Detection of *tstH* Gene in *Staphylococcus aureus* Isolates from Hospitalized Burnt Children

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ABSTRACT

***Background*:** The main cause of toxic shock is TSST-1 toxin which is produced by *S. aureus*. Finding of TSST-1 toxin in burnt children is very important to prevent TSS and its consequences.

***Methods*:** The aim of this study was to investigate the presence of gene encoding TSST-1 toxin in wound specimens by PCR. In this case-control study, 90 children who were admitted to the burn unit, were divided in two groups of 45 patients, namely febrile (cases group) and non-febrile (control group). Samplings were done from the burn wounds and were tested by PCR with specific primers of *tstH* gene. Finally, all data including demographic characteristics, percentage of burnt surface severity and the PCR results were analyzed, statistically.

***Results*:** The positive PCR results indicated the expression of *tstH* gene in 37.7% of the febrile children and 11.1% of the non-febrile children with a statistically significant difference ($p < 0.003$). The means and the standard deviations for the percentage of burnt surfaces (i.e. severity) in the samples with the positive and negative PCR results were 30.9±16.93 and 20.09±11.02, respectively with a statistically significant difference ($p < 0.01$). No difference with respect to age and sex could be detected between positive and negative PCR results.

***Conclusion*:** A direct association between the expression of *tstH* and the occurrence of fever in the burnt children was observed. Furthermore, increased surface area of the wounds was also positively related to the expression of *tstH*.

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Introduction

Staphylococcus aureus is a Gram-positive bacterium that in adults colonizes the skin while in children targets the throat (1, 2). This bacterium produces many virulent factors such as Staphylococcal entrotoxins and toxic shock syndromes type 1 toxin (TSST-1) (3). Toxic shock syndrome that is caused by TSST-1 commences with a brief onset of high fever, diarrhea, conjunctivitis and headache while these may rapidly lead to a reduction in blood pressure, shock, breathing distress syndrome, intravenous coagulation, severe thrombocytopenia and kidney dysfunction (4). TSST-1 is a toxin made up of super antigens with a molecular weight of 22 kDa which is found in 5-25% of *S. aureus* species. This toxin induces toxic shock syndrome which, in turn, stimulates the release of different inflammatory cytokines such as interleukin-1 and 2 as well as the tumor necrosis factor (TNF) (5). TSST-1 is encoded by *tstH* gene which is a part of the mobile genetic elements, found in the pathogenicity islets of *S. aureus* (6).

TSST-1 is a member of the super antigen family that activates a myriad of T lymphocytes, regardless of their specificity. These toxins attach themselves to the Vß domain in T-cell receptors and MHC-II molecules thus activating 5-30% of CD4 T-helper cells (7). One of the most important consequences of the presence of TSST-1 in patients is fever. Also one of the reasons for burn victims to have fever is the colonization of *S. aureus* in their wounds or blood (8). A study conducted in England in 2012 has investigated the lytic and cytolytic activity of a number of TSST-1 positive *S. aureus* species which are sensitive to methicillin. The findings of this study have revealed there is no link between the expression of TSST-1 and the shortage of exotoxin production; therefore, it has been postulated that in case of an infection with *S. aureus* in wounds caused by burns in children, toxic shock syndrome cannot be ruled out (9).

In another study carried out in 1999, antibody levels in children afflicted by burns were measured. The results showed that 50% of 38 children (under the age of 4) had high levels of anti-TSST-1 IgG antibodies, well beyond what had been expected upon their hospitalization (10). In a similar study conducted by Childs et al., the importance of *S. aureus* in the etiology of suspected TSST-1 cases in burn-victim children had been observed. The colonization patterns of *S. aureus* in 53 children were studied where half of the children had antibodies against TSST-1 (11). It appears that by closely studying the gene expression of TSST-1 in burn patients and comparing the results between those the febrile and non-febrile patients, one can attain valuable information regarding the effects of TSST-1 toxin in fever induction as well as more sustainable methods of treatment and fever alleviation for such patients.

Material and method

This study involving case-control subjects was carried out at the Burns Ward of Shahid Motahari Hospital in 2013. After receiving a written consent from the children's parents, sample gathering was conducted .Using the formula for determining the volume of samples, 90 children in total were prepared for this study, in a way that 45 febrile children were considered for the case group and another 45 non-febrile children were selected as the control group. Sampling was done from their burn wounds using two sterile swabs. Blood cultures were taken at the same time. Samples have been collected frequently in the first few days and one week following injury. Sampling was carried out by rotating the swab for several seconds within the wound, removing it, and placing it directly in the swab transport medium. Localization of the wounds was predominantly abdominal, but other locations were also involved. Subsequently, the swab was carefully with drawn to prevent contamination with microflora and placed immediately into the transport tube containing the transport medium. All samples were collected by medical personnel and transported to the microbiology laboratory at the hospital within 2h.

