Original Article

Assessment of the Role of CTLA-4 and CD28 in a Sample of Iraqi Patients with Psoriasis in the Presence of *Staphylococcus aureus* Colonization

Ali Nayyef Umayra, Jabbar S. Hassan, Hasan Nasir Mohsen

Department of Medical Microbiology, College of Medicine, Al-Nahrain University, Baghdad, Iraq

Abstract

Background: There is substantial evidence that the dysregulation of immune cells in the skin, specifically T lymphocytes, plays a crucial role in the development of psoriasis. Objectives: The aim of this study was to investigate the potential role of CTLA-4 and CD28 in the pathogenesis when Staphylococcus aureus is present. **Objectives:** The aim of this study was to investigate the potential role of CTLA-4 and CD28 in the pathogenesis when Staphylococcus aureus is present. **Materials and Methods:** Swabs and blood samples were collected from 225 individuals who enrolled in this case-control study. The patient group that participated in this study was recruited from the Dermatology consulting department at Al-Imamein Al Kadhimein Medical City, Baghdad, Iraq. The swabs were inoculated on blood agar media, then kept at 37ºC for 1 day in the aerobic environment for primary isolates of S. aureus, whereas sera were separated from the blood sample and used in enzyme linked immunosorbent assay (ELISA) methods for the determination of CTLA-4 and CD28 concentrations. **Results:** Out of 225 individuals recruited in this study, there were 112 men whereas the women were 113 with male-to-female ratio of 1:1. Female patients' age ranged from 7 to 70 years with a mean age of 31.67 years, whereas male patients' age ranged from 5 to 52 years with a mean age of 25.27 years. Difference between means (6.405 ± 2.198), *S. aureus* isolates were identified in 82 (36.44%); serum samples from subjects for which *S. aureus* was isolated screened by ELISA for CTLA-4 and CD28. The results of the mean serum level of CTLA-4 demonstrated that there was an interesting highly significant difference ($P \leq 0.0001$) in CTLA-4 level between the psoriatic patient's group versus negative control groups. Regarding CD28, the comparison among the three groups of individuals in this study showed significant differences between the positive control group versus the psoriatic patients' group and the negative control group ($P < 0.05$) in the mean serum level of CD28. **Conclusion:** There was a significant role of toxigenic isolates from *S. aureus* on CTLA-4 and CD28.

Keywords: CD28, cytotoxic T-lymphocyte antigen 4 (CTLA-4), psoriasis, *Staphylococcus aureus*

Introduction

Psoriasis is a prevalent chronic inflammatory skin condition that affects 2%–4% of the general population.[1] The psoriasis disease process is caused by a network of cell types, including T cells, dendritic cells, and keratinocytes, which establish a chronic inflammatory state through the generation of cytokines.[2,3]

Psoriasis, a chronic skin disease, severely impacts patients' lives. Most patients have erythema, papules, and scales. Lesions are most common on the elbows and knees' extensors. To this day, psoriasis's exact origins in the body are a mystery. Genetics, infections, anomalies in the immune system, and endocrine issues are all suspected causes.[4]

There are numerous molecules that can act as co-stimulators, and CD28/CD80 signaling is one of the most important molecules in modulating T-cell response. CTLA4 (cytotoxic

Access this article online

Quick Response Code:

Website: https://journals.lww.com/mjby

DOI: 10.4103/MJBL.MJBL_200_23

Address for correspondence: Dr. Ali Nayyef Umayra, Department of Medical Microbiology, College of Medicine, Al-Nahrain University, Al-Kadhimiyah, Baghdad, Iraq.
E-mail: skyoneskytwomef@gmail.com

Submission: 27-Feb-2023 **Accepted:** 07-Mar-2023 **Published:** 23-Jan-2026

How to cite this article: Umayra AN, Hassan JS, Mohsen HN. Assessment of the role of CTLA-4 and CD28 in a sample of Iraqi patients with Psoriasis in the presence of *Staphylococcus aureus* colonization. Med J Babylon 2025;22:978-83.

