Determination of the Role of Regulatory T-cell in Patients with Psoriasis

Dina M.R. Alkhafaf

College of Dentistry , Al-Qadisyiah University

Dina.alkhafaf@qu.edu.iq

ARTICLE INFO
Submission date: 12/12/2016
Acceptance date: 22/12/2016
Publication date: 1/6/2019

Abstract

Psoriasis is one of most common autoimmune cutaneous diseases of the human communities, current study was aimed to determine the role of T-reg in patients with Psoriasis .Obtained data explained that psorasis is widely distributed disease at Al-Diwanyia city withen different age groups, the most affected group was the group (1-20) years old followed by the group (21-40) years old with tendency to males than females**.**). The relative gene expression in FoxP3 gene showed increment in patients group (7.89±5.82) compared with control group which was (1.91±1.25), NFAT gene showed increment in patients group (7.66±3.27) compared with control group which was (3.002±0.86). AP1gene showed an increment in patients group (5.34±1.08) compared with control group which was (1.88±1.25)**.** Serum profile of TNF-α in patients appeared to be elevated (194±32.9) pg/ml than control group (112±18.6) pg/ml**.** Obtained data also indicated a remarkable increase in serum concentrations of IL-36 (149.6±31.3)pg/ml, in comparison to control group (37.4±9.9) pg/ml**.** Over all findings reflect the abnormal increased activity of both T-reg and the effector cells which result in the progression of psoriasis.

Key words: Psorasis,T-reg, TNF-α, IL-36

Introduction

Psoriasis is one of most common cutaneous diseases which affect about 2-3% of the human communities, Psoriasis is autoimmune disease. Etiology and causes of this disease still unclear, but the activated T cells mainly CD4+ and CD8+ cells play central role in the pathogenicity. Symptoms of the disease appear as cutaneous erythromatous papules and scaly plaques of skin [1].

Several studies includes human xenografts and therapeutic trials of T cells drugs were carried in mice, suggest the role of immune response which mediated by T cells as key role of the disease. Its appeared that the increased expression of some cytokines such as IL-17, IL-12, IL-22,IL-23. IL-1and TNF-α in the onset patients. Molecular findings suggest that IL-23R gene variations are clearly correlated with disease, and the genetic familial contents are associated with psoriasis[2] .

HLA I and HLAII antigens such as HLA-B17,B39,B13,B57,-Cw7,Cw6,-DR7 and –DR4 appeared to be associated the auto inflammatory mechanism of the disease in skin .But the interesting thing in this case still unknown, several studies suggested that

keratinocytes itself is the antigenic target of the T-cells, other studies found that the basement membrane of the epidermis of the patients is ultra-structurally affected than normal individuals which may due to the induction of hyperplastic activity that triggered by effector immune cells[2,3]**.**

Phenotypic assay revealed that the infiltrating cells in epidermis are mainly CD8+ while in dermis are CD4+ in majority. These findings support the significant elevation of both pro-inflammatory such IFN-γ, TNF-α, IL-2 and IL-8 and anti-inflammatory cytokines secretion such as IL-5, IL-4, IL-13 and IL-10[4,5].

As a conclusion of the previous findings it is well known that the T effector cells are responsible for the initiation of psoriasis and persistency of illness**.**

Another line of studies focused on regulatory T-cells and its role in cases of psoriasis , suggesting that the defects of the immune system contribute the pathogenesis because it un able to turn off the abnormal excessive consequences of the immune response[6].

One of the most common mediators of the immune response is the IL-1 family, which play a key role in the inflammatory response, there are 11 member of them including seven ligands with agonist role (IL-1 β and IL-1 α, IL-33, IL-18, IL-36 β, IL-36 α, IL-36γ) and about three receptors antagonists (IL-1Ra, IL-36Ra, IL-38) [7,8].

The releasing of IL-1 triggers the angiogenesis, then increment of expression of adhesion molecules that induce T-cells which produce another types of cytokines especially IFN-γ, IL-8,IL-6, TNF-α and GM-CSF, then trauma of skin by infiltrating of keratinocytes to form new psoriatic lesions[9].

