

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Cytokine Serum Level Association with Superantigen Production by *Staphylococcus aureus* in Psoriasis vulgaris.

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Abstract

Psoriasis is a common, immune mediated, heterogeneous, multifactorial disease. The relationships of serum IFN γ , IL-17 and IL-4 levels and *Staphylococcus aureus* superantigens to severity of psoriasis in patients were evaluated. A total of 60 patients with mild – severe psoriasis and 60 healthy individuals (controls) were enrolled. These patients attended Al-Sadder Medical City, outpatient clinic of Dermatology in Najaf city, Iraq, during the period from May 2013 to October 2014. Serum cytokine levels (IFN γ , IL-17, and IL-4) in controls and patients with psoriasis were measured by ELISA. Superantigens were detected by PCR. Statistical analysis was done by ANOVA test and Pearson correlation. High levels of IFN γ and IL-17 were observed in patients with psoriasis. Mean levels of IFN γ and IL-17 were significantly correlated to disease severity. Moreover, the mean level of IL-4 in psoriatic patient decreased significantly according to graded severity of the disease. Increased numbers of superantigen genes detected in each isolate were associated with higher serum levels of IFN γ and IL-17. The study concluded that the level of IFN γ and IL-17 may play a key role as an indicator in the immunopathogenesis of psoriasis and markers of disease activity.

Keywords: Psoriasis, *Staphylococcus aureus*, superantigens, ELISA.

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INTRODUCTION

Psoriasis is a chronic immunologically mediated inflammatory disease of the skin, which affects 1-2% of the general population [1]. Several overlapping clinical types of psoriasis have been recognized but the chronic plaque form (*Psoriasis vulgaris*) is the most common type [2]. *Psoriasis vulgaris* is characterized by hyper-proliferation and abnormal differentiation of epidermal keratinocytes with a poorly adherent stratum corneum, which results in the characteristic scale or flakes of the lesions [3]. Lymphocyte infiltration consists mostly of T-lymphocytes and is aided by various endothelial vascular changes in the dermal layer, such as angiogenesis, dilatation and high endothelial venule formation [4]. It was confirmed that *S. aureus* colonization and its toxigenic-strains are associated with psoriasis and have a correlation with severity of disease [5].

Bacterial superantigens stimulate subpopulations of T cells by bypassing normal antigenic recognition. They bind directly to class II major histocompatibility complexes on antigen presenting cells (APCs) outside the conventional antigen binding groove. The classical superantigens include staphylococcal enterotoxin (SE) A, B, C, D, and E, as well as TSST-1. Each superantigen complex presented by APCs recognizes only the relevant V β element of the T cell receptor, so any T cell expressing the appropriate V β chain can be stimulated. Subsequently, each superantigen can stimulate many T cells and cytokines are released in large amounts, causing the symptoms [6]. The roles of cytokines in the pathogenesis of psoriasis were investigated. The cutaneous and systemic overexpression of several proinflammatory cytokines, particularly type-1 cytokines such as IL-2, IL-6, IL-8, IL-12, IL-17, IFN- γ and TNF- α , has been demonstrated in patients with psoriasis. These proinflammatory cytokines are considered to be responsible for initiation, maintenance and recurrence of skin lesions. The cellular composition of the inflammatory infiltrate within the plaques as well as the keratinocyte hyperproliferation appears to be directed by these cytokines [7,8]. The presence of IL-2 and IFN- γ in psoriatic lesions indicates that its immunopathogenesis is Th1 mediated [9]. IFN γ induces the expression of the adhesion molecule ICAM-1 on keratinocytes and endothelial cells, influencing the trafficking of T lymphocytes into lesional epidermis [10,11]. When IFN- γ activates keratinocytes, they secrete IL-1, IL-6, IL-8, IFN- γ , TNF- α , and chemokines, which influence both themselves and other cell types including T lymphocytes [12]. The dominant role of IL-23 involves the stimulation of Th-17 cell to produce IL-17, which is a critical component in the establishment and perpetuation of autoimmune inflammation [3].

For routine detection of superantigens, commercially produced kits, such as reverse passive latex agglutination assays and enzyme-linked immunosorbent assays, were most commonly used. However, these methods were, to date, designed only to detect limited types of superantigens. As an alternative to these more traditional methods, the PCR approach can provide detection of toxin genes and is presently designed to detect the majority of superantigen [13].

