

Prevalence of Toxic Shock Syndrome Toxin-1 (TSST-1) Produced by *Staphylococcus aureus* Isolated from Patient Combined Psoriasis with Urinary Tract Infections (UTIs) and Gastroenteritis in Age Groups Between 8-18 Years

Ala'a Abdhusein Abdulzahra¹, Rabehshakir Al-Jameel², Mohammed Abd-Zaid Akool³, Dheyaa Shnan Al-Jameel^{4*}

¹Department of Internal Medicine, Faculty of Medicine, Jabir Ibn Hayyan Medical University, Najaf, Iraq

²Department of Pediatrics, Imam Alsajjad Hospital, Alnajaf Health Directory, Najaf, Iraq

³Department of surgery, Faculty of Medicine, Jabir Ibn Hayyan Medical University, Najaf, Iraq

^{4*}Department of pathology, Faculty of Medicine, Jabir Ibn Hayyan Medical University, Najaf, Iraq

Corresponding Author E-mail: dheyaa.shnan@jmu.edu.iq

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ABSTRACT

Psoriasis lesion may be the source of transmitting the causative bacterial agents to other parts of the body like urinary and digestive systems. *Staphylococcus aureus* is one of the most common bacteria which colonize the lesions of psoriasis and TSST-1 is a super antigen complicates the infections of this bacteria. This study was aimed to investigate the psoriasis lesions as a source for UTIs and gastroenteritis in age groups 8-18 years and the properties of TSST-1 produced by isolated bacteria. In this study total of 60 psoriatic patient and 30 control have been submitted to investigation for combination UTIs or gastroenteritis and screening the patient for *Staphylococcus aureus* and its TSST-1 by PCR technique, furthermore the toxin extracted and purified by affinity chromatography and molecular weight had been determined and the LD₅₀ in mice calculated and study the activity of toxin as a super antigen to induce lymphocyte and other leucocytes in rabbits. Finally the antibiotic sensitivity for isolated bacteria had been achieved by disc diffusion method. The site of psoriasis onset show the dominance for body skin by 25 (41.67 %) then to nares, scalp, and nails respectively and the more affected age groups is 16-18 years with 29 (48.33 %) out of total patients. About 19(31.66 %) and 6(10.00%) of patients combined UTIs and gastroenteritis respectively, from them about 5(8.33 %) *Staphylococcus aureus* had been isolated as a causative agents,

from which 2(3.33 %) produce TSST-1 with molecular weight 23kDa. The LD₅₀ for purified toxin in mice determined about 50 µg/ml which equal to 2mg/kg and the maximum level of lymphocyte and other leucocyte activated in rabbits blood was recorded at the concentration of 100 µg/ml after 48 hours of administration about 24 × 10³/µL and 9 × 10³/µL respectively. Finally the isolated bacteria showed highest level of susceptibility to Ampiclox and Chloramphenicol with (80%) of isolates, while it resist to Lincomycin, Metronidazole, and Bacitracin. Psoriasis lesion is a threatening source for transmission of *Staphylococcus aureus* to urinary and digestive systems and causing UTIs or gastroenteritis and TSST-1 produced by *S. aureus* is high quality super antigen causing TSS.

Keywords: TSST-1, *Staphylococcus aureus*, Psoriasis, UTIs, and Gastroenteritis

Correspondence:

Dheyaa Shnan Al-Jameel
Department of Pathology, Faculty of Medicine
Jabir Ibn Hayyan Medical University
Najaf, Iraq
E-mail: dheyaa.shnan@jmu.edu.iq
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INTRODUCTION

Psoriasis is a disease that attack skin and its accessories like nails, the pathogenesis origin of disease is unknown, may be immunological or genetic or both together¹. Toxic shock syndrome TSS was first described in 1978 by Todd *et al.* who reported the presence of symptom complex in age group of 7 children range from 8 to 17 years with acute febrile illness². Chronic inflammation of skin with plaque which is well-defined scales is the more characteristic of this disease³. T-cells accumulation in the lesion of psoriasis indicating its role in disease which is reported by some studies that aid in stimulating keratinocytes to proliferate⁴. *Staphylococcus aureus* is a Gram- positive bacteria have been known to produce super antigens like toxic shock syndrome toxin 1 (TSST-I), enter toxin A, B, and C (SEA, SEB, SEC), as well to exfoliative toxin (ET), and their role in cellular effects in the psoriasis pathogenesis have been reported⁵. The inflammatory immune mediated disorders associated with psoriasis mostly combined by renal diseases, for examples renal amyloidosis, drug induced renal lesions, and IgA nephropathy⁶.