In the recent studies its thought that IL-36 normally expressed in human body in restricted scales mainly by skin epithelial cells and keratinocytes during certain conditions, by using animal models, the biological crucial role of this cytokine was determined by cross talking of the antigen presenting cells dendritic cells and keratinocytes[4,5]**.**

The present work was amid to focus on the role of regulatory T-cells and the levels of IL-36 and TNF-α cytokines of effector T-cells in the patients with psoriasis.

Materials and methods

Patients and Samples :

Blood sample were collected from 139 patients with psorsis and 40 healthy control individuals in Al-Diwanyia city/Iraq, Serum samples were obtained by taking 5 ml of venous blood and were collected by sterile tubes and then allowed to stand for 20 min at room temperature then centrifuged at 1000 rpm, sera were immediately separated and stored at -20 C in three aliquots to avoid multiple thawing until the time of assay.

Cytokines assay

Levels of IL-36 and TNF-α were assayed by using available commercial enzyme-linked immunosorbent assay(ELISA) kit supplied by (KOMA biotech / Korea), The assay was done according the manufacturer's instructions.

Primers:

All the primers that used in this work which listed in (table -1) .These primers (FOXP3, NFAT & AP-1) had been designed by the NCBI-Gene Bank data base and also the use of the Primer 3 design online, the primers were used to assay the relative gene expression ,quantitation of the gene expression as qRT-PCR technique which use the SYBER Green DNA binding dye (Bioneer, Korea).

Table-1: The Primers, sequences, gene bank accession number, and references

Primer	Sequence		Reference
NFAT Gene	F	GT TGGGGAGT TGGCACTAGC	[10]
	R	GACCCGGGCT T TCTACTGG	
AP1 Gene	F	GGTGGGATAAGACCCCCTCA	[10]
	R	TCCTGCCTGCATAGCAATAGG	
FoxP3 Geng	F	TGTGCTAGGGCGGTATGAGA	[10]
	R	GCTGGGGTGCAACTATGGG	
GAPDH	F	ACGACCACTTTCTCAAGCTC	[11]
	R	T TCCTCT TGTGCTCT TGCTG	

Quantitative Reverse Transcription Real-Time PCR (RT-qPCR):

Real-Time PCR technique (Quantitative Reveres Transcription) was used for measurement(quantitation) of the relative and comparative gene expression analysis. This assay was done depending on the technique of [12].The reaction conditions of Thermocycler protocol were shown in the following (table-2) .

Table -2: Thermocycler protocol

qPCR step	Temperature	Time	Repeat cycle
Initial Denaturation	95 °C	3 min	1
Denaturation	95 °C	20 sec	45
Annealing\Extension Detection(scan)	60 °C	30 sec	
Melting	60-95°C	0.5 sec	1

Data Analysis of qRT-PCR:

All the obtained data of the qRT-PCR of the target and housekeeping genes were subjected for analysis by the relative quantification gene expression levels (fold change). All these results were collected according the reference method of [13].

Statistical Analysis

The results that obtained by this study were analyzed statistically by the using of statistical package SPSS specialized program (Statistical Package for Social Sciences) version 10.0 for windows. All the tested parameters were written as mean ± standard error (S.E.). All the differences between and among the tested parameters were listed in ANOVA (analysis of variance), the least statistical significant differences (LSD) also used to improve the valuable changes in data on the probability (*P*) value was ≤ 0.05 [14].

Results

Obtained data explained that psorasis is widely distributed disease at Al-Diwanyia city withen different age groups, But the most affected group was the group (1-20) years old followed by the group (21-40) years old with tendency to males than females table-3.

