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Research Article

DETECTION OF STAPHYLOCOCCAL SUPER ANTIGEN BY PCR IN PATIENTS WITH PSORIASIS AND TO CORRELATE THE SEVERITY OF PSORIASIS WITH ENTEROTOXIN PRODUCTION

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ABSTRACT

Introduction: *Staphylococcus aureus* is a transient or persistent part of the resident flora in anterior nares of 20-50% of the population. *Staphylococcus aureus* is a major cause of multiple types of infections both in and outside of the hospital setting. Psoriasis is a chronic inflammatory skin disorder affecting 1-2% of the general population. The characteristic lesion of psoriasis is a sharply demarcated erythematous papule or plaque containing hyperproliferating keratinocytes as well as infiltrating neutrophils, monocytes, and T lymphocytes. Patients that harbored toxin-positive *S.aureus* on their skin had a significantly higher Psoriasis Area and Severity Index score than those with toxin negative *S. aureus*. With this background, this study was undertaken to detect the Staphylococcal superantigen in the patient of psoriasis. **Aim & Objective:** Detection of Staphylococcal Super Antigen by PCR in patients with Psoriasis and to correlate the severity of Psoriasis with Enterotoxin production. **Material & Methods:** The present study is a Hospital based prospective study. Clinically diagnosed psoriatic case and *Staphylococcus aureus* isolated from the above mentioned clinical samples were included. 40 clinically diagnosed psoriatic patients were included in the study and 3 swabs from different sites (psoriatic skin lesion, anterior nares and axillary region) were collected from each patient. To correlate the clinical prognosis among the psoriatic patients PASI score was calculated followed by culture & Sensitivity and Polymerase chain reaction was carried out to detect Sec, Sea and Sei genes. **Result:** Of 150 samples processed for culture, 96 *Staphylococcus aureus* were isolated. From Psoriatic patients 39(97.5%) were isolated from diseased skin lesion; 21(52.5%) from axilla; 36(90%) from anterior nares. All 40 psoriatic patients was scored for PASI and maximum range of 5.1-10.0 was seen in 16(40%) followed by 1.1-5.0 in 11(27.5%). Out of 96 *Staphylococcus aureus* isolates, superantigen gene was detected in 75(50%) isolates. 48(40.0%) were positive for SEC gene, 37(30.83%) were positive for SEA gene, 27 (22.50%) were positive for SEI gene.

Conclusion: Psoriasis was found to be more frequent in the third and fourth decades of the life. *S.aureus* seem to play an important role in inducing and triggering of psoriatic lesions. *S. aureus* on the skin of patients with psoriasis is associated with bacterial super antigen that activates the lot of T-cell lymphocytes. Psoriatic lesions inhabited by enterotoxigenic *S. aureus* which harbored enterotoxin Sec gene more than Sea and Sei gene.

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INTRODUCTION

Staphylococcus aureus is a major cause of multiple types of infections both in and outside of the hospital setting. These infections range from superficial skin infections to deeper infections of hair follicles, abscesses, and deep tissue infections.⁴ Psoriasis is a chronic inflammatory skin disorder affecting 1-2% of the general population. The characteristic lesion of psoriasis is a sharply demarcated erythematous papule or plaque containing hyper-proliferating keratinocytes as well

as infiltrating neutrophils, monocytes, and T lymphocytes. Although psoriasis is considered to be an autoimmune disease, increasing evidence suggests an important role for bacteria in its initiation and/or propagation.² *Staphylococcus aureus*, the major virulence factor of hospital and community acquired infections, secretes numerous exotoxins (super antigens), which may affect immunological and inflammatory status in psoriatic skin lesion.³ Staphylococcal superantigens (SAGs) are a unique family of bacterial toxins that activate large populations of both CD4 and CD8. T-cells and can induce polyclonal B-cell

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activation, as well. Unlike nominal peptide antigens, super antigens bind the T-cell receptor (TCR) outside the peptide binding groove on the VL region of the TCR, together with the major histocompatibility complex (MHC) class II molecule of the antigen presenting cell (APC). In contrast, as many as 20% of circulating T-lymphocytes may be activated by superantigens recognizing a particular VL region. This activation of large numbers of lymphocytes leads to the massive release of cytokines that mediate disease processes.¹ *S. aureus* has been demonstrated in approximately 60% of patients with psoriasis compared with 5% to 30% of individuals with a normal healthy skin.² In at least half of the cases, the *S. aureus* isolates were shown to secrete one or more of the staphylococcal enterotoxins; staphylococcal enterotoxin A (SEA), staphylococcal enterotoxin B (SEB), staphylococcal enterotoxin C (SEC) and staphylococcal enterotoxin D (SED), or toxic shock syndrome toxin. Patients that harbored toxin-positive *S. aureus* on their skin had a significantly higher Psoriasis Area and Severity Index score than those with toxin negative *S. aureus*.² With this background, this study was undertaken to detect the staphylococcal superantigen in the patient of psoriasis.

