Journal of Biosciences and Medicines, 2022, 10, 136-149
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

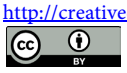
Association between IL-37 and VEGF Gene Expression in Psoriasis Pathogenesis in Egyptian Population

Sherif M. El-Sherbini[1*], Ahmed Kh. S. Salama[2], Laila A. Rashed[3], Mohamed H. Nafady[2], Samir A. M. El-Masry[1]

[1]Genetic Engineering and Biotechnology Research Institute, Al-Sadat University, Al-Sadat, Egypt
[2]Faculty of Applied Medical science, Misr University for Science and Technology, Giza, Egypt
[3]Faculty of Medicine, Cairo University, Cairo, Egypt
Email: *firstsherif_2000@yahoo.com

How to cite this paper: El-Sherbini, S.M., Salama, A.K.S., Rashed, L.A., Nafady, M.H. and El-Masry, S.A.M (2022) Association between IL-37 and VEGF Gene Expression in Psoriasis Pathogenesis in Egyptian Population. *Journal of Biosciences and Medicines*, **10**, 136-149.
https://doi.org/10.4236/jbm.2022.105013

Received: March 28, 2022
Accepted: May 27, 2022
Published: May 30, 2022



Abstract

Background: Psoriasis is considered a common skin disease, marked by the production and elevation of inflammatory plaques that regularly shed scales resulting from extensive skin epithelial cell proliferation. T lymphocytes, neutrophils, and other leukocytes enter the irritated skin, resulting in epidermal keratinocyte hyperplasia, vascular hyperplasia, ectasia, and T lymphocyte, neutrophil other forms of leukocyte infiltration. **Aim of the Work:** the study aimed to investigate the role of IL-37 and VEGF gene expression in the pathogenesis of psoriasis in Egyptian patients. **Methodology:** Polymerase chain reaction techniques (PCR) were applied to detect VEGF in the skin homogenates of psoriasis patients. In addition, the ELIZA technique was applied to investigate IL-37 in skin homogenates. One hundred cases have been divided into two groups: 50 healthy volunteers as the group I healthy control; 50 psoriasis patients as group II. PCR real-time technology was assessed through extracted DNA samples for VEGF amplification in skin homogenate. **Result:** Our results revealed that psoriasis patients significantly had a substantial reduction in IL-37 compared to the control group ($p < 0.001$). However, there was a significant increase ($p < 0.001$) in VEGF in psoriasis patients compared to the control group. **Conclusion:** There is a significant inverse association between IL-37 and VEGF in psoriasis patients. Study findings revealed that IL-37 gene expression decreases while VEGF gene expression increases in psoriatic individuals. Such a measurement may be beneficial in determining the severity of the condition, as well as taking into consideration of the disease's diagnosis.

Keywords

IL-37, VEGF, Gene Expression, Psoriasis, PCR

1. Introduction

Psoriasis is an autoimmune disease that produces a fast accumulation of skin cells on the skin's surface, resulting in scaling [1]. The condition affects 2% of the world's population, with a frequency of around 4.6 percent in affluent countries [2]. The most frequent etiological cause is stress, and individuals with chronic diseases such as Crohn's disease are more prone to get psoriasis [3]. Psoriasis appears to be linked to beta-blockers, synthetic antimalarial, lithium, nonsteroidal anti-inflammatory drugs (NSAIDs), and tetracyclines [4]. Patients who are expected to have cardiovascular comorbidities are more common in patients with severe illnesses [5].

IL-37 is a newly discovered cytokine from the IL-1 family in five different isoforms. IL-37 can inhibit an overactive immune response as an immunosuppressive agent. The protein IL-37 protects against endotoxin shock, autoimmune disorders, ischemia-reperfusion damage, and cardiovascular illnesses. In addition, IL-37 may have anticancer properties. The IL-37 and its receptors might be used to investigate, diagnose, and treat immune-related diseases, including cancers [6]. Moreover, IL-37 is a potent innate immunity inhibitor because it shifts the cytokine balance far from severe inflammation [7]. The function of interleukin 37 (formerly known as IL-1 family 7) is unknown. In macrophages and epithelial cells, IL-37 expression nearly entirely reduced the production of pro-inflammatory cytokines, whereas suppressing endogenous IL-37 in human blood cells enhanced the quantity of these cytokines. On the other hand, anti-inflammatory cytokines remained unchanged [8].

The angiogenic factor is a substance that promotes blood vessel growth. VEGF was first discovered as a vital potent stimulus for vascular endothelium [9]. Vascular endothelial growth factors (VEGFs) are critical regulators for vascular development and blood and lymphatic arterial functioning in health and disease [10]. During embryogenesis, VEGF is considered a crucial regulator of physiological angiogenesis, skeletal development, and reproductive activities. VEGF has also been related to pathological angiogenesis in cancers, ocular nonvascular disorders, and other ailments [11].