This study has been conducted at the microbiology lab in the department of basic science, college of dentistry, Kufa University in Najaf city- Iraq. Over a period from September 2016 to May 2017, total of 60 psoriatic patients and 30 controls have been submitted to this study, all patient aged 8-18 years whom attended to (Imam Alsajjad hospital and Alforat teaching hospital in Najaf city).

Sample collection: the urine and stool sample collected in disposable and sterilized container cups with necessary precautions⁷. Then the samples transported directly to the lab or stored in freeze (-20°C) until use. The culture were done by 0.1 ml of urine sample or swab streaking in the mannitol salt agar (Himedia, India), after incubation for 24h at 37°C, the identifications of positive mannitol fermentation colonies submitted to Gram stain, catalase test, coagulate test, production of clumping factor, and to the Api Staph identification kit (Bio-Merieux France). the isolated positive colonies of staphylococcus aureus submitted to confirmatory tests^{8,9}.

Molecular detection of TSST gene by PCR
Molecular TSST gene detection was done by PCR technique by amplified primer, Forward:

MATERIALS AND METHODS

5'GAAATTTTTCATCGTAAGCCCTTTGTTG 3' and Reverse: 5'

TTCATCAATATTTATAGGTGGTTTTTCA 3' ¹⁰. The reaction of PCR achieved in 50 µl reaction tube. The template of DNA (50 ng) mixed with master mix contain 5 µlTaq buffer (10x), 2 µldNTPs (10 mM), 2 µl of both primers (100 ng), 0.2 µlTaq of the DNA polymerase (3U/µl) then complete the volume to 50 µl with PCR double distilled water. The reactions tubes transferred to the thermo cycler (Biometra, Germany). The initial denaturation applied for 5 min at 94 °C and then denaturation for 30 sec at 94 °C, annealing for 30 sec at 55 °C and extension for 1.30 min at 72 °C, the cycle about Thirty-five, final extension step for 5 min at 72 °C. The product of PCR was loaded on agarose gel (1.0 %) along with 1500 bp DNA ladder. Then the result was visualized in a gel electrophoresis system (Biometra, Germany).

Extraction and purification of cell-free culture TSST-1 toxin

Toxin extraction and purification was achieved by affinity chromatography ¹¹. Bacterial strain defined by PCR as a toxin producer has been cultured on try tone soy broth with 0.6 % yeast extract supplement at 35°C with shaking at (150 rpm)for 24h, then centrifuged at (12,000 x g for 60 min) and the supernatant concentrated to third of its original volume by dialysis in 20 M polyethylene glycol then 0.02 M phosphate buffer with (pH 6.2) repeated four time. The resulted supernatant was applied in the column of Sephacryl S-200 (Sigma-Aldrich, Germany) in phosphate buffer, and then washed with buffer in 3 volume of column, and the toxin eluted by 2 volumes of 0.15 M sodium chloride resolved with 0.05 M phosphate buffer with pH 7.2. Column fractions were assayed for toxin by reversed passive latex agglutination TSST-1 by TST-RPLA toxin detection kit (Oxoid, UK). The positive fractions pooled and 10 % glycerol was added to the final concentration and stored in -20°C, the molecular weight estimated about 23kDa¹².

Evaluation of LD₅₀ for TSST-1

LD₅₀ for TSST-1 were determined in white mice weight about (20-25) g, divided in five groups each of them consist of five mice ¹³. Each group has been injected in the intraperitoneal with the concentration of (20, 40, 60, 80, and 100) µg/ml prepared by the toxin dissolved in PBS solution with injection volume 0.5ml for each mice. The negative

control consists of five mice injected with 0.5 ml of PBS. After 5 days the LD₅₀ has been determined ¹⁴.

TSST-1 lymphocyte Activation in Rabbit

A total of five groups of rabbits each of them with three rabbits have been injected intramuscular with serial dilution of toxin (20, 40, 60, 80, and 100) µg/ml and the another control negative group injected with PBS. After (6, 12, 24, 48) hours amount of 2.5ml of blood collected in anticoagulant tube (Dispo-EDTA) to determine the account of (WBCs and lymphocytes) by automated hematology analyzer.