Objective

Detection of Staphylococcal Super Antigen by PCR in patients with Psoriasis and to correlate the severity of Psoriasis with Enterotoxin production.

MATERIALS AND METHODS

The present study is a Hospital based prospective study. 40 clinically diagnosed psoriatic patients were included in the study and 3 swabs from different sites (psoriatic skin lesion, anterior nares and axillary region) were collected from each patient and 15 healthy groups were included in the study to correlate the clinical prognosis among the psoriatic patients with healthy controls. 2 swabs were collected from different sites (anterior nares and axillary region) in healthy controls.

Clinically diagnosed psoriatic case and *Staphylococcus aureus* isolated from the above mentioned clinical samples were included. Patient on immunosuppressive agents, topical steroids and antibiotics followed by Pregnant and lactating women were excluded from this study. This study was approved by the Institute Ethics Committee and an informed consent was obtained from the patients or respective guardians accompanying the patient before collecting swabs from anterior nares, axillary and skin lesion.

PASI score among the psoriatic patients was calculated through clinical examination and severity of the infection. PASI score is defined as the Psoriasis Area Severity Index (PASI) which is used to express the severity of psoriasis. It is the combination of severity (erythema, induration, desquamation) and percentage of affected area.

All the samples were collected in the Department of Dermatology under clinical supervision and the samples were immediately transported to the Department of Microbiology for laboratory procedures. All *S. aureus* isolates were tested for routine culture and antibiotic susceptibility testing by Kirby–Bauer disk diffusion method on Mueller–Hinton agar as per standard protocol.

All the *S. aureus* isolates both from psoriasis patients and healthy controls were subjected to Molecular detection of Staphylococcal super antigen gene. DNA extraction, PCR and gel electrophoresis was done according to manufacturer's instructions.

DNA extraction was carried out for all *Staphylococcus aureus* isolates. DNA was extracted from overnight broth culture of *Staphylococcus aureus*, using HiPurA™ Bacterial Genomic DNA Purification Kit (#MB505 HIMEDIA) and extraction carried out as per manufacture protocol. The Purified DNA was then stored at -20 °C until being used for future research.

All the DNA extracted from *Staphylococcus aureus* were first validated by using 16S rDNA bacterial universal primer in a PCR reaction. Predesigned primer mix for *Staphylococcus aureus* enterotoxins genes namely Sea gene, Sec gene and Sei gene were procured from Helini Biosciences. (Table 1).

Table 1 Gene name and Primer sequence

S No.	Gene Name	Primer Sequence	Size (bp)
1.	16SrDNA	F-5'-CCAGCAGCCGCGGTAATACG-3' R-3'- ATCGGTTACCTTGTACGACTTC-5' F- 5'-GCAGGGAACAGCTTTAGGC-3'	996bp
2.	Sea gene	R- 5'- GTTCTGTAGAAGTATGAAACACG-3' F- 5'-	520bp
3.	Sec gene	AGATGAAGTAGTTGATGTGTATGG-3' R-5'- CACACTTTTAGAATCAACCG-3' F- 5'-	451bp
4.	Sei gene	CAACTCGAATTTTCAACAGGTAC-3' R-5'-CAGGCAGTCCATCTCCTG-3'	465bp

All the PCR components were thawed on ice and briefly spin down using micro-centrifuge. PCR master mix was prepared for detection of four genes from each DNA sample, which includes 16SrDNA gene for detection of *Staphylococcus aureus* in the given DNA sample. PCR reactions were carried out for Sea, Sec and Sei gene for 30µl volume.

All the PCR reaction mixer components were added to the PCR tube, depending on the number of reactions, the volume of each constituent was calculated and added in the sequence as mentioned below.