Table-3: Distribution of cases in different age groups

Gender	Age (year)				Healthy human control
Age groups	(1-20)	(21-40)	(41->60)	total	
Female	32	23	7	62	20
Male	44	24	9	77	20
Total	76	47	16	139	40

Relative gene expression:

The relative expression of target genes (NFAT gene , FoxP3 gene and AP1gene) in patients' blood samples were tested by the use of Livak Method (2^-ΔΔCT) which based on the normalization of the (CT value) of RT-qPCR for the tested target genes in comparison to the housekeeping gene (GAPDH) which was used as reference gene in the patients and the control groups (fig-1)

The results of relative gene expression in FoxP3 gene showed increment in patients group (7.89±5.82) compared with control group which was (1.91±1.25) as shown in figure5, result of NFAT gene showed increment in patients group (7.66±3.27) compared with control group which was (3.002±0.86) as shown in figure6 and result of AP1gene showed increment in patients group (5.34±1.08)compared with control group which was (1.88±1.25) as shown in figure7 .The statistical analysis of the relative expression of FoxP3 gene , NFAT gene and AP1gene were showed significant differences in patients groups in comparison with control groups at level ($P \leq 0.05$) (Table.4) .

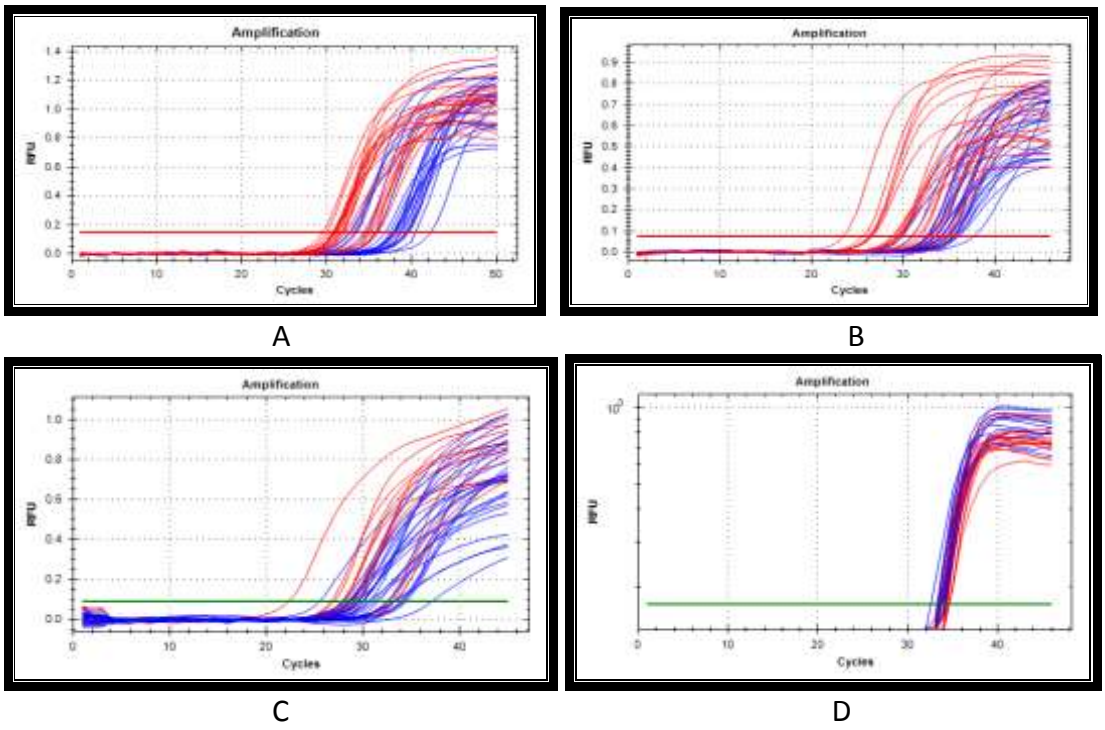

Figure1: Real-Time PCR amplification plots of:

A Left:FoxP3 gene patients group samples (red plot) and healthy control group samples (blue plot).B Right: NFAT gene in patient's group samples (red plot) and healthy control group samples (blue plot).C Left: API gene in patient's group samples (red plot) and healthy control group samples (blue plot).D Right: GAPDH gene in patients and healthy control groups. Where, blue plot patient's samples and red healthy control samples.

