

## Streptococcal super antigens and LL-37 antimicrobial peptide as an immune mediator among psoriasis patients in Iraq

Ifad Kerim Al-Shibly<sup>1\*</sup>, Hussein Abbas Al-Sultany<sup>2</sup>

<sup>1,2</sup> College of Medicine, University of Babylon/Iraq

\*Corresponding author: Ifad Kerim Al-Shibly ([medimmunity@yahoo.com](mailto:medimmunity@yahoo.com))

### Abstract:

Psoriasis is a polygenic skin illness with pathogenesis still not well clarified. Guttate psoriasis, one of a modified type of psoriasis headed by respiratory infection by *Streptococcus pyogenes* one of group A beta-hemolytic streptococcus, types of streptococcal exotoxin (spe a, spe b, spe c) that stimulate T lymphocytes. We aimed to investigate the relation between streptococcal super antigens, antimicrobial peptide and the immune system among psoriasis patients. A cross-sectional study project conducted on 38 acute guttate type psoriasis patients. The throat swabs were taken for bacterial culture. DNA was extracted for the detection of three targeted genes for superantigens (speA, speB, and speC), by conventional PCR technique using specific primer pairs. Blood samples collected for the evaluation of serum level of LL-37 by ELISA method. Psoriasis patients mean age  $18 \pm 2.7$  years. Among 38 psoriatic patients, 17 patients (44.73%) were males and 21 patients (58.3%) were female. 29 patients gave a positive history forming 76.31% out of total psoriasis patients in the study in the last one month most patients gave an acute onset within less than 10 days with a mean onset of  $8 \pm 1.4$  days. Out of the total of 38 patients in the study, 31 patients (81.57%) showed positive results for the isolation of *S. pyogenes* bacteria. The genetic study established that SpeA, SpeB, and SpeC genes were identified in a frequency of 17(54.83%), 23(74.19%), and 15(48.38%) respectively. Finally, the LL-37 level in the healthy control group was significantly lower than its level among psoriasis patients in the study and founded a significant difference between the two groups ( $P=0.004$ ). In conclusion, the results of the present study reach a conclusion that Streptococcal infection is a recognized trigger of psoriasis, especially guttate psoriasis, their pyrogenic exotoxins; superantigens are potent immunostimulators and play important role in the pathogenesis of psoriasis.

**Keywords:** guttate psoriasis, streptococcal super antigens, LL37, SpeA, SpeB, and SpeC genes

**How to cite this article:** Al-Shibly IK, Al-Sultany HA (2020): Streptococcal super antigens and IL-37 antimicrobial peptide as an immune mediator among psoriasis patients in Iraq, *Ann Trop Med & Public Health*; 23(S18): SP231811. DOI: <http://doi.org/10.36295/ASRO.2020.231811>

### Introduction:

Psoriasis is a skin illness describe as an immune-mediated polygenic. Numerous environmental causing reasons, e.g. trauma, contagions, drugs, may provoke illness in susceptible persons. Psoriasis is a T cell-mediated skin illness described by inflamed, scaly, and finely defined plaques on the skin, nail deformities, and, in some patients, critical arthritis<sup>[1, 2]</sup>. Guttate psoriasis is lesion looks like a water drop, 2-5mm. this lesion occurs suddenly after recent infection, like streptococcal pharyngitis, Guttate psoriasis happens mostly in patients under age 30. The association of streptococcal pharyngitis with guttate psoriasis is well established. *Staphylococcus aureus* and *Streptococci secreta* exotoxins that act as superantigens, producing massive T-cell activation, and pharyngeal colonization should be addressed as previously noted<sup>[3]</sup>. An association between tonsillitis and psoriasis first noted nearly 100 years ago. Oligo clonal development of T cells happens in the tonsils in reply to the colonization of streptococcal, and the similar T-cell list is found in the blood and skin of guttate psoriasis patients<sup>[4, 5]</sup>.