Thermocycling Temperature Sea, Sec and Seigene: PCR tube consisted of 29 µl of Master Mix + 1 µL of DNA. The conditions included an initial denaturation step of 5 min at 94°C, followed by 35 cycles of Holding Temperature:94°C for 2 minutes, Annealing Temperature:48°C for 2 minutes and Extension for 1 minute at 72°C, Final extension step of 7 min at 72°C. Isolates were screened for acquired *bla*KPC, *bla*NDM-1 and *bla*OXA-48 gene by PCR using primers and conditions described previously.

Evaluation of PCR Products by Agarose Gel Electrophoresis: PCR Product generated after PCR amplification of specific gene of interest from *Staphylococcus aureus* were further assessed using agarose gel electrophoresis. The gel was documented using gel documentation unit (BIORAD).

RESULT

A total of 150 swabs were collected. Among which 120 swabs were from 40 psoriatic patients and 30 were collected from 15 healthy carriers. The swabs were subjected to bacterial culture, antibiotic susceptibility test and Staphylococcal super antigen gene detection. Of 150 samples processed for culture, *S.aureus* was grown in 101 samples (96 in psoriasis patients and 5 in healthy controls). Out of 96 Staphylococcus aureus isolates from psoriatic patients 39(97.5%) were isolated from diseased skin lesion; 21(52.5%) from axilla; 36 (90%) from anterior nares, whereas in healthy carriers 05 (33.33%) isolated from anterior nares. (Table 2). P Value=<0.001.

Table 2 Showing the different site of S.sureus isolation from psoriatic patients and healthy carriers

Sample site	Positive culture Number (%)		Negative culture Number (%)	
	Psoriatic patient	Healthy carriers	Psoriatic patient	Healthy carriers
Diseased skin lesion	39(97.5%)	00	01(2.5%)	00
Anterior nares	36(90%)	05(33.33%)	04(10%)	10(66.66%)
Axilla	21(52.5%)	00	19(47.5%)	15(100%)
Total	96(80%)	05(16.66%)	24(20%)	25(83.34%)

Among 40 psoriatic patients ,26(65%) were male and 14(35%) were female. Out of 15 healthy carriers 10 (66.66%) were from male and 5 (33.33%) were from female.

All 40 psoriatic patients was scored for PASI and maximum range of 5.1-10.0 was seen in 16(40%) followed by 1.1-5.0 in 11(27.5%), 0.1-1.0 in 5(12.5%) and 10.1-15.0 and 15.1-20.0 scoring was seen in 4 patients each.

Majority of the psoriatic patients in our study belonged to the age group of 21-40 years-- 14(35%), followed by 41-60 years-- 11(27.5%), 61-80 years-- 9(22.5%) and 81 and above years 01(2.5%) respectively, whereas in Healthy controls majority of them belonged to the age group of 21-40 years—11(73.33%), followed by 1-20 years—04(26.66%).

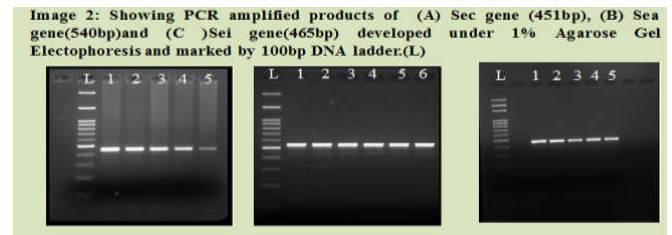
Clinical history from all the 40 psoriatic patients was collected from the case records. Co-morbidities such as hypertension 16(40%) followed by diabetes mellitus 12(30%), hypothyroidism 2(5%) was seen in our subjects and no predominant clinical history was seen in 10 (25%) patients respectively.(Table3)

Table 3 Clinical History In Psoriatic Patients

Clinical features	Number	Percentage
Dm	12	30%
Htn	16	40%
Hypothyroidism	02	5%
No history	10	25%

A total of 101 staphylococcus aureus isolates were subjected to superantigens(SEC, SEA and SEI) detection using gel electrophoresis. Of 101 isolates 96 were from psoriatic patients and 5 were from healthy controls. Out of 96 staphylococcus aureus isolates, superantigen gene was detected in 75(50.00%) isolates. And all the 5 isolates from healthy controls did not detect the presence of genes for super-antigens. Of 96 S.aureus isolates, 48(40%) were positive for SEC gene, 37(30.83%) were positive for SEA gene, 27(22.50%) were positive for SEI gene (Table 4). Few isolates have shown the presence of two

or more genes. 16(13.33%) of the isolates detected both SEC and SEA genes, 11(9.16%) showed the presence of SEA and SEI, 14(11.66%) detected SEI and SEC. 4 (3.30%) of the isolates detected all the three genes SEC, SEA and SEI in Lesion, axilla and Nasal samples.