Table -4 : gene expression of FOXP3,NFAT and AP-1 gene by RT-qPCR

Gene	Patients group	Control group
FOXP3	7.89±5.82	1.91±1.25
NFAT	7.66±3.27	3.002±0.86
AP-1	5.34±1.08	1.88±1.25

Serum profile of TNF-α in patients appeared to be elevated significantly $p \leq 0.05$, 194±32.9 pg/ml than control group 112±18.6 pg/ml (fig.2)

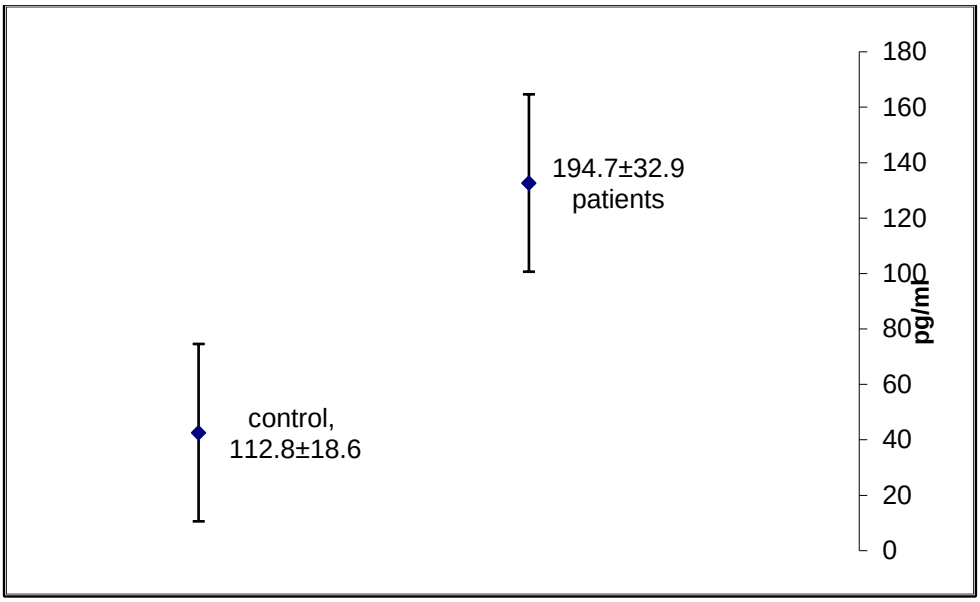

Figure2: Serum levels of TNF-α

Obtained data also indicated a remarkable increase in serum concentrations of IL-36 149.6±31.3pg/ml, in comparison to control group 37.4±9.9 pg/ml (fig.3).

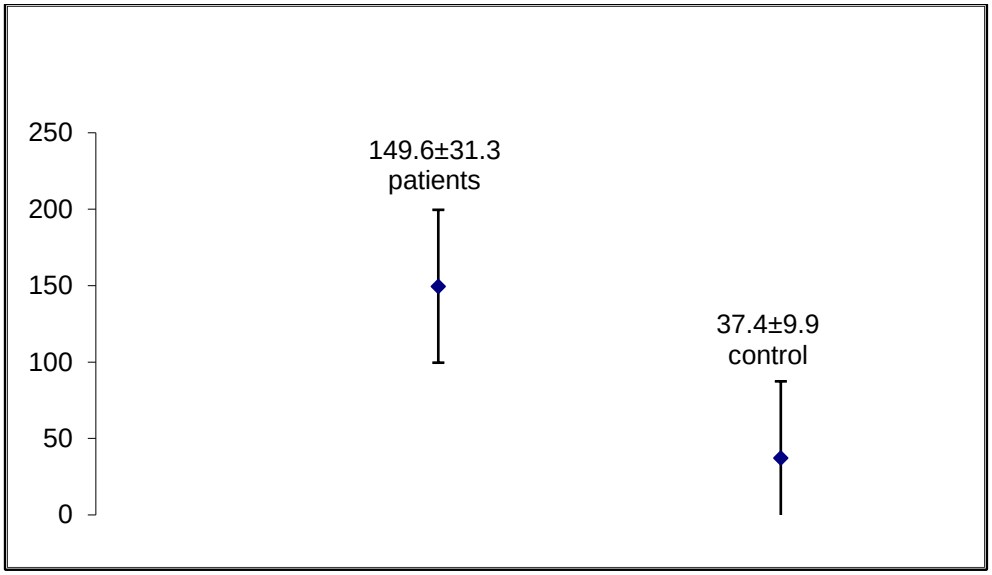

Figure -3: Serum levels of IL36

Discussion

From the observations of the current study, its clear that the age category 1-20 showed the most affected group especially in males. And there was significant elevation in sera levels of TNF-α and IL-36, molecular study results indicate increased gene expression in FOXP3, NFAT & AP-1 genes.

