Ko KS. Comparison of genotypes and enterotoxin genes between Staphylococcus aureus isolated from blood and nasal colonizers in a Korean hospital. J Korean Med Sci 2009; 24: 585-91.