Antibiotic susceptibility

Antibiotic susceptibility tests achieved on Mueller-Hinton agar (Himedia, India) with the procedure of disk diffusion method described by the standards of Clinical and Laboratory Standards Institute (CLSI). The disks of antibiotics were added to the plate after 15 minutes of bacterial streaking. The CLSI standard used to read the results. The types of antibiotics used in this study include: Chloramphenicol, (C: 10µg), Oxacillin, (OX: 5µg), Lincomycin, (L: 10µg), Bacitracin, (B: 10 units) Ampicillin and cloxacillin (APX: 25µg and 5 µg), Carbenicillin, (PY: 25µg), Nitrofurantion, (F: 300µg), Metronidazole, (MET: 30µg), Clindamycin, (DA: 10µg), and Amoxicillin, (AX: 25µg).

Statistical analysis

All the Statistical analysis in this study was achieved by using Statistical Package for Social Sciences (SPSS) version 25. The used test was Chi-square, *P*-value which less than 0.05 has been considered as statistically significant.

RESULTS

The result showed in table (1) indicates the distribution the ages of patients between 8-18 years that have been classified into five groups (two years interval). The site of psoriasis onset show the dominance for general body skin which is more affected by 25 (41.67 %) from total number then to nares 14 (23.33 %) while scalp and nails by 11 (18.33 %) and 10 (16.67 %) respectively. All result is significant compared with control groups. The more affected age groups is 16-18 years by 29 (48.33 %) versus 8-10 and 10-12 years by recording minimum infection ratios 4 (6.67 %).

Table 1: Distribution of site of psoriasis onset among age groups ranging from 8-18 years

Age groups* (years)	Site of psoriasis onset (n = 60)				
	Body skin n (%)	Scalp n (%)	Nail n (%)	Nares n (%)	Total n (%)
8-10	2 (3.33)	0 (0.00)	2 (3.33)	0 (0.00)	4 (6.67)
10-12	2 (3.33)	1 (1.67)	1 (1.67)	0 (0.00)	4 (6.67)
12-14	4 (6.67)	0 (0.00)	0 (0.00)	2 (3.33)	6 (10.00)
14-16	6 (10.00)	4 (6.67)	3 (5.00)	4 (6.67)	17 (28.33)
16-18	11 (18.33)	6 (10.00)	4 (6.67)	8 (13.33)	29 (48.33)
Total	25 (41.67)	11 (18.33)	10 (16.67)	14 (23.33)	60 (100.00)

* *P*-value=0.625, the result significant at the level of *P*≤0.05

According to site of psoriasis onset the most affected area is the skin combined UTIs in about 12(20.00 %) patients, then to nares and scalp by 5(8.33 %) and 2(3.33 %) respectively while no nail infection has been recorded , while gastroenteritis records 3(5.00 %) for body skin, 2(3.33 %)

for nails, 1(1.66 %) for nares, and no infection for scalp. Total growth of bacteria is 5(8.33 %) with dominance for body skin which 2(3.33 %), the TSST-1 production only two isolates 2(3.33 %) in body skin and nares.

Table 2: Prevalence of *Staphylococcus aureus* as causative agent of UTIs and gastroenteritis combined with psoriasis

Site of psoriasis* (n=60)	No of psoriasis combined UTI patients n (%)	No of psoriasis combined gastroenteritis patients n (%)	<i>Staphylococcus aureus</i> positive culture n (%)	Presence of Staphylococcal TSST-1 n (%)
Body skin	12(20.00)	3(5.00)	2(3.33)	1(1.66)
Scalp	2(3.33)	0(0.00)	1(1.66)	0(0.00)
Nail	0(0.00)	2(3.33)	1(1.66)	0(0.00)
Nares	5(8.33)	1(1.66)	1(1.66)	1(1.66)
Total	19(31.66)	6(10.00)	5(8.33)	2(3.33)

* P-value=0.455 the result significant at the level of $P \leq 0.05$

LD₅₀ for TSST-1 toxin

LD₅₀ dose has been evaluated through the route of intraperitoneal in BALB/c mice. The five serial

concentrations of TSST-1 (20, 40, 60, 80, and 100) µg/ml used in this study show that the LD₅₀ is 50 µg/ml which equal to 2mg/kg (Table 3 fig. 1).