The objective of this study was to measure the incidence of some superantigens (*sea*, *seb*, *sec*, *sed*, *see*, *tst*, *eta*, and *etb*) in *S. aureus* isolates obtained from psoriasis patients and attempt to identify a possible role for the superantigens and their relationship with level of cytokines (IFN γ , IL-17 and IL-4) in association with the disease severity.

However, the present study was attempted to evaluate the role of *S. aureus* superantigens and their relationship with IFN γ , IL-17 and IL-4 in association with the disease severity.

MATERIALS AND METHODS

This study approved by the ethical committee of the faculty of medicine -University of Kufa.

Clinical Assessment

This study comprised sixty consecutive patients with psoriasis who attended Al-Sadder Medical City, outpatient clinic of Dermatology in Najaf city, Iraq, during the period from May 2013 to October 2014 and had not received any treatment, topical or systemic for at least 3 weeks. Patients were diagnosed clinically and the disease severity was evaluated by using the Psoriasis Area and Severity Index (PASI) [14]. The patients were grouped according to their mild, moderate, and severe psoriasis. Twenty healthy volunteers matched by age and sex and with no family history of psoriasis were included as a control group. The demographics and antibiotic usage of the patients and healthy controls were tabulated.

For isolation of *S. aureus*, skin swab was taken from psoriatic lesion of all patients by using a sterile cotton swab.

Blood Sample

Five ml of venous blood were collected from 60 patients with psoriasis and 60 controls. Blood samples were allowed to form clots. Serum was separated by centrifugation at 600 g, collected, divided into 3 tubes (200 µl for each aliquot) and stored in the freezer (- 20°C) for subsequent investigations.

Serum cytokines measurements

Serum levels of IFN γ , IL-17 and IL-4 (Peprotech, USA) were measured by enzyme-linked immunosorbent assay (ELISA), according to the manufacturers’ instructions. Briefly, the ELISA development kits provided the key components for quantitative sandwich immunoassays that measured natural and/or recombinant human IFN γ , IL-17, and IL-4 within the range of 47-1500 pg/ml, 16-2000 pg/ml, and 16-2000 pg/ml, respectively.

Isolation and identification of Staphylococcal superantigens

The staphylococcal isolates were identified morphologically and biochemically by standard laboratory procedures [15]. Staphylococcal superantigens (enterotoxin A, B, C, D, E and TSST) were detected by conventional PCR. The DNA extract of *S. aureus* isolates were subjected to genes primers. The protocol used depends on manufacturer's instruction. Primers 1 and 2 designated in this study and primers (3,4,5 and 6) were used according to Balci *et al* [16]. Primers sequences are listed in (Table-1).

Table-1: Primers of PCR

Primers	Oligo sequence (3'-5')	Product size (bp)	Origin
1- <i>Sea</i>	F:TTGGAAACGGTAAAAC AA R:GAACCTTCCCATCAAAAACA	370	BioNeer (Korea)
2- <i>Seb</i>	F:TCGCATCAAAGTACAAACG R:GCAGGTAAGTCTATAAGTCC	436	BioNeer (Korea)
3- <i>Sec</i>	F:GACATAAAAGCTAGGAAT TT R: AAATCGGATTAACATTATCC	257	Biocorp (Canada)
4- <i>Sed</i>	F:CTAGTTTGTAATATCTCCT R:TAATGCTATATCTTATAGGG	317	Biocorp (Canada)
5- <i>See</i>	F:AGGTTTTTTTCACAGGTAAGTCC R:CTTTTTTTTCTCGGTACATC	200	Alpha DNA (Canada)
6- <i>Tsst</i>	F:ATG GACGACTCA GCT TGA TA R:TTTCCAATAACCCCGTTT	350	Biocorp (Canada)

Statistical analysis was done by using SPSS (statistical package for social sciences) version 17 in which T-test and ANOVA test were used for continuous data and chi- square (X²) test for categorical data.