2. Subject and Methods

2.1. Subjects

The current study is a case-control study conducted at a hospital (El-Kser El-Any hospital) from 2018 to 2020. Approval was received from local health authority legal and scientific committees. Individuals under study were classified into two groups.

2.2. Detection of Psoriasis by ELISA

According to the manufacturer instructions of cloud-clone Corp, USA, the method was done. Principle of the assay: The sandwich enzyme-linked immunosorbent test (ELISA) kit is based on the conventional sandwich technique. 96-well plates have been pre-coated with a goat polyclonal antibody specific for IL-37. After adding the standards (*E. coli* expression system; Immunogen sequence: V46-D218) and test samples to the wells, a biotinylated detection polyclonal antibody from goat specific for IL-37 is added, followed by washing with TBS buffer. Unbound Avidin-Biotin-Peroxidase Complex is added.

TBS buffer is used to wash away the conjugates. TBS buffer is used to wash away the conjugates Substrate for horseradish peroxidase (HRP). The HRP enzymatic process is seen using TMB. TMB is catalyzed by HRP, resulting in a chromogenic reaction that produces a blue color product that becomes yellow when acidic TMB Stop Solution is added. The absorbance of the chromogenic reaction that results is measured. The amount of Human IL-37 sample caught in the plate is related to the density of yellow.

2.3. Molecular Study

Stored skin homogenate samples were used in molecular techniques such as genomic DNA extraction from skin homogenate samples. Then extracted DNA for PCR amplification for VEGF was assessed using PCR real-time technique. IL-37 was analyzed using estimation by the ELIZA technique.

According to the manufacturer's instructions, total RNA was extracted using a Qiagen tissue extraction kit (Qiagen, USA). The total RNA (0.5 - 2 g) was utilized to convert to cDNA using Ferments' high-capacity cDNA reverse transcription kit.

2.4. Primer Sequence

Table 1 refers to the sequence of the primers.

3. Statistical Analysis

The statistical program SPSS version 22 was used to code and input the data. Data were analyzed using the average, standard deviation, and frequency for quantitative and categorical variables. The independent t-test is also used to compare data across the groups, and chi X^2 compares categorical variables. Statistically, a significance value was defined as a probability value (P-value) less than 0.05 [12].

Table 1. Refer to the primer sequence.

BAX Forward primer	5'-CCCTGTGCACTAAAGTGCCC-3'
Reverse primer	5'-CTTCTTCACGATGGTGAGCG-3'
NFKB Forward primer	5'-CATTGAGGTGTATTTCACGG-3'
Reverse primer	5'-GGCAAGTGGCCATTGTGTTC-3'
βeta actin Forward primer	5'-TGTTTGAGACCTTCAACACC-3'
Reverse primer	5'-CGCTCATTGCCGATAGTGAT-3'

4. Results

The present study was conducted on a group of patients consisting of 100 subjects, of matched age; the patients were enrolled from Dermatology dep., Faculty of Med., Cairo Uni. The participants were split into two groups, Group (I): 50 non-patient volunteers as control and Group (II): 50 psoriasis patients. For all studied groups, the following were estimated: 1) IL-37 by ELISA technique; 2) VEGF gene expression by RT-PCR technique.

4.1. Demographic Data

Demographic data of all groups are shown in Table 2. It has been shown that the healthy control group was 100 (26 males and 24 females). However, the psoriasis patients group includes 88 individuals (28 males and 22 females). Results in Table 2 and Figure 1 showed no significant variance in the age in the control and psoriasis patients ($p = 0.9$). Moreover, the difference was non-significant in sex between the control and psoriasis patients ($p = 0.6$).

4.2. Clinical Data

Regarding the clinical data of psoriasis patients Table 3, the PSA ranges from

Table 2. Demographic data among the studied groups.

Groups		Control group N = 50	Psoriasis group N = 50	P-value
Age		45.38 ± 7.96	45.56 ± 12.9	0.9
Sex	Male	26 (52%)	28 (56%)	0.6
	Female	24 (48%)	22 (44%)	

The data were presented as a number, a percentage, and a mean, standard deviation (SD), with a p-value of 0.05 considered significant.