T lymphocyte antigen-4) is a physiologic antagonist of CD28. Psoriatic disease, which can affect the skin or the joints, is thought to be maintained in part by abnormal activation of T cells. It appears that CD28/CD80 plays a critical role in stimulating T-cell activation and inflammation in psoriasis, and blocking this pathway with CTLA4 analogs is effective against psoriasis. T-cells express the physiological antagonist CTLA-4 to CD28, which is triggered by the antigen-presenting cell (APC) counter receptors CD80/CD86. CTLA4 binds to CD80/86 with high affinity and suppresses T-cell activation by inhibiting intracellular CD28 downstream actions.[5,6]

In the past few years, many authors reported that psoriatic patients, who have *Staphylococcus aureus* infection experience worsening of their psoriatic lesions, and that is due to the fact that this bacterium possesses many antigens, which act as bacterial superantigens.[7,8] Bacterial superantigens have been linked to the etiology of psoriasis, and one of the most lethal/potent ones is staphylococcal enterotoxin. T cells in psoriatic skin lesions contain high numbers of major histocompatibility complex class II molecules, which are high-affinity receptors for Staphylococcus superantigen that could explain how *S. aureus* colonization contributes to the development of psoriatic lesions.[9,10]

Objectives

The aim of this study was to investigate the potential role of CTLA-4 and CD28 in psoriasis pathogenesis when *S. aureus* is present.

Materials and Methods

Study design

The present case-control study was conducted on 225 individuals divided into three groups. The first group comprises 75 individuals with different types of psoriasis (Vulgaris, Guttate, and Inverse), each of them varying in his/her signs and symptoms (as a patient group). According to the psoriasis area and severity index, out of 75 psoriatic patients enrolled in this study, as many as 28 (37.33%) of cases presented with mild disease activity, and 32 (42.66 %) of cases presented with moderate disease activity furthermore 15 (20.01%) of cases presented with severe disease activity. The second group comprises 75 individuals of burn patients suspected to have bacterial burn wound infection as a positive control, and the third group comprises 75 individuals (apparently healthy people) as a negative control. The patient group that participated in this study was recruited from the dermatology consulting department in Al-Imamein Al Kadhimein Medical City, Baghdad, Iraq, the positive control group was recruited from Burns Specialized Hospital, Medical City, Baghdad, Iraq, and the negative control group were recruited from blood donating center in Al-Imamein Al Kadhimein Medical city, Baghdad, Iraq. Female patients' age ranged from 7 to 70 years with a mean age of 31.67 years, whereas patients' age ranged from 5 to 52 years with a mean age of 25.27 years.

Inclusion criteria

All psoriatic patients diagnosed clinically by dermatologists from different age groups with different types of psoriasis were included in this study.

Exclusion criteria

Patients who received any treatment, topical or systemic for at least 3 weeks before sample collection were excluded from this study.

Sample collection and processing

After rotating a sterile cotton swab stick as deeply as possible for 10 s over the psoriatic lesion within any body part that is affected with psoriasis, while for positive control from burn patients from any injured part through the body; for negative control the swab was taken from the nasal cavity. After that, the swabs were inserted into sterile transport tube media, 225 swab samples were collected aseptically and inoculated on blood agar media, then stored at 37ºC in an aerobic environment for one day. Isolates thought to be *S. aureus* based on beta hemolysis on blood age media were gram stained and inoculated on mannitol salt agar, where they spent 24 h at 37ºC. The bacteria that formed characteristic yellow colonies on this media were then further confirmed by using VITEK-2 ID System according to the manufacturer's instructions (bioMérieux). Additionally, a peripheral venipuncture was used to collect a venous blood sample equal to 5 mL from each participant. The blood sample was then transferred to a plane tube to obtain the serum, which was stored at –20ºC until the enzyme linked immunosorbent assay (ELISA) (Elabscience Corporation, China) method was used to measure the level of the biomarkers CTLA and CD28.

Calculation of result by constructing a standard curve for CD28

A standard curve was drawn by plotting the optical density (OD) values of the standard dilutions (on the *Y*-axis) against the known concentration of the standard (on the *X*-axis). The concentration of CD28 was obtained by applying the OD of the sample on the *Y*-axis and a perpendicular line was drawn to cross the standard curve at a point, from which another line was drawn to the *X*-axis to abstract the concentration of the sample from it [Figure 1].