RESULTS

Serum Levels of Cytokines among Psoriatic patients

The patients comprised 31 (51.6%) females and 29 (48.4%) males. According to PASI score classification, the severity of 60 patients with psoriasis were graded into mild, moderate, and severe. Results revealed that 23 (38.4%) patients had mild, 31 (51.6%) had moderate, and 6 (10%) had severe grade (Table 2). There was no significant difference in the severity of the disease between males and females (P> 0.05).

High levels of IFN γ and IL-17 were observed in patients with psoriasis (358.129 \pm 180.504 pg/ml) and (346.026 \pm 273.469 pg/ml), respectively compared with control group (64.984 \pm 21.3571pg/ml) and (22.7 \pm 4.96662 pg/ml). A significant difference was found between the mean concentration of IFN γ and IL-17 level with control group (p <0.05). In this investigation, IL-4 mean level (45.364 \pm 42.981 pg/ml) found higher than mean level of control group (8.063 \pm 3.96282 pg/ml) (Figure-1). In addition, serum levels of both IFN γ and IL-17 significantly correlated with PASI score. Serum level of IFN γ was increased in severe degree of psoriasis and the level found to be (701.1167 \pm 91.7329 pg/ml) as compared with that in moderate (250.3960 \pm 83.92543 pg/ml) and in mild degree (122.8759 \pm 36.44595 pg/ml). The mean levels of IL-17 in psoriatic patients were also found to increase according to severity of disease (Table 3). Furthermore, the mean serum levels of IFN γ and IL-17 in psoriatic patient were found to be correlated to disease severity (r = 0.849, P< 0.001) and (r = 0.433, P < 0.001) respectively (Figures 2 and 3). According to IL-4 serum levels, the study found that the mean level in severe group of psoriatic patients (3.0167 \pm 2.17522 pg/ml) was lower than that of mild (79.9759 \pm 97.82719 pg/ml) and moderate groups (15.3784 \pm 23.88903 pg/ml) (Table-3). The mean concentrations of IL-4 in psoriatic patient were found to be decreasing significantly according to graded severity of the disease. The severe stage appeared lower mean concentration level than other stages. The correlation of these cytokine mean concentration between grads severity of disease was appeared (r = 0.416, P < 0.001) (Figure-4).

Table 2: Number of psoriasis patients according to gender and severity grades of disease

Severity	Male	Female	Total	P value
Mild	13	16	29 (41.4)	0.858
Moderate	17	18	35(50.0)	
Sever	4	2	6 (8.6)	
Total	34	36	70	

Table 3: Relationship between cytokine level (INF γ , IL-17, and IL-4) with the severity of psoriasis patients

Cytokine type	Study group	Mean	Std. Deviation	Std. Error	P value
INF γ	Mild	122.8759	36.44595	6.76784	<0.001
	Moderate	250.3960	83.92543	16.78509	
	Sever	701.1167	91.73290	37.44980	
	control +ve	48.0600	12.08564	3.82181	
	control -ve	16.9240	9.27146	2.93189	
IL-17	Mild	72.6862	26.39197	4.90087	<0.001
	Moderate	265.3920	806.76233	161.35247	
	Sever	702.2500	878.6832	35.87209	
	control +ve	14.4900	2.71844	0.85964	
	control -ve	8.2100	2.24818	0.71094	
IL-4	Mild	79.9759	97.82719	18.16605	<0.001
	Moderate	15.3784	23.88903	4.77781	
	Sever	3.0167	0.46655	0.19047	
	control +ve	4.2640	1.78760	0.56529	
	control -ve	3.7990	2.17522	0.68786	

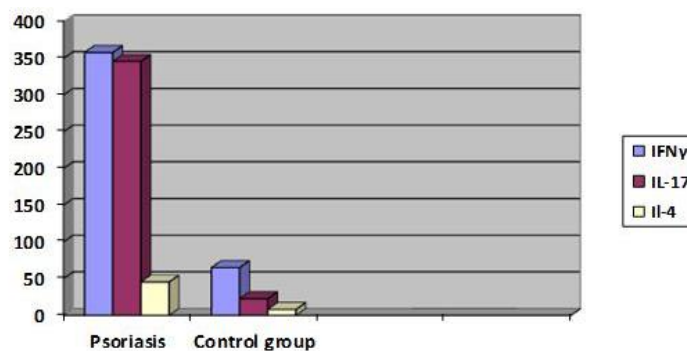


Figure-1: Mean distribution of IFN γ , IL-17 and IL-4 levels (pg/ml) among psoriatic patients and healthy control group