The causes of psoriasis not well defined, but many mechanisms involved psoriasis antigen, streptococcus illness considered severe disease that causes health harms, exotoxin type A (speA), type B (speB), type C (speC), are mitogen that causes stimulation of T lymphocytes, lead to release T mediators and cytokines that lead toxic shock disease then psoriasis. Now, around 11 types of antigens of *Streptococcus pyogenes* are defined<sup>[2,6]</sup>. Compared to a normal antigen-induced T-cell response, these S. antigens are capable of activating up to 20% of the T-cells<sup>[7, 8]</sup>.

**Methods:**

A cross-sectional revision design conducted on 38 acute guttate type psoriasis patients who were referred to Merjan teaching hospital /dermatology clinic, Babylon province, from January 2019 - September 2019. Gender distribution among psoriasis patients was 21 females and 17 males, while patients' age range was 13-46 years with mean age  $18\pm 2.7$  years. A full information data from each patient taken in a questionnaire regarding age, gender, history of onset of psoriasis, history of recent throat infection, medical and drug history. A specialist dermatologist accomplished the diagnosis of acute guttate psoriasis, and the severity of the disease recorded for all patients according to the PASI score.

**Inclusion and exclusion criteria**

Patients with an acute guttate type of psoriasis any age and gender were included in the study. Any patient with chronic disease, or have any condition that may interfere with the immunological evaluation of LL-37 serum level excluded from the study.

**Bacteriological study**

The throat swabs taken from the tonsils and post pharyngeal region by the physician for bacterial culture and were inoculated on blood agar plates and brain heart infusion agar. The plates incubated at 37°C for 18 to 24 hours under 5 – 10 % Co<sub>2</sub>, and then subjected for further conventional laboratory diagnosis. A pure colony was taken from a positive culture. Its identification depended on the cultural characteristic, colony morphology,  $\beta$ -hemolysis on blood agar, and Bacitracin susceptibility<sup>[9]</sup>.

**Genetic study**

For genomic DNA isolation, an overnight culture was prepared. DNA was extracted according to the manufacturer's instructions and was stored at  $-20^{\circ}\text{C}$ . *S. pyogenes* isolates were subjected to the detection of their three targeted genes for superantigens A, B, and C by conventional PCR technique using specific primer pairs. The amplification was conducted in a Thermal Cycler (ESCO, India) with the programs described by (Baidya *et al.*)<sup>[10]</sup> or the three selected genes (SpeA, SpeB, and SpeC). Agarose gel electrophoresis used to visualize the tested amplicons on 1.8% agarose gel. Electrophoresis was performed for about 1 h at a constant voltage of 80 V in running buffer containing 0.5 $\times$  TBE and ethidium bromide (0.5  $\mu\text{g}/\text{ml}$ ) (Sigma Aldrich Corp., St. Louis, MO, USA).

**Serological study**

Blood samples collected by drawing 3ml of blood from each subject included in this study using sterile 5ml syringes with sterile needle G-22. The blood samples for the evaluation of serum levels of LL-37 were collected after phlebotomy in evacuated tubes (without anticoagulant) and were centrifuged at 4000 rpm for 5 min after the blood had clotted then all samples were stored at deep freezer for ELISA study. This ELISA kit uses the Sandwich-ELISA principle according to the manufacturing procedure provided by Human LL-37 (Antibacterial Protein LL-37) ELISA Kit, Elabscience, USA, and Lot number E-EL-H2438.

**The ethical approval**

The verbal agreement took for each patient before starting an oral vitamin D regimen after a full explanation about the nature and target of this study. Statistical analysis was done by SPSS-20. Data presented in simple measures of mean and standard deviation with P-value < 0.05.