Among 150 samples collected (120 Psoriatic samples(lesion, axilla and nasal from each patient) & 30 samples from 15 healthy controls. All healthy control were negative for superantigen genes. From total of 120 Psoriatic samples(lesion, axilla, nares) together showed, Sec gene was detected in 48(40%) followed by 37(30.83%) Sea gene and 27(22.50%) Sei gene. Followed by combinations showed 16(13.33%)for sec+sea gene, 11(9.16%) for sea+sei gene and 14(11.66%) for sei+sec gene. All three gene combination showed 4(3.30%) respectively (table4).

In 40 Lesion sample in psoriatic patients, 29(72.50%) sec gene was detected, 20(50.00%) sea gene, 15(37.50%) sei gene. Followed by combination genes, sec+sea showed 12(30.00%), sea+sei showed 7(17.50%), sei+sec showed 9(22.50%) and all three genes showed 3(7.50%)(table 4).(p=0.001)

Among 40 Anterior nares swabs, Sec gene was detected in 19(47.50%) followed by 17(42.50%) Sea gene and 12(30.00%) Sei gene respectively.(Table 4) combination genes showed sec+sea showed 4(10.00%), sea+sei showed 4(10.00%), sei+sec showed 5(12.50%) and all three genes showed 1(2.50%)(table 4). And no sample from Axilla showed the presence of superantigen genes. (p=0.001)

Lesion and nasal samples together showed the presence of same gene in individual patients showed 12(15.00%) in sec gene, 6(7.50%) in sea gene, 5(6.25%) in seigene followed by sec+sea gene showed 1(1.25%) positive rate. But none of the patients showed combination of sea+sei / sei+sec gene and all 3 genes combinations respectively.(Table4)(p=0.001).

Table 4 Genotypic results of Staphylococcal Superantigen gene

Samples	Genes	No. of Positives	Percentage
Total of Lesion, Nasal & Axilla (n=120)	SEC	48	40.00%
	SEA	37	30.83%
	SEI	27	22.50%
	(SEC+SEA)	16	13.33%
	(SEA+SEI)	11	9.16%
	(SEI+SEC)	14	11.66%
	(SEC+SEA+SEI)	04	3.30%
	SEC	29	72.50%
	SEA	20	50.00%
	SEI	15	37.50%
LESION (n=40)	(SEC+SEA)	12	30.00%
	(SEA+SEI)	07	17.50%
	(SEI+SEC)	09	22.50%
	(SEC+SEA+SEI)	03	7.50%
NASAL (n=40)	SEC	19	47.50%
	SEA	17	42.50%
	SEI	12	30.00%

	(SEC+SEA)	04	10.00%
	(SEA+SEI)	04	10.00%
	(SEI+SEC)	05	12.50%
	(SEC+SEA+SEI)	01	2.50%
Axilla (n=40)	All 3 genes	00	00.00%
	SEC	12	15.00%
	SEA	06	7.50%
	SEI	05	6.25%
In each patient- Both Lesion & Nasal (n=80)	(SEC+SEA)	01	1.25%
	(SEA+SEI)	00	00.00%
	(SEI+SEC)	00	00.00%
	(SEC+SEA+SEI)	00	00.00%
Health samples (n=30)	All 3 genes	00	00.00%

DISCUSSION

Accumulating evidences indicate that psoriasis is a multifactorial disorder triggered by environmental factors (Elder *et al.* 2001).¹⁰ As with many complex diseases, both genetic and environment play a role in the development of psoriasis (Peter *et al.* 2000; Langley *et al.* 2005).^{11,12}

Regarding the environmental factors, staphylococcal and streptococcal infections are among the most common triggering factors that initiate and exacerbate the disease (Kotzin *et al.* 1993).¹³

Many studies reported that psoriasis can appear at any age and has been reported at birth and in elderly (Smith *et al.* 1993; Ferrandiz *et al.* 2002)^{14,15}, but the most common onset is in the second to fourth decades of the life (Babu 2001)¹⁶. Studies have shown that 75 - 90% of 126 patients have their psoriasis before the age of 40, with a peak of case onset around puberty and a smaller peak around 50-60 years of age (Melski&Stern, 1981; Swanbeck *et al.* 1995).^{17,18} In our study, majority of the psoriatic patients belonged to the age group of 21-40 years--14(35%).