Table 3: LD₅₀ for TSST-1 toxin produced by *staphylococcus aureus* isolated from psoriasis patients

TSST-1 concentration (µg/ml)	Mortality ratio	Died	Survived	Accumulation values			
				Died (D)	Survived (S)	Mortality ratio $\frac{D}{D+S}$	Percent % $\frac{D}{D+S} \times 100$
100	3/3	3	0	9	0	9/9	100
80	3/3	3	0	6	0	6/6	100
60	2/3	2	1	3	1	3/4	75
40	1/3	1	2	1	3	1/4	25
20	0/3	0	3	0	6	0/6	0

Proportional distance	=	50 - Mortality below 50 %	
		Mortality above 50 % - Mortality below 50 %	
=		50 - 25	= 0.5
		75 - 25	
Proportional distance 50 %	=	Proportional distance	× (Concentration above 50 % - Concentration below 50 %)
=		0.5	× (60-40) = 10
LD ₅₀	=	Proportional distance 50 % + Concentration below 50 %	
LD ₅₀	=	10 + 40 = 50 (µg/ml)	
=		2 mg/kg	

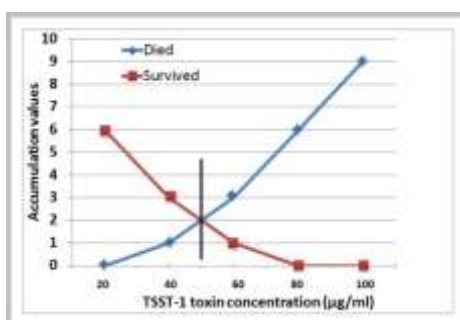


Figure 1: LD₅₀ for TSST-1 toxin produced by *staphylococcus aureus* isolated from psoriasis patients

The result of lymphocytes counts after intramuscular administration the serial concentration of TSST-1 (20, 40, 60, 80, and 100) $\mu\text{g/ml}$ to the rabbits explains the significant rising lymphocyte counts. The maximum level of lymphocyte recorded at the TSST-1 concentration of 100 $\mu\text{g/ml}$ after 48 hours of administration $24 \times 10^3/\mu\text{L}$

compared with control treatment $5 \times 10^3/\mu\text{L}$, then significant increase recorded for the concentration of 80 $\mu\text{g/ml}$ After 48 hours of administration $16 \times 10^3/\mu\text{L}$ compared with control treatment. All the increasing level of lymphocytes counts with time elapsed have been illustrated in figure (2).

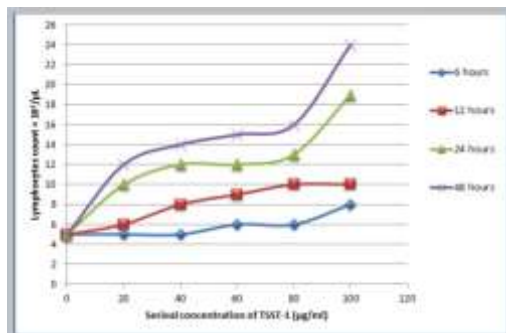


Figure 2: lymphocytes counts after administration serial concentration of TSST-1 (20, 40, 60, 80, and 100) $\mu\text{g/ml}$ to the rabbits, the estimated counts occur four times (6, 12, 24, and 48 hours) after administration of toxin, Lymphocyte counts $\times 10^3/\mu\text{L}$. $P\text{-value}=0.999$ The result significant at the level of $P \leq 0.05$

Total WBC count other than Lymphocyte after intramuscular administration the serial concentration of TSST-1 (20, 40, 60, 80, and 100) $\mu\text{g/ml}$ to the rabbits explain the significant rising WBC counts. The maximum level of lymphocyte recorded at the TSST-1 concentration of 100 $\mu\text{g/ml}$ after 48 hours of administration $9 \times 10^3/\mu\text{L}$ compared

with control treatment $3 \times 10^3/\mu\text{L}$, then significant increase recorded for the concentration of 80 $\mu\text{g/ml}$ After 48 hours of administration $8 \times 10^3/\mu\text{L}$ compared with control treatment. All the increasing level of WBC counts with time elapsed have been illustrated in figure 3.

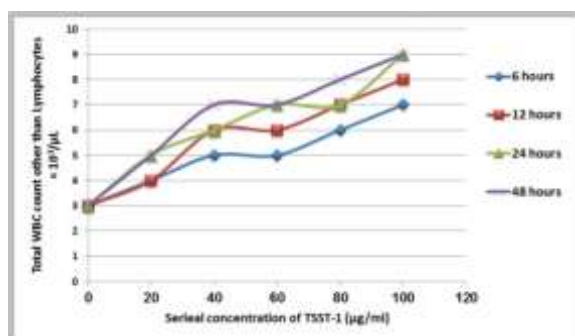


Figure 3: Total WBC count other than Lymphocyte after administration serial concentration of TSST-1 (20, 40, 60, 80, and 100) $\mu\text{g/ml}$ to the rabbits, the estimated counts occur four times (6, 12, 24, and 48 hours) after administration of toxin, WBC counts $\times 10^3/\mu\text{L}$. $P\text{-value}=0.996$ The result significant at the level of $P \leq 0.05$