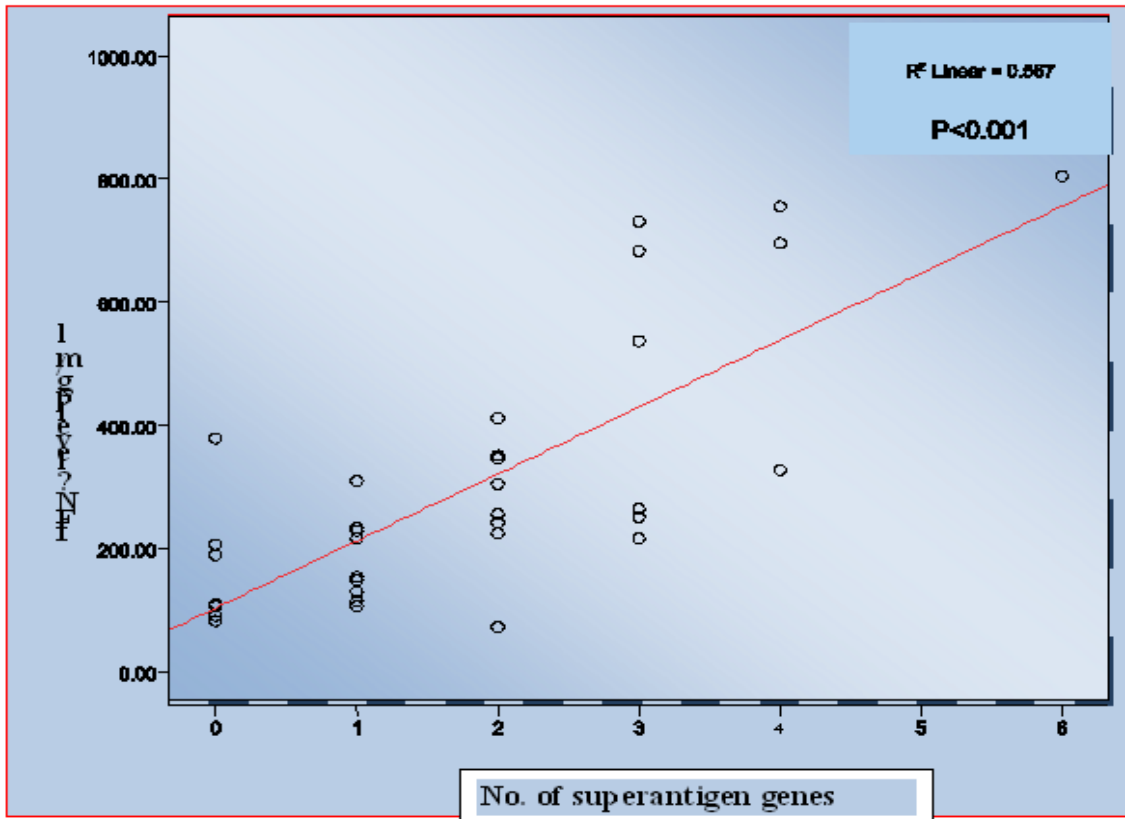


Figure 2: Correlation between levels of IFN γ and numbers of superantigen genes in *S. aureus* isolates recovered from psoriatic patients