Our study revealed that males were affected by psoriasis more than females in most age groups. This result is in line with Obasi (1986)¹⁹ and Babu (2001)¹⁶.

In the present study, various degrees of severity were associated with psoriatic patients. All 40 psoriatic patients was scored for PASI and maximum range of 5.1-10.0 was seen in 16(40%) Such findings are parallel to the study of Inerot *et al.* (2005) in which 70% of psoriatic patients were mild rather than severe and moderate (25% and 20%) respectively.²⁰

One of the most important clinical features associated with psoriasis is the presence of the hypertension 16(40%) followed by diabetes mellitus 12(30%).

In a study by Balci *et al.* ⁸, 64% of the diseased and 14% of healthy skin cultures obtained from psoriasis patients were found to be positive for *S. aureus*. They also found a significant relationship between toxin production of the strains isolated from skin lesions and disease grades.⁸ 96 *Staphylococcus aureus* isolates from psoriatic patients in our study were significantly isolated from diseased skin lesion 39(97.5%) when compared with isolates from axilla 21(52.5%) and from anterior nares 36(90%), whereas in healthy group only 05 (33.33%) isolates from anterior nares.

Tomi *et al.*⁶ showed that 36% of the *S. aureus* strains isolated from skin lesions of psoriasis patients secreted toxins. In the same study, psoriasis patients who carried toxin-negative and toxin-positive *S. aureus* strains were compared, and disease

grades of the patients with toxin-positive strains were found to be higher. These results show that there is a relationship between toxin positive *S. aureus* colonization and psoriasis activation.⁶

On the other hand, Sayama *et al.*⁹ could only demonstrate the presence of enterotoxin (*seb*) and *tst-1* in 5 of the 100 *S. aureus* strains isolated from diseased skin swabs of psoriasis patients, and they concluded that Sags do not have a role in the development of psoriasis.⁹ Recently *Staphylococcus* superantigens are proposed as a possible antigen in psoriasis Leung *et al.* 1993b.⁷

Of 101 *S.aureus* isolates 75 detected the presence of superantigens and 26 detected none and the results of molecular methods are as follows: 22(22.91%) were positive for SEC gene, 14 (14.58%) were positive for SEA gene, 06 (6.25%) were positive for SEI gene. Few isolates have shown the presence of two or more genes. 12 (12.5%) of the isolates detected both SEC and SEA genes, 07 (7.29%) showed the presence of SEA and SEI, 10 (10.41%) detected SEI and SEC. 4 (4.16%) of the isolates detected all the three genes SEC, SEA and SEI.

Among 96 *S.aureus* isolates, 41(42.70%) were MRSA and 55(57.29%) were MSSA. 20 isolates of MSSA showed presence of SEC and SEA genes each and 12 isolates showed the presence of SEI gene. Among 41 MRSA isolates, SEC gene was predominantly detected in 28, followed by SEA in 17 and SEI in 15. Combination of few genes were majorly seen in MRSA, SEC+SEI in 6, SEC+SEA in 9, SEA+SEI in 4 and SEC+SEA+SEI in 2 isolates of MRSA, this is high when compared to the strains of MSSA where SEC+SEI in 4 followed by SEC+SEA in 3, SEA+SEI in 3 and SEC+SEA+SEI in 2 isolates of MSSA.

Jülide Sedef Göçmen *et al* demonstrated the presence of toxin genes in 20 (35.71%) of the 56 MSSA strains. Among diseased skin isolates (n = 7), 6 carried the *sei* and 1 the *seg* gene. Four of the 11 healthy skin isolates carried the *sei* gene, and of the 9 nasal isolates, 8 carried the *sei* gene and 1 the *seb-sec* genes.⁵

CONCLUSION

Psoriasis was found to be more frequent in the third and fourth decades of the life. *S.aureus* seem to play an important role in inducing and triggering of psoriatic lesions. Adding antibiotic against *S. aureus* to other conventional treatment might be helpful. *S. aureus* on the skin of patients with psoriasis is associated with bacterial super antigen that activates the lot of T-cell lymphocytes. Quantitatively the *S. aureus* density was higher in psoriatic lesions than uninvolved healthy skin of psoriatic patients and control group. Psoriatic lesions inhabited by enterotoxigenic *S. aureus* which harbored enterotoxin Sec gene more than Sea and Sei gene. In our study Sec, Sea and Sei gene were detected from the psoriatic lesion and anterior nares swabs from the same patients. This may support the role of super antigens in the exacerbation of psoriasis.

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