Antibiotics susceptibility test

The test has been achieved by the method of disc diffusion and the aimed of this test is to investigate the resistant and susceptibility of 5 isolates of *staphylococcus aureus* for antibiotics where the results showed that Ampiclox and Chloramphenicol, are the highest level of susceptibility with

(80%) of isolates then Carbenicillin (60%) and for Nitrofurantion, Clindamycin, Amoxicillin, and Oxacillin which are (40%) then with (20%) susceptibility and finally Lincomycin and Metronidazole. Bacitracin was all five isolates of bacteria completely resistant. All the results of susceptibility and resistant has been illustrated in figure (5).

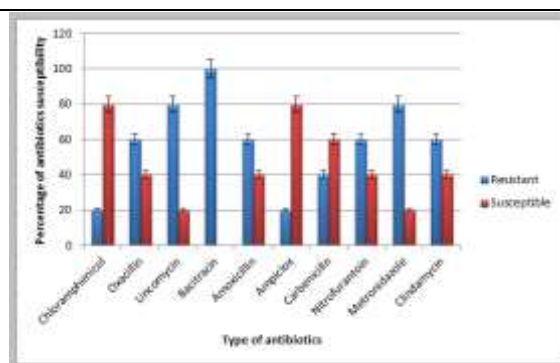


Figure 4: The percentage of antibiotics susceptibility and resistance to *staphylococcus aureus* isolated from psoriasis patients

DISCUSSION

Skin and joint is the mostly affected organs in psoriasis patients, renal and GIT involvements has been reported previously in the literatures. Although there is no prevalence of kidney diseases among patients of psoriasis, diseases of amyloidosis, drug induced renal lesions, and IgA nephropathy has been reported^{15,16}. Isolation of *Staphylococcus aureus* from about 60% of patients of psoriasis, out of them about 36% were diagnostic as atoxin producer like staphylococcal enter toxins A, B, C, exfoliative toxin, and toxic shock syndrome toxin 1 (SEA, SEB, SEC, ET, and TSST-I)^{17, 18}. Super antigen produced by *S. aureus* and *streptococcus spp.* estimated as the causative agents of psoriasis severity¹⁹. *Staphylococcus aureus* TSST-1 considered a leader of super antigens which capable to stimulate T cell many time than other types of antigen and 2-20% of all T cells in the body^{20, 21}. The measurement of toxin carried out by various methods which include ELISA, immunodiffusion, and agglutinations while the methods of amplification such as PCR for detection of gene which responsible for the production of TSST-1 is very highly specific and sensitive^{22, 23, 24, 25}. application of dose contain 200 µg/kg of staph TSST-1 then followed by dose of 400 µg/kg of LPS show fatality rate about 50% in mice. Nevertheless, administration of 10 ng/kg TSST-1 with 10 µg/kg LPS lead to fatality rate about 100% in rabbits. Developing lethality show approximately similar peak of circulating TNF-α IN both mice and rabbits, where they both sensitive to lethal dose of circulating TNF-α. Lymphocyte activation have been studied in the rabbits because its obtain high sensitivity to the TSST-1²⁶. Furthermore, the exposure to toxin or the bacteria that produce toxin lead to a disease sign in rabbits similar to that in human toxic shock syndrome, so that the rabbit model of experimental animals has been used to study the immunological activation of lymphocytes and other WBC in toxic shock syndrome^{27, 28, 29}. Organ affected by the toxin include liver, spleen, and lungs. After exposer to toxin, most of the organs have different degrees of responding and scientific cause is more important to explain. For example TNF-α produced by spleen may be difficult to detect it in the periphery blood, and lymphocyte may have been leave the circulation while the storm of cytokine after exposure to TSST-1^{30, 31}. Extravasations mechanism is not completely

understood, the migrating cell which present MHC II in the surface of it, which include (T cells, B cells, and monocyte macrophage lineage), uncertain whether the direct activation by interaction between TSST-1 and MHC II is sufficient for trigger the extravasations process³². The source of infection may be eliminated by effective sterilization procedures or by effective treatment with types of antibiotics which is highly active on strains of *Staphylococcus aureus* toxin producers^{33, 34, 35, 36}.

CONCLUSION

Psoriasis lesion is a threading source for transmission of *Staphylococcus aureus* to urinary and digestive system and causing UTIs or gastroenteritis and TSST-1 produced by *S. aureus* is high quality super antigen causing TSS.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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None

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

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