Calculation of result by constructing a standard curve for CTLA-4

A standard curve was drawn by plotting the OD values of the standard dilutions (on the *Y*-axis) against the known concentration of the standard (on the *X*-axis). The concentration of CTLA-4 was obtained by applying the OD of the sample on the *Y*-axis and a perpendicular line was drawn to cross the standard curve at a point, from which another line was drawn to the *X*-axis to abstract the concentration of the sample from it [Figure 2].

Statistical analysis

GraphPad Prism (GraphPad Software, Inc., San Diego, CA, USA) version 8 software was used for the statistical analysis of data. Quantitative results are indicated as mean ± standard error of the mean (SEM). The significance between all groups that enrolled in this study was assessed using *t* test. A value of $P < 0.05$ was considered significant ($*P < 0.05$, $**P < 0.01$, and $***P < 0.001$).

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with the patients' verbal and analytical approval before the sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to document number 202011126 in January 10, 2021.

Results

Distribution of psoriatic patients according to gender and age groups

The distribution of psoriatic patients who participated in this study, according to gender revealed that out of 75 individuals, there were 48 (64%) male patients, whereas 27 (36%) were the female patients [Figure 3].

Distribution of *Staphylococcus aureus* isolates among the study groups

Out of a total of 225 individuals recruited in this study, *S. aureus* isolates were identified in 82 (36.44 %). Most *S. aureus* isolates were from the positive control group (35) followed by the negative control group (25), and from psoriatic patients' group (22) [Figure 4].