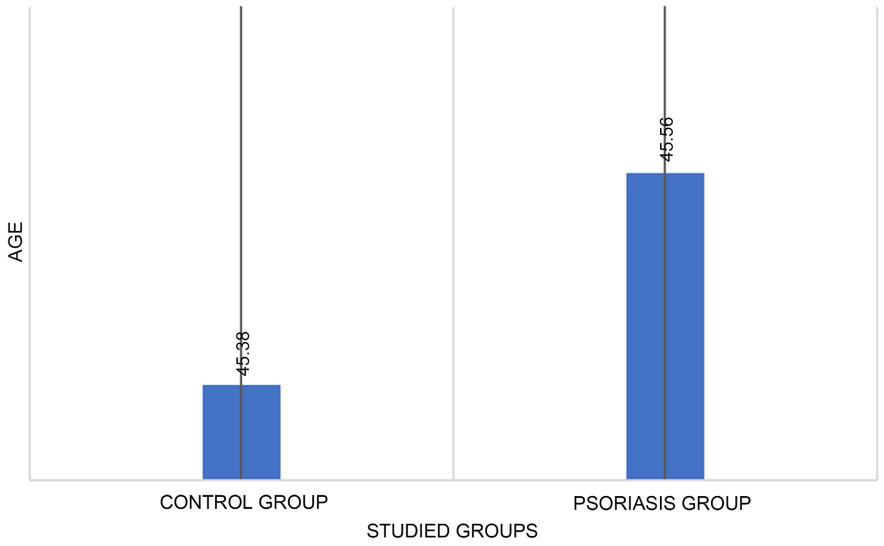


Figure 1. Age of the studied groups.

Table 3. Clinical data among psoriasis patients.

Variable	N (%)
PASI	
minimum	0.9
maximum	28.5
median	6.5
mean ± SD	8.24 ± 6.7
Duration (month)	
minimum	100
maximum	260
median	120
mean ± SD	113.5 ± 82.68
Onset	
gradual	45 (90%)
sudden	5 (10%)
Course	
progressive	12 (24%)
regressive	2 (4%)
remission & exacerbation	29 (58%)
stationary	7 (14%)
FH	
No	43 (86%)
Yes	7 (14%)
PPT factors	
cold	21 (42%)
stress	16 (32%)
stress, cold	11 (22%)
sun	2 (4%)

(0.9 - 28.5) with a median value of 6.5. The disease duration ranges from (100-to 260) months with a median value of 120. 58% of psoriasis patients suffer from remission and exacerbation course, 24% have a progressive course, 14% stationary, and 4% regressive course. A 4% of psoriasis patients have a positive family history. 42% of psoriasis patients reported that cold is precipitating factor, 32% reported stress as a precipitating factor, 22% said both stress and cold, and 4% reported that exposure to the sun is a precipitating factor.

4.3. Molecular Study

An assessment of VEG using PCR real-time technique and IL-37 using by ELIZA technique has occurred.

4.3.1. Interleukin-37 Gene

Data in **Figure 2** show a significant decrease in the Interleukin-37 gene (IL-37) in psoriasis patients in comparison to the control ($p < 0.001$). Results revealed the behavior of IL-37 gene expression with its relationship with the age of the studied groups, which showed that IL-37 gene expression tends to increase as

participants get old in the control group. Conversely, IL-37 gene expression decreases as participants get old in the in-patient group Figure 3.

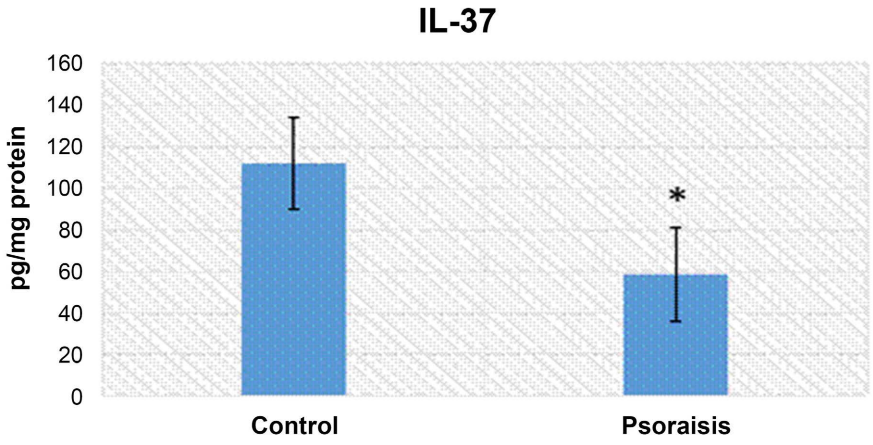


Figure 2. Interleukin-37 gene (IL-37) among studied groups by ELISA technique.