The swabs were then placed in a tube containing the necessary culture Tryptic soy broth (TSB) for the bacteria's growth. These tubes were then sent to Hazrat –e- Rasoul Akram Educational and Treatment Center for Research of Infectious Diseases Afflicting Children. At the laboratory, one swab for isolation bacteria has been cultured on blood agar (BA), McConkey Agar (MCA in sterile conditions.

Identification of isolates Bacteria

Based on colonial morphology, gram stain, catalase test and oxidase test (Test oxidase, UK), Bacteria were isolated in different genus. Further identification to the species level in all streptococci (Vitek GPI, UK) was isolated using analytical profile index. A slide coagulase test (Microgen Staph, Microgen Bioproducts Ltd, Camberley, UK) differentiated staphylococcal isolates into *S. aureus* and coagulase-negative staphylococci. Further confirmation was by tube coagulase test.

DNA extraction and tstH gene amplification

At the laboratory, from another swab the bacterial DNA were extracted using DNA extraction Kit (Roche Diagnostics GmbH, Mannheir, Germany) and PCR was performed using specific primers to amplify *tstH* gene. The specific forward and reverse primers were: 5'-TGTAGATCACAAACGATAATATAAAGGA T-3' and 5'-ATTAAGCTTAATTAA TTTCTGC TTCTATAGTT-3', respectively (12). For each PCR, 5 µl master mix (Qiagen, Germany), 2 µl deionized water, 1 µl DNA template and 0.5 µl of each specific primers (5 Pmol) in one micro-tube were mixed. PCR was performed in a thermal cycler (SenQuest, Germany). The cycling conditions were: 94 °C for 5 minutes, then 35 cycles of denaturation at 94 °C for 30 sec, annealing at 72 °C for 50 sec and elongation at 53 °C for 30 sec. and a final extension of 72°C for 50 sec. The amplicons were then stained by DNA safe stain (Cinnagen, Iran) staining following 1% agarose gel electrophoresis and visualized using a trans-illuminator (UVTEC- UK) device.

Data including sex, age, severity and the level of burns and the PCR were gathered and analyzed statistically by SPSS software. For determination of the frequency of the research variables, descriptive statistics were used including mean, standard deviation and frequency percentage. The trial X2 and Independent sample t-test were used to compare the variables between the febrile and no febrile groups ($p < 0.05$ was considered to be significant).

Results

Blood culture of 90 children participated in this study *S. aureus* was grown. If other bacteria had grown from this study were excluded (Example five *Pseudomonas aeruginosa* strains were isolated).In total, there were 90 children who participated in this research (two groups of 45, one febrile, one non-febrile). Sixty percent of them (i.e. 54 children) were male and 40% of them (i.e. 36 children) were female.

In the case group (febrile) 30(66.7%) were male and 15(33.3%) were female and the mean age of the patients was 3.9 ± 2.53. In this group, the severity of the burns was between 6- 66% with the mean and standard deviation of 25.58 ± 15.01.

In the control group (no febrile) 24(53.3%) were male and 21(46.7%) were female with the mean age of 3.9 ± 2.53. In this group, the severity of the burns was between 4-45% with the mean and standard derivation of 20.14 ± 11.40.

While Table1 shows the frequency of different PCR test results according to the gender of the patients, Table 2 factors in age and the severity of the burns as effective variants on the frequency of PCR test results in the two aforementioned groups.

Our observation showed that a significant difference in levels of TSST-1 toxin was present between the groups. To be precise, in the case group (febrile) 17 subjects out of 45 (i.e. 37.7%) had TSST-1 toxin while in the control group (no febrile) only 5 subjects out of 45(i.e. 11.1%) harbored that toxin ($p = 0.003$). Table 3 shows the

frequency of positive PCR test results (expression of TSST-1) in the control and the case groups. The results indicated no significant difference between the mean age and gender of the children and the amount of TSST-1 expression (i.e. a positive PCR; p>0.5 and p > 0.6, respectively.

In all the samples gathered, there was a significant difference between the severity of the burns and the amount of TSST-1 produced ($p < 0.01$) Furthermore in positive PCR cases, the burns were more severe compared to negative PCR cases (30% as compared to 20% burn severity). In the case group (febrile), PCR results for TSST-1 turned up positive for 17 subjects (37.8%) while for the other 28 (3.62%) the results had been negative. In the control group (no febrile), PCR results were positive in 5 subjects (11.1%) while in other 40 (88.9%) the results were negative. In this group, the severity of the burns and the amount of toxin was statistically significant. ($p < 0.003$).