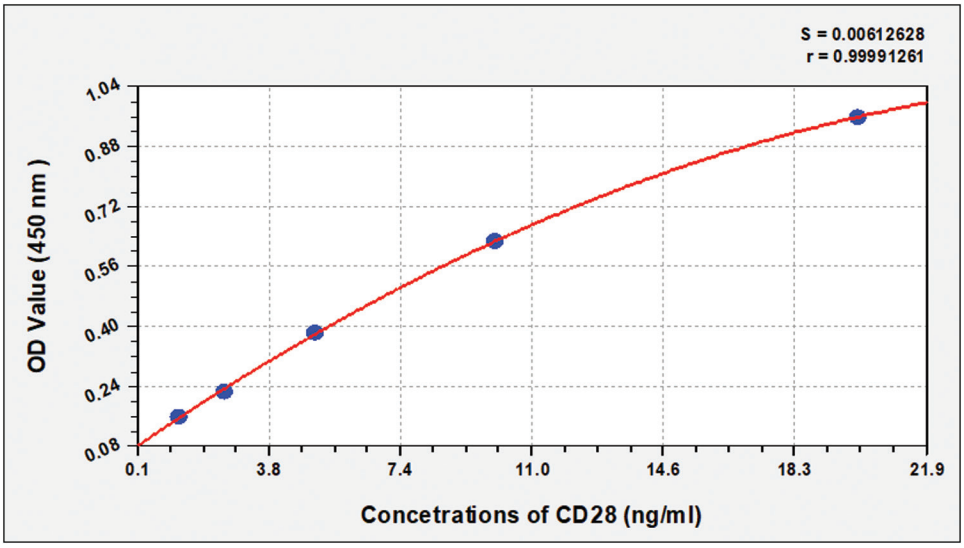

Figure 1: Standard curve of CD28

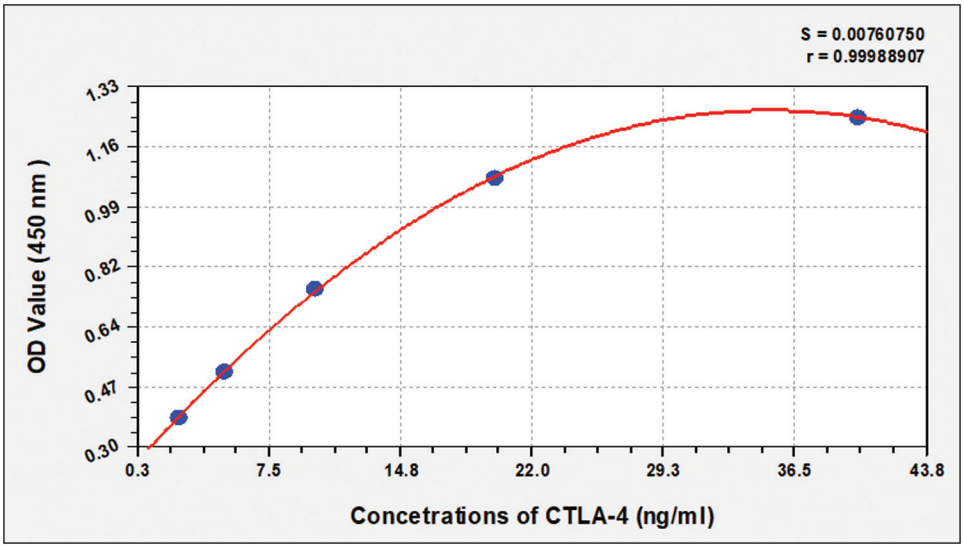

Figure 2: Standard curve of CTLA-4

Estimation of serum CTLA-4 and CD28 levels in study groups by ELISA

In this study, out of 225 individuals' sera, 82 sera samples were screened by ELISA for CTLA-4 and CD28. The results of the mean serum level of CTLA-4 demonstrated that there was an interesting and highly significant difference ($P \leq 0.0001$) in CTLA-4 level between the psoriatic patients' group versus negative control groups; apart from that, there was also a significant difference between the psoriatic patient group and positive control group ($P \leq 0.01$); besides that there were significant differences in CTLA-4 level between the negative control group and positive control group ($P \leq 0.05$) [Figure 5].

Regarding CD28, the comparison among the three groups of individuals in this study showed significant differences between the positive control group versus the psoriatic patients' group and the negative control group ($P < 0.05$) in the mean serum level of CD28. Conversely, there were no significant differences ($P > 0.05$) in the mean serum level of CD28 among psoriatic patients' groups as opposed to the negative control group [Figure 6].

Figure 3: Distribution of psoriatic patient's group according to gender, $N = 75$

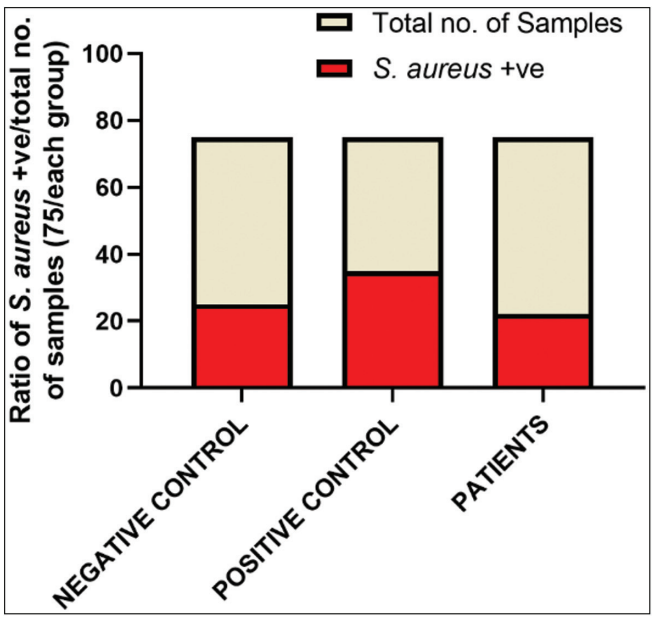

Figure 4: Distribution of *Staphylococcus aureus* isolates among study groups

Discussion

This study was conducted with some limitations. For example, the difficulty of finding newly diagnosed cases of psoriasis, as well as find psoriatic patients with *S. aureus* infection. The importance of this study is that it dealt with immune checkpoints in psoriatic patients and a positive and negative control group in the presence of *S. aureus*.