Due to its amazing characteristics, TNF-α was the first cytokine that had been gain attention especially at the period of research that included the Th1 response studies, this attention was increased directly after the discovery of anti TNF-α mAb which was considered as the golden alternative treatment to the patients with psoriasis who were not respond to the traditional chemotherapy. Th1 cells response in patients with psoriasis show atypical immune activation, so at the present time and after investigating

another types of cytokines in scientific research, it it very clear that psoriasis as a disease result from a complicated interactions between Th1, Th17 and the T-reg[15,16].

All these observations reflect the active inflammatory response in the patients with psoriasis and can give a good hint on the interacting activity of T-effector cells such as Th1 and Th17 and the activity of T-reg[16].

The complex network of many immune mediators especially IL-23 , IL-17 and recently IL-36, Its believed that these cytokines play a central role in the development of psoriasis, therefor they represent a convenient candidate for designing new targets as new therapies. One of the key cytokines of the innate immunity is the IL-36 which involved in the dysfunctions of this type of immune response in the patients with psoriasis. IL-36 also able to manipulate the cross-talk of antigen presenting cells (dendritic cells) and keratinocytes then activate these cells to produce TNF-α, and have the ability to affect the functions of IL-17, IL-22 and IL-23[19,20].

IL36 one member of IL-1 family its contain three types α, β and γ all these types bind to heterodimer receptor IL-36R. Now it's believed that IL-36 produced in some human cells, mainly in skin by keratinocytes and other epithelial cells during the activation by a given pathogen of any other immune stimulus[4,5].

From the studies that had been carried out on mice and human, it's clear now that IL-36 and its receptor play an important role in the pathological inflammatory immune response in the skin in the patients with psoriasis suggesting that the signals between the dendritic cells and keratinocytes in the skin. The expression of IL-36 is up regulated by the IL-17 which is a cytokine produced by Th17, then IL-36 in turn enhance the IFN-γ producing T cells in the inflammatory area[8,16].

Upon inflammation, T-reg play crucial role by reducing the deleterious impacts of the immune response, FOXP3 is well known transcription factor mainly expressed in these cells, the expression many anti-inflammatory cytokines including IL-10 that affect and modulate other pro-inflammatory cytokines such as IL_12, IL-2, and IL-6[20,21].

NFAT + AP-1 form a complex and are very important in activation of CD4+ and CD8+ T cell, and are responsible for the completing the reaction that is started by FOXP3 , in which T-helper cells and T-reg orchestrated in suitable manner that would not be harmful in normal conditions. When imbalance occurred in some cases such as autoimmune disease this balance impaired resulted in a different types of diseases such as psoriasis[16,17,18,19].
As shown in the obtained data from the gene expression, it was clear that the expression of the three genes increased which may explain the confected inflammatory response of patients this increment can be explaned as an attempt to reduce the deleterious effect of the abnormal excesive response of Th-1 and Th-17[20,21,22,23].

Conclusion

The remarkable increment in the gene expression of the genes of T-reg cells explain the uncontrollable effective mechanisms which reduce the immune-pathogenicity that resulted in patients due to the elevation of certain cytokines produced by effector cells such as Th-1 or Th-17 such as TNF-α and IL-36 which affect the keratinocytes proliferation.

Conflict of Interests.

There are non−conflicts of interest .

References

[1] S. Di Nuzzo, C. Feliciani, C. Cortelazzi, G. Fabrizi, and C. Pagliarello, "Immunopathogenesis of psoriasis: Emphasis on the role of Th17 cells," *Int Trends Immun*, vol. 2, no. 3, p. 111, 2014.