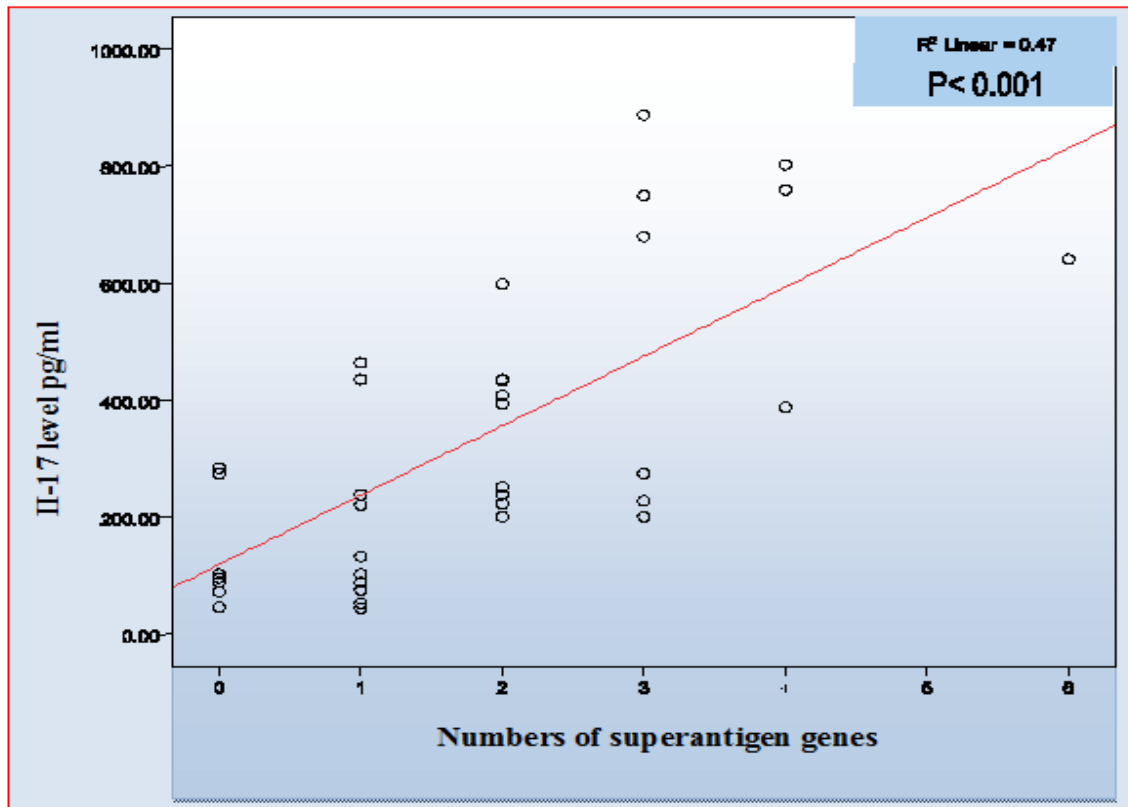


Figure 3: Correlation between levels of IL-17 and number of superantigen genes in *S. aureus* isolates recovered from psoriatic patients