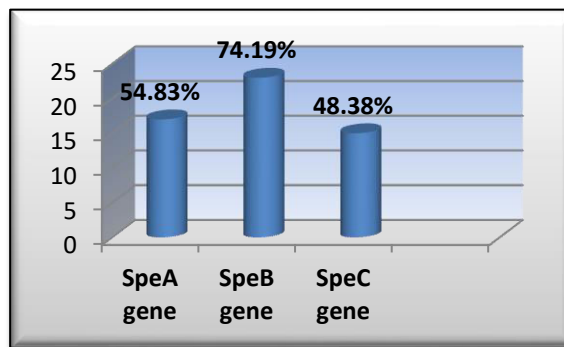
**Results**

In this study, we assessed the frequency of isolation of *S. pyogenes* and SpeA, SpeB, and SpeC genes, and the serum level of LL-37 antimicrobial peptide from psoriasis patients in a cross-sectional study conducted in Babylon province / Iraq from January 2019 - September 2019. Psoriasis patients' age range was 13-46 years with a mean age of  $18\pm 2.7$  years. Among 38 psoriatic patients, 17 patients (44.73%) were males and 21 patients (58.3%) were female. All the 38 psoriatic patients scored for PASI in a range of 4.6-12.5 and a mean PASI of  $7.3\pm 2.2$ . Regarding the history of recent throat infection, 29 patients gave positive history forming 76.31% out of total psoriasis patients in the study while 9 patients (23.68%) ignored any occurrence of a throat infection in the last one month. Otherwise, regarding the history of onset of the disease most patients gave an acute onset within less than 10 days with a mean onset of  $8\pm 1.4$  days. These demographic data illustrated in the table (2).

**Table (2):** Demographic information scattering among psoriasis patients.

Demographic data	Recordings
Mean age / years	18±2.7
Gender : males females	17 (44.73%) 21 (58.3%)
Mean PASI score	7.3±2.2
History of recent throat infection	29 (76.31%)
History of onset of disease / days	8±1.4

Out of the total of 38 patients in the study, 31 patients (81.57%) showed positive results for the isolation of *S. pyogenes* bacteria by conventional laboratory tests confirmed by the Vitek 2 System. The genetic study established that SpeA, SpeB, and SpeC genes were identified in a frequency of 17(54.83%), 23(74.19%), and 15(48.38%) respectively among psoriasis patients as presented in figure (1).



**Figure (1):** Distribution of SpeA, SpeB, and SpeC genes among psoriasis patients in the study

In the existing study, the antimicrobial peptide cathelicidin (LL-37) serum level was evaluated as an immune parameter to deliberate its role in the pathogenesis of guttate psoriasis, using an ELISA kit the results in table (3) displayed that the LL-37 level in the healthy control group was significantly lower than its level among psoriasis patients in the study and significant difference between the two groups ( $P=0.004$ ).

**Table (3):** The distribution of serum level of LL-37 among psoriasis patients and healthy controls.

Factor	Psoriasis patients	Healthy control	P-value
LL- 37	60.43 ± 11.21	31.52 ±13.42	0.004

**Discussion:**

Accumulating evidence indicates that psoriasis is a multifactorial illness caused by environmental factors. As with many complex diseases, both genetic and environment play a role in the development of psoriasis [11]. In the present study psoriasis, patients’ age range was 13-46 years with a mean age of 18±2.7 years. Despite this relatively wide age range, most patients in this study were adolescents and this result was confirmed by previous researches about the age of onset of guttate psoriasis who indicated that guttate psoriasis regularly occurs in children plus adolescents especially those individuals younger than 30 years [12]. Likewise, Lee *et al.*, 2016 noticed that guttate psoriasis is more frequent in children than adults, particularly under the young age in whom it may be the first presentation of chronic plaque psoriasis [13].