[2] H. S. Song, S. J. Kim, T.-I. Park, Y. H. Jang, and E.-S. Lee, "Immunohistochemical comparison of IL-36 and the IL-23/Th17 axis of generalized pustular psoriasis and acute generalized exanthematous pustulosis," *Ann. Dermatol.*, vol. 28, no. 4, pp. 451–456, 2016.

[3] M. Keermann, S. Kõks, E. Reimann, E. Prans, K. Abram, and K. Kingo, "Transcriptional landscape of psoriasis identifies the involvement of IL36 and IL36RN," *BMC Genomics*, vol. 16, no. 1, p. 322, 2015.

[4] A. M. Foster *et al.*, "IL-36 promotes myeloid cell infiltration, activation, and inflammatory activity in skin," *J. Immunol.*, vol. 192, no. 12, pp. 6053–6061, 2014.

[5] M. Chu *et al.*, "Elevated expression and pro-inflammatory activity of IL-36 in patients with systemic lupus erythematosus," *Molecules*, vol. 20, no. 10, pp. 19588–19604, 2015.

[6] D.C. Soler and T. S. McCormick, "The dark side of regulatory T cells in psoriasis," *J. Invest. Dermatol.*, vol. 131, no. 9, pp. 1785–1786, 2011.

[7] A.M. Brotas, J. M. T. Cunha, E. H. J. Lago, C. C. N. Machado, and S. C. da S. Carneiro, "Tumor necrosis factor-alpha and the cytokine network in psoriasis," *An. Bras. Dermatol.*, vol. 87, no. 5, pp. 673–683, 2012.

[8] J.H. Eysteinsdóttir *et al.*, "The role of Th17/Tc17 peripheral blood T cells in psoriasis and their positive therapeutic response," *Scand. J. Immunol.*, vol. 78, no. 6, pp. 529–537, 2013.

[9] Y. Wu, H. Li, Z. Jiang, and Y. Lai, "The interleukin-1 family: a key regulator in the pathogenesis of psoriasis," *Austin J Clin Immunol*, vol. 5, p. id1023, 2014.

[10] Z. A. M. Alqassemi and Z. M. F. Alkhozai, "Immune Modulation in Patient with Varicella Zoster Virus Treated with Phototherapy and Chemotherapy," *J. ARC Dermatol*, vol. 1, no. 1, pp. 1–9, 2016.

[11] H. Hayase *et al.*, "Aberrant gene expression by CD25+ CD4+ immunoregulatory T cells in autoimmune-prone rats carrying the human T cell leukemia virus type-I gene," *Int. Immunol.*, vol. 17, no. 6, pp. 677–684, 2005.

[12] G. Wang and M. P. Hardy, "Development of leydig cells in the insulin-like growth factor-I (igf-I) knockout mouse: effects of igf-I replacement and gonadotropic stimulation," *Biol. Reprod.*, vol. 70, no. 3, pp. 632–639, 2004.

[13] A. Radonić, S. Thulke, I. M. Mackay, O. Landt, W. Siegert, and A. Nitsche, "Guideline to reference gene selection for quantitative real-time PCR," *Biochem. Biophys. Res. Commun.*, vol. 313, no. 4, pp. 856–862, 2004.

[14] J. H. McDonald, *Handbook of biological statistics*, vol. 2. Sparky house publishing Baltimore, MD, 2009.

[15] A. Nakajima *et al.*, "TNF, but not IL-6 and IL-17, is crucial for the development of T cell-independent psoriasis-like dermatitis in Il1rn−/− mice," *J. Immunol.*, vol. 185, no. 3, pp. 1887–1893, 2010.

[16] M. H. Kagen, T. S. McCormick, and K. D. Cooper, "Regulatory T cells in psoriasis," in *Cytokines as Potential Therapeutic Targets for Inflammatory Skin Diseases*, Springer, 2005, pp. 193–209.

[17] A. Arvey, J. Van Der Veeken, R. M. Samstein, Y. Feng, J. A. Stamatoyannopoulos, and A. Y. Rudensky, "Inflammation-induced repression of chromatin bound by the transcription factor Foxp3 in regulatory T cells," *Nat. Immunol.*, vol. 15, no. 6, p. 580, 2014.