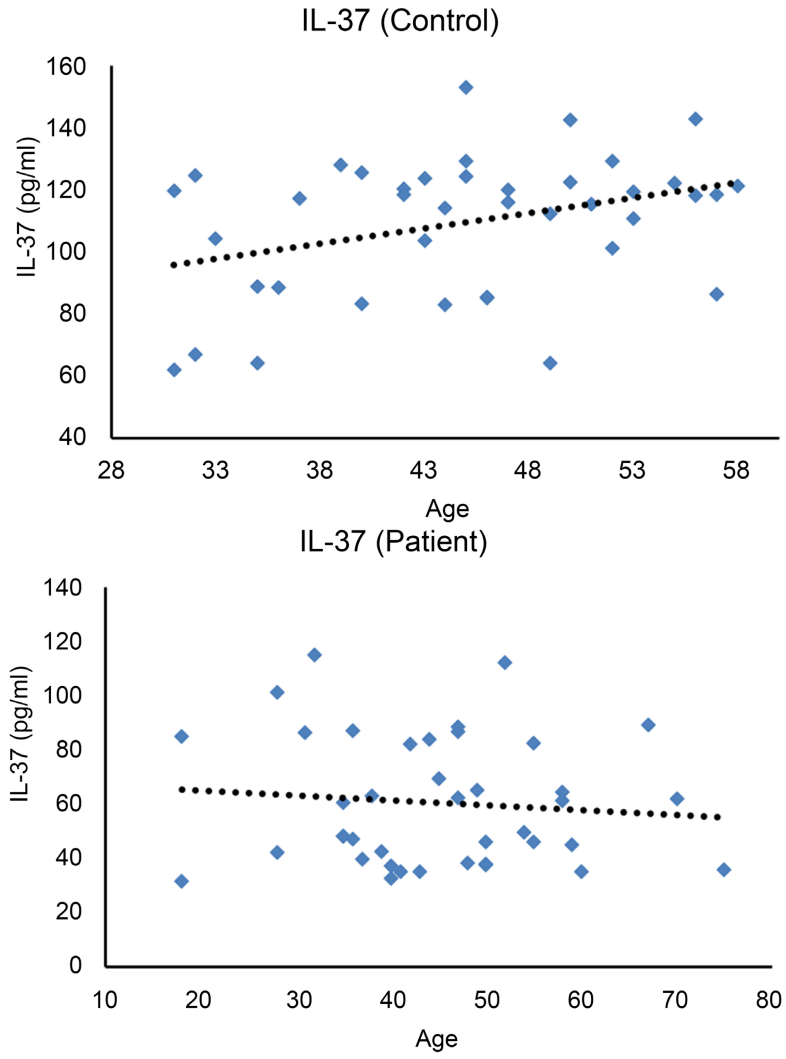


Figure 3. IL-37 gene expression behavior at different ages of the studied groups.

4.3.2. Vascular Endothelial Growth Factor (VEGF)

Psoriasis patients had a substantial rise in VEGF compared to the control group ($p < 0.001$) Figure 4. Data shows the behavior of VEGF gene expression and its relationship with the age of the studied groups, which revealed that VEGF gene expression tends to be steady as participants get old in the control group. VEGF gene expression tends slightly to increase as participants get senior in-patient group Figure 5.

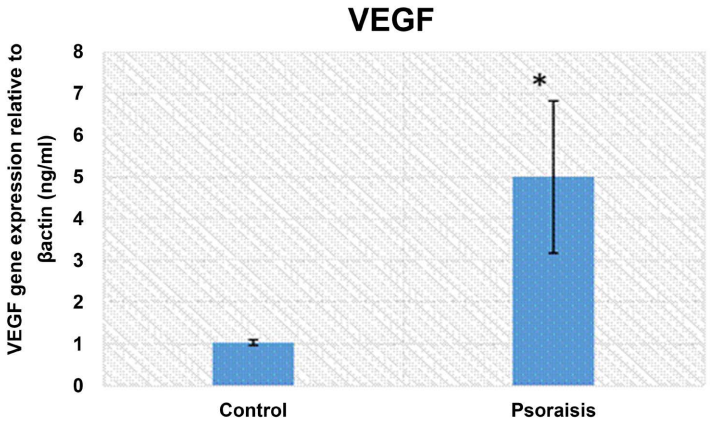


Figure 4. VEGF among the studied group by RT-PCR.