Regarding gender distribution study in this work, results showed that among 38 psoriatic patients, 21 patients (58.3%) were female and only 17 patients (44.73%) were males. These results augmented by previous study stating that guttate psoriasis seems to be marginally extra predominant amongst females than males although men's psoriasis is often more serious which explains a gender gap, with more men than women seeking psoriasis treatment. This study may give an explanation to our study results about the somewhat low severity level of psoriasis which was assessed by the PASI score (7.3±2.2) that indicated mild-moderate overall severity among most of the patients in our study [14].

Study of the history of onset of guttate psoriasis in the present study most patients gave an acute onset within less than 10 days with a mean onset of  $8 \pm 1.4$  days. Many previous researchers established these results, typical psoriasis lesions characteristically found as sudden lesion after recent infection, Guttate psoriasis looks like acute slight erythematous plaques occur more in child and adolescent, activated by group-A streptococcal that infected the tonsils. 1/3 of patients that had guttate psoriasis lead to progress to plaque psoriasis during their life. Studying acute or early forms of psoriasis may provide insights into the initiating inflammatory events driving the development of chronic disease <sup>[10, 15]</sup>.

Group streptococci have a worldwide distribution. GAS is the most predominant cause of pharyngitis and is associated with the non-supportive sequelae, psoriasis. The genes that are more commonly associated with psoriasis SpeA, SpeB, and SpeC in the clinical isolates. These pyrogenic exotoxins are superantigens that are potent immunostimulators that results in activation of a large number of T cells leading to increased secretion of proinflammatory cytokines. The SpeB gene chromosomally encoded, whereas the exotoxin encoding genes SpeA and SpeC carried by lysogenic phages, and easily disseminated to other GAS. 31 confirmed isolates of *S. pyogenes* were obtained from the above-mentioned samples in the study period. The overall prevalence of the SpeB gene was 80.6% followed by SpeC that was found to be 35.5%. The SpeA gene was detected only in a single isolate <sup>[10]</sup>.

Our results recognized that the LL-37 level in the healthy control group was significantly lower than its level among psoriasis patients in the study and there was a highly significant difference between the two groups ( $P=0.004$ ). Psoriasis categorized to Th1 and Th17, Th17 more important in the study that has a role antimicrobial peptides in addition to dendritic cells in the pathology of psoriasis. Cathelicidin LL-37 causes stimulation of dendritic cells and encourages interferons secretion. Patients with psoriasis (plaque and guttate) had an increase in cathelicidin LL-37 than normal peoples. In the existing study, LL37 investigated in order to identify its possible role as an immune modulator among patients with guttate psoriasis. Morizaneet *et al.*, 2012 who established that LL-37 not only has the capacity to kill a wide variety of microbes but also can modify host immune and growth responses, supported the present study results. In their study, they have shown that keratinocytes will respond to microbial stimulus in an LL-37 reliant on the way. I IFNs considered important in the pathology of psoriasis, profuse keratinocytes stating LL-37 in psoriatic skin lesion and make this lesion critical <sup>[16]</sup>.

Similarly, in accordance with our results about the LL37 level among psoriasis patients, other research outcomes display that (LL37) is certainly documented as an autoantigen mediated by T cells in the blood stream especially in moderate and severe psoriasis patients (75% and more had PASI > 10). IL-17 secreted by specific LL37 T lymphocytes that lead to yield pathogenic cytokines pass to the blood stream. So LL37 acts as autoantigen exciting together innate and adaptive immune cells <sup>[6]</sup>. In conclusion, the results of the present study reach a conclusion that Streptococcal infection is a recognized trigger of psoriasis, especially guttate psoriasis, their pyrogenic exotoxins; superantigens are potent immunostimulators and play important role in the pathogenesis of psoriasis. Furthermore, LL37 documented as an autoantigen mediated by T cells among patients with psoriasis stimulating both innate and adaptive immune cells in autoimmune settings.