Distribution of psoriatic patients according to gender and age groups

Gender and age variations in disease appearance, comorbidities, and treatment results have been seen in a variety of illnesses and are becoming more and more important in medical research. Concerning the psoriasis disease's presentation, severity, possible treatments, how the condition is perceived by the patient, and how it affects the quality of life, it is also connected with these two factors.[11,12]

On the basis of current data, out of 75 psoriatic patients, there were 48 (64%) men, and 27 were female patients (36%). With male-to-female ratio of 1.7:1, this observation is in agreement with a study done in Iraq by Adnan Abd Oun Hashim 2012, who reported that male dominance on female patients out of the 115 psoriatic patients.[13] This study disagrees with a study carried out by Mallbris

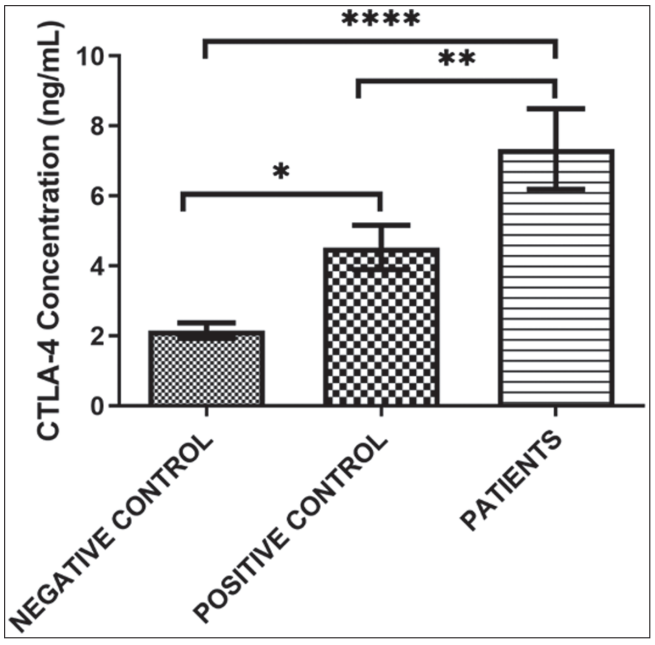

Figure 5: Serum CTLA-4 level in study groups

Data are means ± SEM (*t* test), $N = 82$, *$P < 0.05$, **$P < 0.01$, and **** $P < 0.0001$

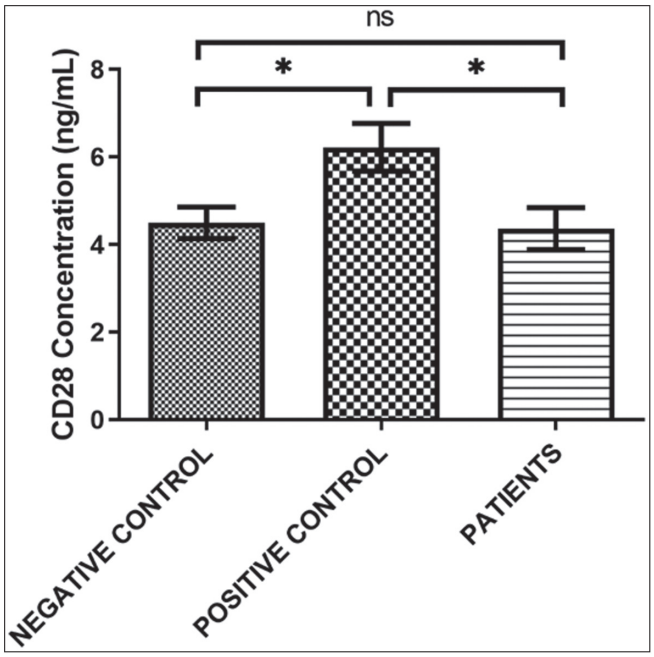

Figure 6: Serum CD28 level in study groups

Data are means ± SEM (*t* test), $N = 82$, $^*P < 0.05$, and ns P value > 0.05

2005, who found female dominance (approaching 1.3:1) as compared with male counterpart.[14] However, Guillet *et al.*,[11] mentioned that the incidence, prevalence, and manifestation of psoriasis are similar between male patients and female patients.