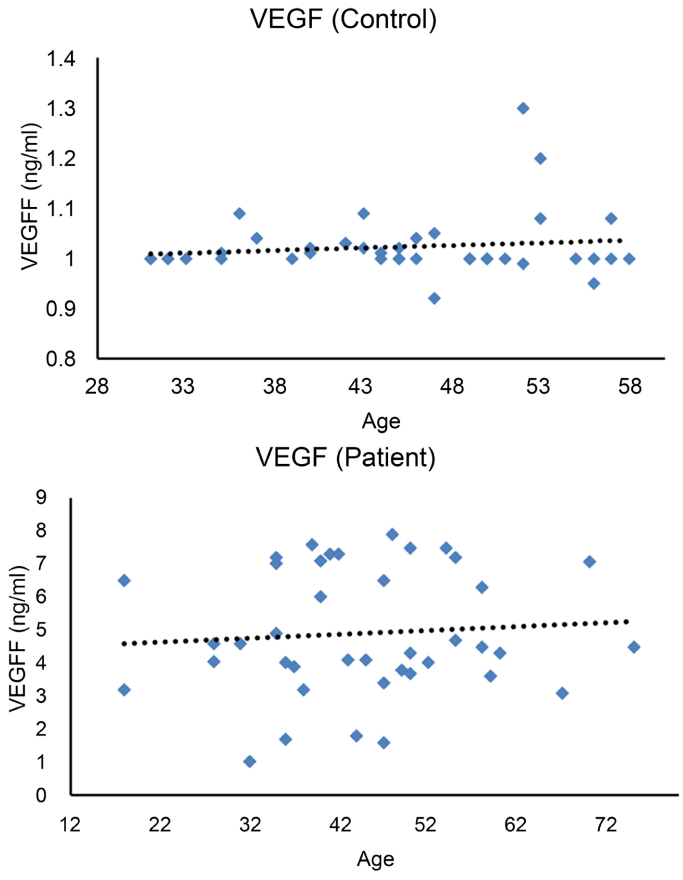


Figure 5. VEGF gene expression behavior at different ages of the studied groups.

4.3.3. Psoriasis Area Severity Index (PASI)

PASI tends to decrease as Psoriasis patients get old Figure 6.

4.4. Regression Analysis

4.4.1. IL-37 Gene Expression

IL-37 gene expression shows homogeneity with PASI and psoriasis duration regarding the patient's age (categorized into seven age groups with five years intervals), revealing the direct impact of PASI and psoriasis duration on the IL-37 gene expression in the psoriasis patient Figure 7. Regression analysis shows a good correlation between IL-37 gene expression versus PASI and duration (month) regarding the age with r^2 valued at 90.2% ($p < 0.05$). The regression equation is represented as:

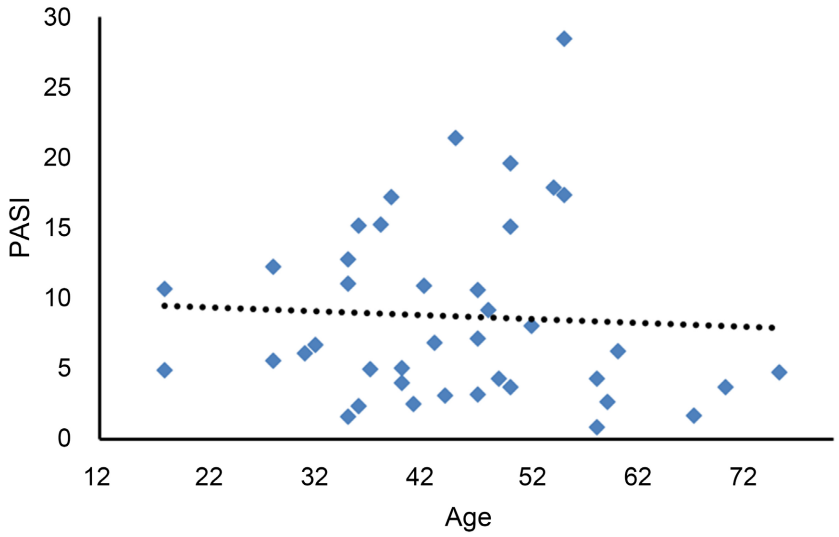
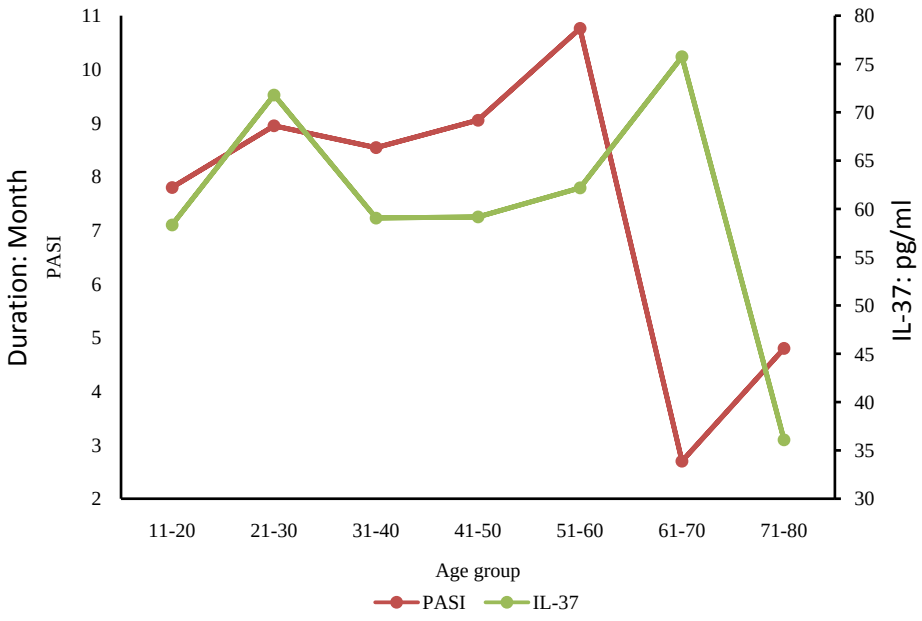


Figure 6. PASI at different ages of the psoriasis group.