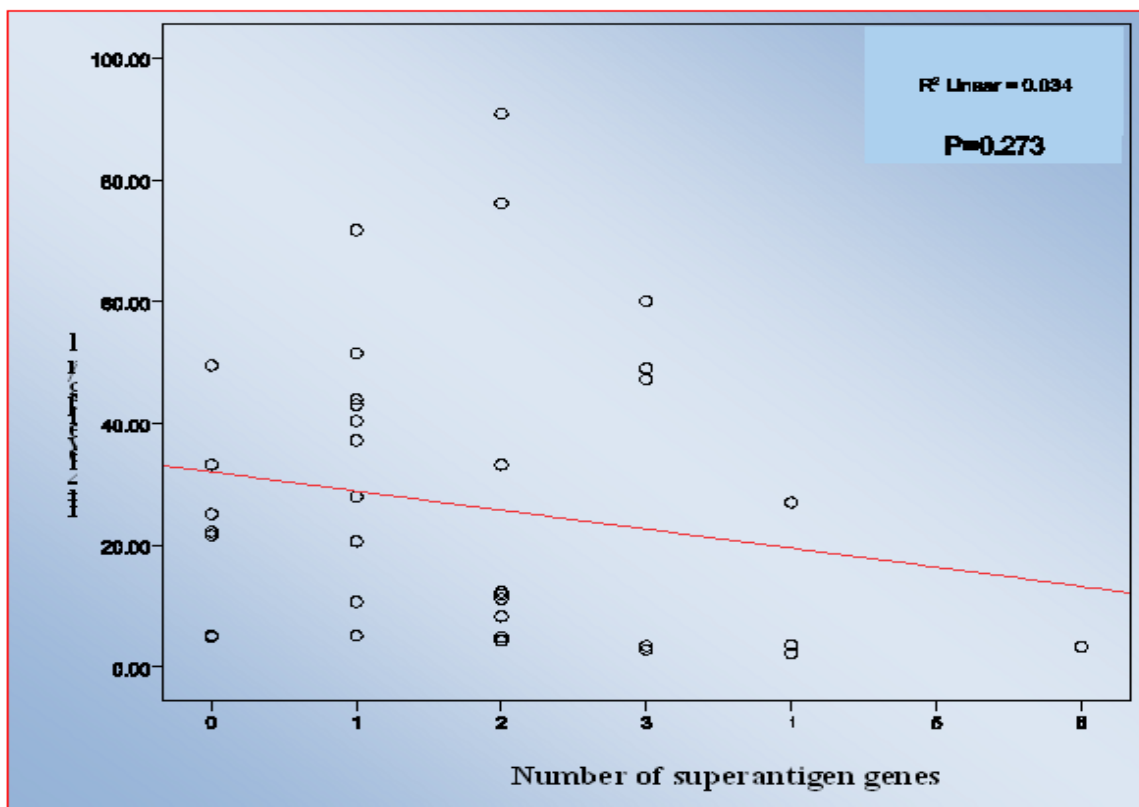


Figure 4: Correlation between levels of IL-4 and numbers of superantigen genes in *S. aureus* isolates recovered from psoriatic patients

Cytokine serum level association with superantigen production

In the group of psoriatic patient, whose lesion cultures contained *S. aureus* (37/60), 29 of the isolates recovered from the lesional skin produced diversity of superantigens. Patients who were *S. aureus* positive in lesional skin had significantly higher mean level of IFN γ than patients who were cultivation-negative (296.83 \pm 201.7 pg/ml vs. 89.21 \pm 25.88pg/ml) respectively, (P< 0.001). In correspondence, patients with cultivation-positive for *S. aureus*, IL-17 levels were significantly higher (330.37 \pm 241.7pg/ml) than patients who were negative cultivation for *S. aureus* (71.7 \pm 25.43 pg/ml), there was a significant difference in serum level of IL-17 between carrier of *S. aureus* isolates and non-carrier *S. aureus* patients (P< 0.001) (Table 4).

Table 4: Mean concentration of cytokines (IFN γ , IL-17 and IL-4) in psoriasis patients with and without *S. aureus*

Psoriasis patients	Total	IFN γ pg/ml Mean \pm SD	IL-17 Mean \pm SD	IL-4 Mean \pm SD
<i>S. aureus</i> present	37	296.83 \pm 201.7	330.37 \pm 241.7	26.4 \pm 23.6
<i>S. aureus</i> absent	23	89.21 \pm 25.88	71.7 \pm 25.43	37.09 \pm 28.67
P value		<0.001	<0.001	0.122

On the other hand, mean level of IL-4 in serum psoriasis patients cultivation-negative higher than in patients cultivation positive with *S. aureus* (37.09 \pm 28.67 pg/ml, 26.4 \pm 23.6pg/ml, respectively, (P> 0.05), (Table 4).

This study also focused on the correlation between level of the three cytokines and the numbers of superantigen genes for each isolate of *S. aureus*. The results revealed a significant correlation (r = 0.752, P < 0.001) between increased numbers of superantigen genes (detected in each isolate) and increased level of IFN γ (Figure 5).

Figure (6) showed the relationship between concentration levels of IL-17 and number of superantigen genes. Based on these results, there was a significant difference between increased levels of IL-17 with increased numbers of genes of each isolate, correlation was recorded ($r = 0.685$) with ($P < 0.001$).

According to serum level of IL-4 in psoriasis patients that cultivated with *S. aureus* and the correlated with increased number of superantigen gene. The result was referred that no association between mean concentration level of IL-4 and increase number of gene, there was a weak correlation between them ($r = 0.184$) ($P > 0.05$) (Figure 7).

DISCUSSION

Staphylococcal infection has been suspected as triggering and exacerbating factors in psoriasis [17]. Cytokine release by activation T- lymphocytes stimulated by staphylococcal superantigens and initiated the proliferation then augment psoriatic severity [16]. In this study we attempted to clearance the potential role of superantigen in associated with elevated of some cytokines in serum of psoriasis patients.