[18] S.D.P. Ellis, J.L. McGovern, A. van Maurik, D. Howe, M.R. Ehrenstein, and C.A. Notley, "Induced CD8+ FoxP3+ Treg cells in rheumatoid arthritis are modulated by p38 phosphorylation and monocytes expressing membrane tumor necrosis factor α and CD86," *Arthritis Rheumatol.*, vol. 66, no. 10, pp. 2694–2705, 2014.

[19] F. Macian, "NFAT proteins: key regulators of T-cell development and function," *Nat. Rev. Immunol.*, vol. 5, no. 6, p. 472, 2005.

[20] R.V. Luckheeram, R. Zhou, A.D. Verma, and B. Xia, "CD4+ T cells: differentiation and functions," *Clin. Dev. Immunol.*, vol. 2012, 2012.

[21] S. Sakaguchi, M. Miyara, C. M. Costantino, and D. A. Hafler, "FOXP3+ regulatory T cells in the human immune system," *Nat. Rev. Immunol.*, vol. 10, no. 7, p. 490, 2010.

[22] Y. Kim, X. Liu, S. Tanaka, D. Tran, and Y. Chung, "Regulation of germinal center reactions by B and T cells," *Antibodies*, vol. 2, no. 4, pp. 554–586, 2013.

[23] R.S. M. AlOmari , M.F. Alkhozai Ziad. Determination of the Gene Expression of CD25 and CD29 in Albino rates immunized with Vi antigen of Salmonella typhi coupled to chitosan nanoparticle and Tetanus toxoid. *J. International Journal of Natural Sciences*. vol.2, no.4, pp.33-43, 2014.

الخلاصة

الصدفية احد امراض المناعة الذاتية الجلدية المعروفة في المجتمعات البشرية,حيث هدفت الدراسة الحالية لتحديد الدور الذي تلعبه الخلايا التائية المنظمة. بينت النتائج التي تم الحصول عليها ان مرض الصدفية من الامراض المنتشرة بشكل ملحوظ في فئات عمرية مختلفة مدينة الديوانية, حيث كانت الفئة العمرية (20−1) سنة هي الاكثر تسجيلا للمرض تلتها الفئة (40−20) سنة, ويلاحظ فيها وجود ميل للاصابة عند النساء اكثر مما عند الرجال. سجلت البيانات التي تم الحصول عليها ان التعبير الجيني النسبي للجين FoxP3 زيادة معنوية ملحوظة في مجموعة المرضى حيث بلغت (7.89±5.82) بينما كانت قيم تعبي الجين في مجموعة السيطرة الاصحاء (1.91±1.25). أما بالنسبة للتعبير الجيني للجين NFAT فقد اظهر هو الاخر زيادة معنوية في قيم التعبير في مجموعة المرضى بلغت (7.66±3.27) مقارنة بمجموعة السيطرة التي سجلت في حين كانت بمجموعة السيطرة (3.002±0.86). كذلك ومن الملاحظ ايضا وجود زيادة عالية المعنوية للتعبير الجيني للجين AP1 في مجموعة المرضى , اذ بلغت (5.34±1.08) في حين كانت في مجموعة السيطرة (1.88±1.25) . بينت الدراسة المصلية للحركيات الخلوية ان قيم عامل تنخر السرطان TNF−α ارتفعت بمجموعة المرضى (194±32.9 pg/ml) عما هو عليه في مجموعة السيطرة (112±18.6 pg/ml). كذلك فان قيم IL−36 هي الاخرى سجلت ارتفاعا لتبلغ (149.6±31.3pg/ml) بالمقارنة مع السيطرة(37.4±9.9 pg/ml). من مجمل النتائج المحصل عليها هي وجود ارتفاعا غير طبيعي لنشاط الخلايا المؤثرة المناعية مثل الخلايا المساعدة يقابلها زيادة في فعالية الخلايا التائية المنظمة الامر الذي يقود لتطور مرض الصدفية عند المصابين.

الكلمات الدالة: TNF−α, IL−36 T−reg, Psorasis .