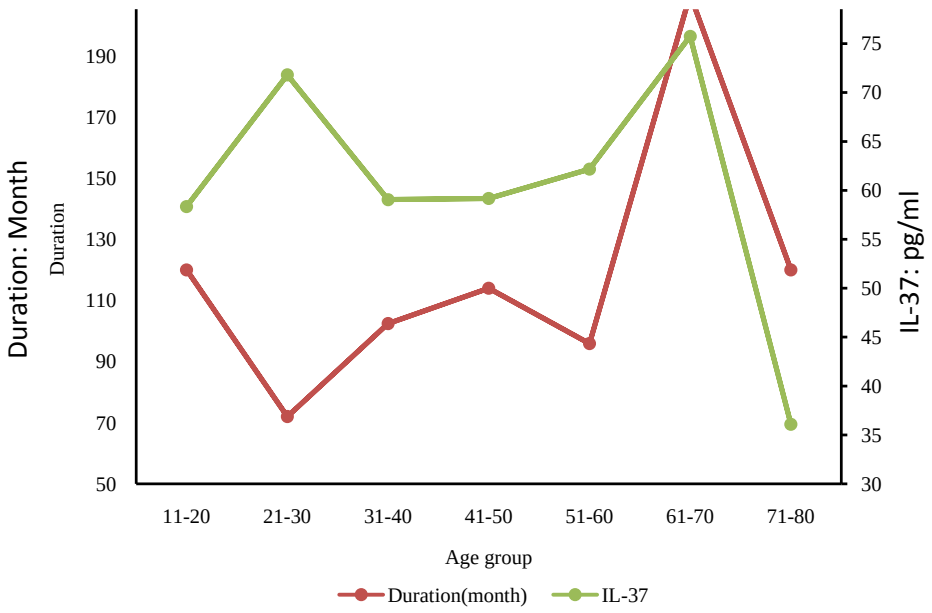


Figure 7. IL-37 gene expression relation to (PASI and psoriasis duration) regarding the Psoriasis patient's age as a categorical variable.

$$\text{IL-37} = 142.5 - 3.83\,\text{PASI} - 0.4480\,\text{Duration}\left(\text{Month}\right)$$

4.4.2. VEGF Gene Expression

VEGF gene expression shows homogeneity with PASI and psoriasis duration regarding the patient's age (which has been categorized into seven age groups with five years intervals), revealing the direct impact of PASI psoriasis duration on the VEGF gene expression in the psoriasis patient **Figure 8**. Regression analysis shows a good correlation between VEGF gene expression versus PASI and duration (month) regarding the age with r^2 valued at 83.48% ($p < 0.05$). The regression equation is represented as:

$$\text{VEGF} = 2.082 + 0.1694\,\text{PASI} + 0.01231\,\text{Duration}\left(\text{Month}\right)$$

4.4.3. IL-37 versus VEGF Gene Expression

Polynomial regression analysis **Figure 9**, IL-37 versus VEGF gene expression showed a good correlation with r^2 85.24% ($p < 0.05$). The regression equation is represented as:

$$\text{IL-37} = -41148 + 26027\,\text{VEGF} - 5459\,\text{VEGF}^2 + 380.3\,\text{VEGF}^3$$

IL-37 gene expression shows homogeneity with VEGF gene expression regarding the PASI of the patient (which has been categorized into 11 groups). This reveals the correlation between IL-37 and VEGF gene expression in psoriasis patients **Figure 10**.

5. Discussion

Psoriasis is a skin condition marked by the development of elevated, inflammatory that continually excessive epidermal epithelial cell growth causes scales to