Distribution of *Staphylococcus aureus* isolates among the study groups

Our study implied that a considerable number of *S. aureus* were isolated mainly from the positive control group. However; out of 225 individuals recruited in this study, *S. aureus* isolates were identified in 82 (36.44 %); most *S. aureus* isolates were from positive control group 35 (42.68%) followed by negative control group 25 (30.48%), and from psoriatic patients' group was 22 (26.82%). This study supports the idea that *S. aureus* skin colonization is enhanced by reducing the acidic pH of human perspiration by producing ammonia (NH4+) from the catabolism of L-arginine. This makes it able to quickly adapt to host damage in order to increase survival under various circumstances. The bacteria are notable for their capacity to infect sensitive tissues such as skin through the addition of genes and modifications to regulatory.[15]

Estimation of serum CTLA-4 and CD28 levels in study groups by ELISA cytotoxic T-lymphocyte antigen-4

This study showed that CTLA-4 serum level, there is a statistically significant difference. The results showed a highly significant difference ($P < 0.0001$) in CTLA-4 serum levels between the psoriatic patients' group and the negative control, as well as a significant difference between the psoriatic patients and the positive control group ($P \leq 0.01$); besides that there was a significant difference in CTLA-4 level between negative control group and positive control group ($P \leq 0.05$).

Our results showed a highly significant difference between the psoriatic group and the negative control group ($P < 0.0001$). Staphylococcal enterotoxins in psoriatic patients cause polyclonal activation of T-cells, so CTLA-4 also became highly activated to control and downregulate them. There is a study carried out by Liu *et al.*,[16] whose findings agreed with our results and they reported that after T cell activation, CTLA-4 is significantly induced, whereas CD28 is downregulated by internalization.

Another study by Al-Mousawi,[17] agreed with our results. He reported that CTLA-4 protein is induced on T cells following antigen stimulation, and his finding supports our finding that CTLA-4 concentration highly increased in psoriatic patients in the presence of staphylococcal enterotoxins. Daroszewski *et al.*[18] found that CTLA-4 molecule maintains peripheral tolerance and terminates T-cell responses, but CTLA-4 did not prevent T-cell super activation in psoriatic patients.

This study found a significant difference between the psoriatic patients' group and the positive control ($P < 0.01$). Psoriatic patients have polyclonal T-cell activation and CTLA-4 increases in concentration to downregulate them, while the positive control group (burn patients with staphylococcal enterotoxins) has less activation than T-cell activation in psoriatic patients. Also, our results showed a significant difference between the positive control group and the negative control group ($P < 0.05$).

Impact of CD28 on psoriasis patients

Our results showed that are no significant differences between the psoriatic group and the negative control group (P value > 0.05). CD28 down modulated in our finding and this result agreed with a study done by Liu *et al.*,[16] who reported that after T cell activation, CTLA-4 is significantly induced, whereas CD28 is downregulated by internalization.

Another study supported our findings that CD28 is downregulated by CTLA-4 in psoriasis. That study, conducted by Iannone and Lapadula,[5] reported that CD28 appears to be crucial in stimulating T cell activation and inflammation in psoriasis, and its inhibition by CTLA4 is effective against psoriasis. This finding explains why CD28 is downregulated in order to reduce T-cell activation that is already super activated because of *S. aureus* superantigens. CD28 signaling is triggered by its counter receptors CD80/CD86 [expressed on APC] and is regulated by a physiological antagonist (cytotoxic T lymphocyte

antigen-4, CD152; CTLA4) expressed by T cells. CTLA4 binds to CD80/86 with high avidity and prevents T-cell activation by blocking intracellular CD28 downstream events. The inhibition of CD28-driven co-stimulation can be the ideal target to downregulate T cell activity.