Several researchers reported that psoriasis occurs in any gender or race with equal amount of male and female [18,19]. The results showed a slightly female predominance which yet again may reflect a sex-related preference in psoriatic patients. T cells were classified as Th1 or Th2 cells by production of defining cytokines, IFN γ and IL-4, respectively. However, Th17, has been linked to autoimmune inflammation [20,21]. However, the study observed that the mean concentration of IFN γ was significantly higher in patients with psoriasis compared with control groups. This result was also confirmed by other studies [22-25] which found that IFN γ and other cytokines were elevated in psoriatic patients. It was agreed with the hypothesis that psoriasis considered as a T-cell-mediated inflammatory skin disease. Th1-lymphocytes produce IFN γ and induce cellular reactions, resulting in marked increases of keratinocyte proliferation, abnormal patterns of keratinocyte differentiation, concomitant inflammation, and dermal proliferation of small vessels [26].

The study reveals that the level of IL-17 was higher in patients with psoriatic than that of control groups. This observation supports the hypothesis that the high level of IL-17 may be critical mediators of the persistently altered epidermal growth and differentiation and local inflammation that was characteristic of psoriasis. and Th17 cells may be proximal regulators of psoriatic skin inflammation [19] However, many studies which support this result that found the IL-17 level were increased in lesional tissue and serum of psoriatic patients [25,27,28]. Th17 cells may be proximal regulators of psoriatic skin inflammation [29]. IL-17 serum concentration were increased also in psoriatic patients [5,27,28].

However, this investigation showed that the serum level of IL-4 in psoriatic patients were higher than those of the control groups. The result agreed with another study with psoriasis as compared to that of controls [29].

The serum levels of both IFN γ and IL-17 in patient found to be significantly correlated to disease severity index PASI of psoriasis. Jadali *et al.* [30] found that a significant correlation between the level of IFN- γ and psoriatic disease severity [22]. However, the mechanisms by which T cells, keratinocytes, and macrophages achieve and maintain their activated state in psoriatic skin lesions were poorly understood.

Usually, diseases caused by *S. aureus* can elicit a systemic or topical inflammatory response syndrome. The innate immune response was important to the up regulation of cytokine production [23]. Although multiple factors may contribute to the exacerbation of psoriasis, there were many reports suggesting that *S. aureus* infection can trigger this illness [31,32].

Staphylococcal superantigens were secreted toxins that induce a strong activation of large T-cell subpopulation [33].

Increasing evidence suggests an important role for superantigen in the initiation of psoriasis. *S. aureus* infection was shown to activate psoriasis via release of their superantigenic toxin [31]. The superantigens bind to major histocompatibility complex class II molecules on APCs and to T-cell-receptor-

bearing specific V β elements. This tri-molecular interaction leads to a massive proliferation of T cells and the elaborate systemic release of proinflammatory cytokines [34].

The study was observed that high levels of IFN- γ and IL-17 in serum of psoriasis patients who had toxigenic *S. aureus* isolates compared to serum of patients who had non-toxigenic *S. aureus* isolates and suggested that superantigenic *S. aureus* might be strong inducers of IFN- γ and IL-17 from Th1 and Th17 cells. This is in agreement with several studies which showed that application of staphylococcal superantigens on the skin of psoriasis patients induced a greater inflammatory response compared to non-subjects [35,36].

The serum level of IFN- γ and IL-17 were elevated with increased number of superantigen genes detected in each isolates of patients with psoriasis. This observation responded with further study that supported increase level of epidermal IFN- γ in lesional skin of psoriasis patients colonized with toxigenic *S. aureus* which were strong inducer of IL-17 [37,38].

This study found that a correlation between increase level of these two cytokines with severity of disease. These findings corresponded to other results that demonstrated a significant relationship between PASI scores and toxin production [16].

The serum levels of IL-4 were significantly lower in patients with toxigenic *S. aureus* isolates than those from patients who had non-toxigenic *S. aureus* isolates. Furthermore, the IL-4 levels were not associated with the number of superantigen genes. This result was in agreement with several studies that reported low levels of IL-4 in lesional skin of psoriasis [38-41].

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