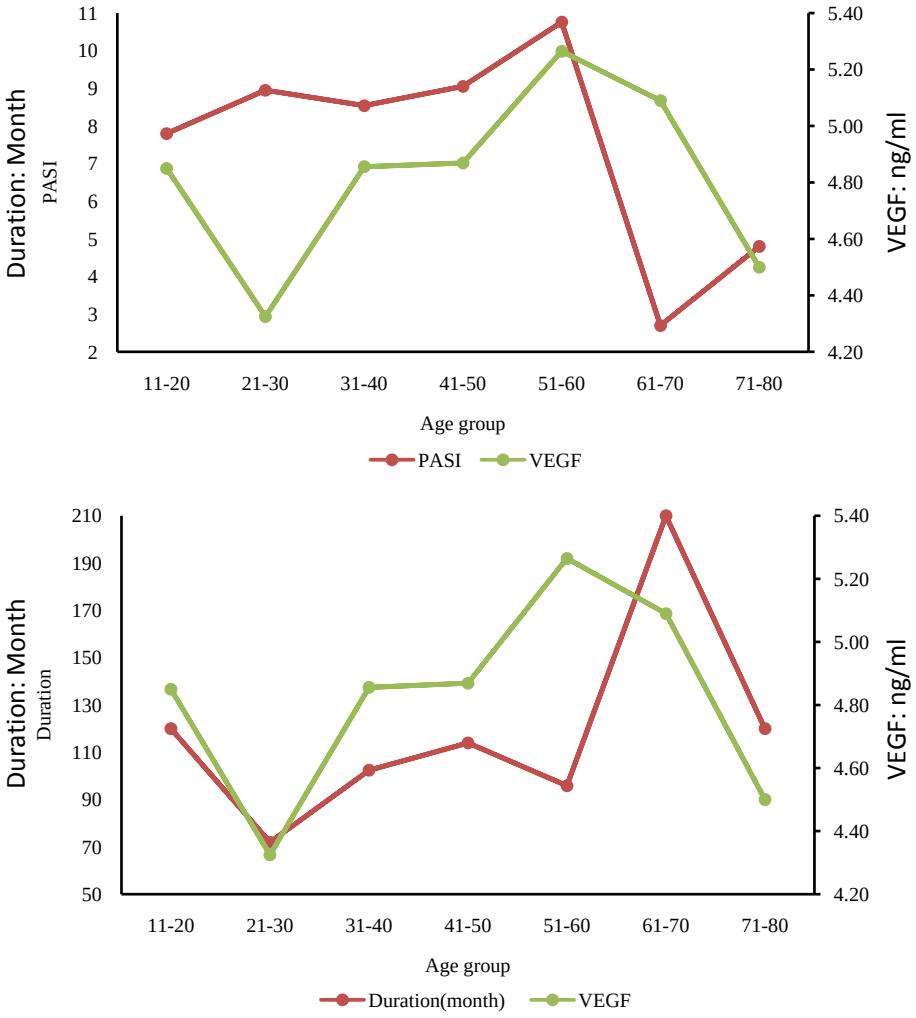


Figure 8. VEGF gene expression relation to (PASI and psoriasis duration) regarding the Psoriasis patient's age as a categorical variable.

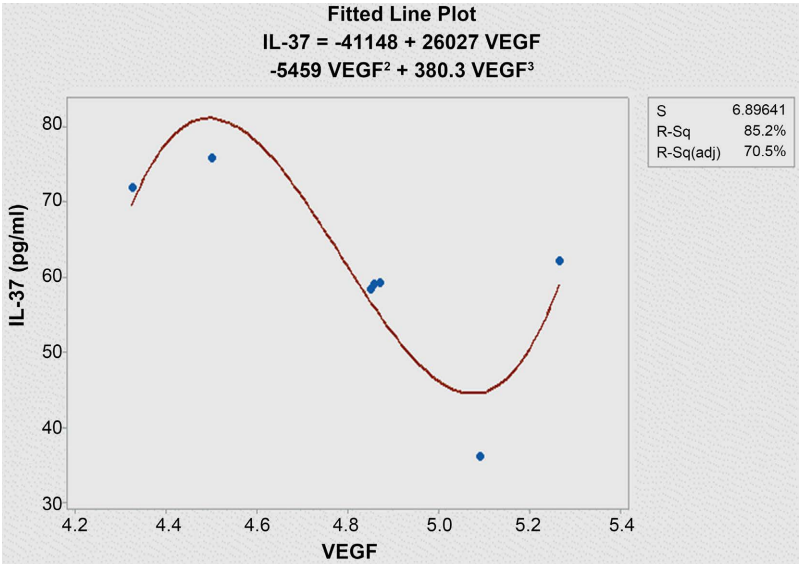


Figure 9. Polynomial regression analysis between VEGF and IL-37 gene expression.

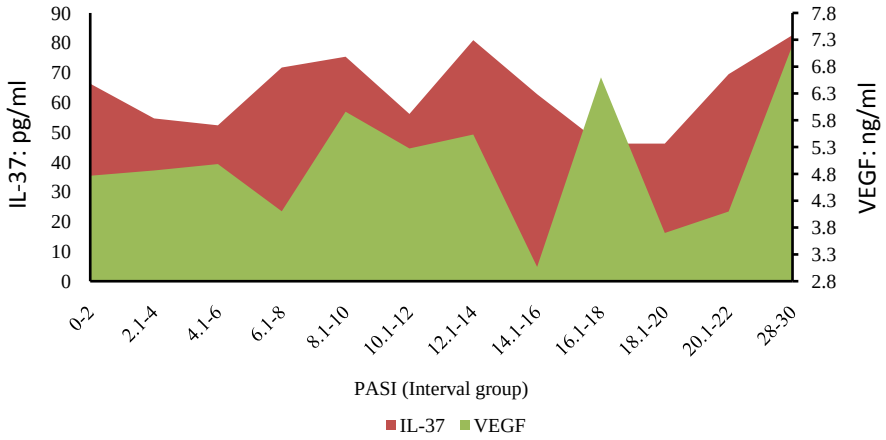


Figure 10. VEGF gene expression in relation to VEGF gene expression in regarding the PASI as a categorical variable.