It has been shown by Berg and Zavazava[19] that CTLA-4 expression promotes the downregulation of CD28 on the T cell surface as a result of enhanced internalization and degradation of CD28. These data suggest that apart from the established competition for B7.1 and B7.2 by CTLA-4, inhibition of T cells by CTLA-4 might be additionally explained by the reduction of CD28 on the cell surface.[20,21]

Conclusion

There was a significant role in regarding the colonization of *S. aureus* in psoriatic patients and the mean level of CTLA-4 and CD28 compared to negative and positive control groups.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Jensen P, Skov L. Psoriasis and obesity. Dermatology 2016;232: 633-9.
2. Obaid EM, Baiee HA. Epidemiological and clinical characteristics of burn injuries among hospitalized patients in Babylon Province. Med J Babylon 2022;19:9-14.
3. Hugh JM, Weinberg JM. Update on the pathophysiology of psoriasis. Cutis 2016;102:6-12.
4. Yu M, Zhang R, Ni P, Chen S, Duan G. *Helicobacter pylori* infection and Psoriasis: A systematic review and meta-analysis. Medicina (Kaunas) 2019;55:645.
5. Iannone F, Lapadula G. The inhibitor of costimulation of T cells: Abatacept. J Rheumatol 2012;39:100-2.
6. Zhou S, Yao Z. Roles of infection in psoriasis. Int J Mol Sci 2022;23:6955.
7. Tuffs SW, Goncheva MI, Xu SX, Craig HC, Kasper KJ, Choi J, *et al*. Superantigens promote *Staphylococcus aureus* bloodstream infection by eliciting pathogenic interferon-gamma production. Proc Natl Acad Sci USA 2022;119:1-11.
8. Ardern-Jones MR, Black AP, Bateman EA, Ogg GS. Bacterial superantigen facilitates epithelial presentation of allergen to T helper 2 cells. Proc Natl Acad Sci USA 2007;104:5557-62.
9. Mohammed HA, Sahi NM, Ahmed RT, fauzi Al-Rubaye A. Antimicrobial activity of some nanoparticles synthesized by laser ablation technique against some bacteria isolated from oral cavity. Med J Babylon 2022;19:601.
10. Guillet C, Seeli C, Nina M, Maul LV, Maul JT. The impact of gender and sex in psoriasis: What to be aware of when treating women with psoriasis. Int J Women's Dermatol 2022;8:e010.
11. Sarkar R, Chugh S, Bansal S. General measures and quality of life issues in psoriasis. Indian Dermatol Online J 2016;7:481-8.
12. Abd A, Hashim O. Serum zinc in psoriatic patients. Mustansiriya Med J 2012;11:20-3.
13. Mallbris L. Studies of phenotype at onset and of associated cardiovascular morbidity. Doctoral thesis, Karolinska Institutet, Stockholm, 2005.
14. Alonzo F, Torres VJ. A lesson in survival: *S. aureus* versus the skin. Cell Host Microbe 2013;13:3-5.
15. Basile MS, Bramanti P, Mazzon E. The role of cytotoxic t-lymphocyte antigen 4 in the pathogenesis of multiple sclerosis. Genes (Basel) 2022;13:1319.
16. Liu S, Xu J, Wu J. The role of co-signaling molecules in psoriasis and their implications for targeted treatment. Front Pharmacol 2021;12:1-11.
17. Al-Musawi AAR. Immunogenetic Study of Iraqi Women with Breast Cancer. M. Sc. Thesis, College of Education for Pure Science, University of Baghdad (in Arabic); 2018.
18. Daroszewski J, Pawlak E, Karabon L, Frydecka I, Jonkisz A, Slowik M, *et al*. Soluble CTLA-4 receptor an immunological marker of Graves' disease and severity of ophthalmopathy is associated with CTLA-4 Jo31 and CT60 gene polymorphisms. Eur J Endocrinol 2009;161:787-93.
19. Berg M, Zavazava N. Regulation of CD28 expression on $CD8^+$T cells by CTLA-4. J Leukoc Biol 2008;83:853-63.
20. Mahmood NS, Omran R, Abdulla AA. Estimation of Serum Prolactin Level in Patients with Chronic Plaque Psoriasis and Their Association with Severity. Med J Babylon 2024;21(4):757-65.
21. Al-Hattab MK, Warwar AH, Al-Kalidi JH. Estimation of Serum Prolactin Level in Patients with Chronic Plaque Psoriasis and Their Association with Severity. Med J Babylon 2025;22(3):837-43.