#### References:

1. Johnston A., X. Xing, A.M. Guzman, M.B. Riblett, C.M. Loyd, N.L. Ward. IL-1F5, -F6, -F8, and -F9: A Novel IL-1 Family Signaling System That Is Active in Psoriasis and Promotes Keratinocyte Antimicrobial Peptide Expression. *J Immunol* 2011; 186:2613-2622.
2. Proft, T. & Fraser, J. D. Streptococcal Superantigens: Biological properties and potential role in disease. *Streptococcus pyogenes : Basic Biology to Clinical Manifestations* (2016). P:1-16.
3. James, WD, DM, Elston, TG, Berger. (2016). *Andrews diseases of the skin clinical dermatology*. Elsevier. Twelfth Edition. 217-225.
4. Griffiths, CE, J Barker, T Bleiker, R Chalmers, and D Creamer. (2016). *Rook's Textbook of Dermatology*. 9th ed. Oxford: Wiley-Blackwell; 2016. pp. 1130–1160.
5. Ferretti, J. J., Stevens, D. L. & Fischetti, V. a. *Streptococcus pyogenes: Basic Biology to Clinical Manifestations*. *Streptococcus pyogenes: Basic Biology to Clinical Manifestations* 1–15 (2016).
6. Baranska-Rybak, W. et al. Activity of Antimicrobial Peptides and Conventional Antibiotics against Superantigen Positive *Staphylococcus aureus* Isolated from the Patients with Neoplastic and Inflammatory Erythrodermia. *Chemotherapy Research and Practice* **2011**, 1–6 (2011).
7. Rasooly, R., and Hernlem, B.: Auto-presentation of Staphylococcal enterotoxin A by mouse CD4+ T cells. *Open Journal of Immunology*, 1, 8-14. (2011).
8. Wu PCh., WT. Lo, ShJ. Chen, ChCh. Wang. Molecular characterization of Group A streptococcal

- isolates causing scarlet fever and pharyngitis among young children: A retrospective study from a northern Taiwan medical center. *Journal of Microbiology, Immunology and Infection* . (2014) 47, 304-310.
9. Holt, J., Krieg, N., Sneath, P., Staley, J. & Williams, S. *Bergey's manual of determinative microbiology*, 9th edn. Lippincot, Williams Wilkins, Balt. (2000).pp 787.
  10. Baidya A., B. Bimal, K. Das, A. Kabil. Characterisation of *Streptococcus pyogenes* on the basis of pyrotoxin exotoxin genes in north India. *J Med Trop* 2017;19:104-9.
  11. Alshobaili, H. A. et al. Genetic background of psoriasis. *International journal of health sciences* 4, 23–9 (2010).
  12. Bologna, JL, JV. Schaffer, L. Cerroni. (2018). *Bologna dermatology*. Fourth edition. Elsevier.168-191.
  13. Lee., A.-J. and H. Benign Cutaneous Adverse Reaction. In:Griffiths CE, Barker JN, Bleiker T, et al. *Rook's textbook of Dermatology*. 9th ed. Oxford, England: Willy-Blackwell, 2016:181.1-2.
  14. Hägg, D., Sundström, A., Eriksson, M. & Schmitt-Egenolf, M. Severity of Psoriasis Differs Between Men and Women: A Study of the Clinical Outcome Measure Psoriasis Area and Severity Index (PASI) in 5438 Swedish Register Patients. *American Journal of Clinical Dermatology* 18, 583–590 (2017).
  15. Ko H.C., Jwa S.W., Song M., Kim M.B., Kwon K.S. Clinical course of guttate psoriasis: Long-term follow-up study. *J. Dermatol.* 2010;37:894–899.
  16. Morizane Sh., K. Yamasaki, B. Mu'hleisen, PF. Koto, M.Murakami, Y. Aoyama, K. Iwatsuki, T. Hata and R.L. Gallo. Cathelicidin Antimicrobial Peptide LL-37 in Psoriasis Enables Keratinocyte Reactivity against TLR9 Ligands. *Journal of Investigative Dermatology* (2012) 132, 135–143.