Discussion

Most of the species of *S. aureus* isolated from patients with toxic shock syndrome produce TSST-1. This toxin leads to fever, reduction in blood pressure and symptoms indicating the involvement of different organs and skin lesions. In different studies, immunodiffusion, ELISA and agglutination are some of the methods which have been used for definitive diagnosis of Staphylococcus super antigens. Recently, amplification techniques such as PCR have been successfully used to identify enterotoxins and TSST-1. However, due to their high cost and general non-availability, their public use has been limited at best (13). In a study by Nourouzi and colleague, the identification of *S. aureus* harboring enterotoxin A-E and TssT-1 genes by PCR was conducted. They have reported that a high percentage of *S. aureus* bacteria isolated from the clinical samples, material and healthy individuals, genes for TSST-1 and enterotoxin could be detected. (13). The coding gene for TSST-1 (i.e. *tstH*) could be detected using PCR but not all strains containing *tstH* gene express TSST-1. The frequency of the *tstH* gene in various blood and nose specimen studies performed in Germany (14), Iran (15), Korea (16), Czech Republic (17) and Colombia (18), were 14%, 26.41%, 72.2%, 50% and 2% , respectively. The frequency of *tstH* gene in our study was 37.7%.

A link has been observed between the serum levels of anti TSST-1 antibodies and the progression of age in children suffering from burns. For example, in a study that had been carried out since 2006 to 2007 with the aim of determining the anti TSST-1 antibody titers in Japanese children, 119 patients hospitalized at the reconstruction and cosmetic surgery ward had been tested upon (19). The results showed that the age group of 7 months to 2 years had the lowest titer of anti TSST-1 antibody, in a way that the antibody titer in age groups of 7 months to 12 and12 to 24 months had been 30.8% and 33.3% respectively. There are two groups which had the most hospitalization cases due to medical symptoms. This fact shows a high propensity of contracting toxic shock syndrome in this children. In the above-mentioned study, 78.6% of children under the age of 6 months had a positive titer of anti TSST-1 antibody which was the highest titer possible for that age group. This is due to the reality that the children had received anti TSST-1 antibodies from their mother. The antibody titer increases after the age of 3 and until 6 years of age, 54.5% of the children had been shown to have positive antibody titters (19). However in the current study, the non-febrile group subjects who had a positive PCR test result were of an older age range.

Table 1. Frequency variable results with respect to sex and PCR test between the two groups.

group		Case group		Control group		*p* value
variable		PCR positive	PCR negative	PCR positive	PCR negative	
Sex	male	11	19	3	21	0.99
	female	6	9	2	19	

Table 2. Frequency variable results with respect to age, the severity of burns and PCR test between the two groups.

group	Case group(Fever)		Control group(no fever)		*p* value
variable	PCR positive	PCR negative	PCR positive	PCR negative	
	μ ± SD	μ ± SD	μ ± SD	μ ± SD	
Age(year)	3.70 ± 2.98	4.01 ± 2.26	7.50 ± 1.91	3.86 ± 2.33	0.005
Burn (%)	29.93 ±18.86	22.80 ±11.52	34±9.19	18.35 ±10.46	0.003

Table 3. Frequency result of positive PCR test between the two groups.

group	Result PCR test		*p* value
	frequency	percentage	
Case group (Fever)	17	37.7	0.003
Control group (no fever)	5	11.1	

Antibody protection against vigorous T cell activation by TSST-1 increases with age. There are some other researches in which similar results to our have been obtained. For instance, the distribution of TSST-1 antibody levels among the different age groups showed that the lowest TSST-1 antibody level was observed in the age group of 19-40 years while the highest value was measured in the age group of 41-81 years (19).

Conclusion

As a conclusion, the febrile groups of children in this study were shown to have more positive PCR cases than that of the non-febrile group which can indicate that anti TSST-1 antibodies can prevent fever in children afflicted by burns. Also a positive PCR test was associated with the level of burns but had no obvious relation with either age, sex or fever induction. This finding attests to the role of TSST-1 in fever induction of children suffering from burns. Due to the restrictions regarding the number of samples and the presence of parameters such as race and different genetic backgrounds, one cannot generalize the obtained results to all the children. Therefore, research in different populations is recommended in order to confirm these results. Furthermore, possible links between anti-TSST-1 antibody levels and other factors related to immunologic responses in the body could be further investigated.

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Ethical Considerations

The present study was accepted by the ethics committees of the children hospital of Tehran, Iran (IRB.IUMS.REC.1393.24487). Written informed consents were obtained from all the study patients or their parents.

Conflict of interest

The authors declare that they have no conflicts of interest.

Financial disclosure

The authors declared no financial disclosures.

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