slough [3]. Psoriasis is an autoimmune disease that produces a fast accumulation of skin cells on the skin's surface, resulting in scaling [1]. Psoriasis is mainly diagnosed by clinical examination. Psoriasis comes in various clinical manifestations. Chronic plaque psoriasis is the most prevalent, affecting 80 percent to 90 percent of psoriasis patients. Plaques can develop everywhere on the body, but the most prevalent sites are the scalp, trunk, buttocks, and extremities. Psoriasis can sometimes manifest as an isomorphic reaction, in which new lesions appear on previously healthy skin that has been damaged. The severity of the condition, which is categorized as mild, moderate, or severe, can assist guide therapy [8].

IL-37 was discovered in the year 2000. The five isoforms of IL-37 that have been found so far are IL-37a-e, with IL-37b being the longest and most thoroughly studied [6]. IL-37 has been discovered in the colon, muscle, testis, breast, lung, and others [13]. PCR and subsequent DNA sequencing revealed that IL-37b was the most expressed isoform in human keratinocytes, followed by IL-37. In psoriasis, IL-37 has only been studied in a limited manner. To our knowledge, only two prior researches have looked at the anti-inflammatory properties of IL-37 in psoriasis [7].

In psoriasis, IL-37 has only been studied in a limited number of cases, with mixed outcomes. Using just the immune-fluorescence test, Teng *et al.*, (2014) [7] prove that IL-37 was higher in human LP skin than in healthy control skin, which was related to increasing psoriasis severity was localized to memory CD4+ T cells in the epidermis and macrophages in the dermis. In the current study, IL-37 gene expression was significantly lower in LP skin than in NP skin. IL37 mRNA was also shown to be lower in LP skin than in healthy controls, which is consistent with Rnholt *et al.*'s (2020) findings [14]. In addition, a new study has backed up the current qPCR findings [15] and [16].

IL-37 protects against inflammation in a variety of different ways [17]. Injections of plasmid-coding human IL37 sequence-formulated cationic liposomes treat Keratin 14 vascular endothelial growth factor. Teng *et al.* (2014) [7] dis-

covered that IL-37 has protective properties. IL- 37 gene expression is decreased in LP patients. Because IL-37 is restricted to the stratum-granulosum, psoriasis patients' cytokine levels are likely to be low due to the loss of this layer of the epidermis. Th17-pathway cytokines blocked TNF-a-induced IL-37 overexpression in NHEKs, showing that cytokine signaling is involved [14].

IL-37 gene expression was much lower in psoriatic patients than in healthy people. In contrast, VEGF gene expression was much higher in psoriatic patients than in healthy ones. More importantly, our data indicated a link between IL-37 and disease severity, consistent with previous research [18]. Based on the inflammatory/anti-inflammatory effects of IL-37, it is possible to conclude that such cytokines are related to the regulation of inflammation in psoriasis. According to a growing number of studies, IL-37 inhibits inflammation responses in autoimmune diseases, and it is frequently associated with disease severity [19] and [16]. IL-37 can reduce the symptoms of psoriasis [8] by preventing the production of inflammatory cytokines, including IL-33. In our psoriatic patients, IL-37 gene expression is lower. Still, VEGF gene expression is higher, linked to disease severity should be seen as a compensatory reaction that is insufficient to relieve the disease's inflammatory condition [7]. This link might indicate that compensation begins at the intracellular level and continues non-interfering at gene expression. Because gene expression of the anti-inflammatory marker IL-37 was shown to have favorable correlations with psoriasis severity in our study, it indicates that such a marker might help patients' skin function by counteracting the inflammatory effect of other IL indicators [1].

6. Research Limitation

The IL-37 and VEGF gene expression exhibited poor strength in diagnosing psoriasis utilizing different models in the current investigation. Statistical methods such as discriminant analysis, generalized linear models, and latent profile analysis must be employed to investigate the combinatory influence of such variables in the diagnosis of psoriasis. Furthermore, we should use multivariate ordinal logistics to examine the impact of any variable on psoriasis severity. We suggest further investigations to overcome the IL-37 and VEGF gene expression limitation for psoriasis diagnosis.

7. Conclusion

There is a significant inverse association between IL37 and VEGF in psoriasis patients. Study findings revealed that IL-37 gene expression decreases while VEGF gene expression increases in psoriatic individuals. Such a measurement might be used to determine the severity of the condition, as well as a reflection of the disease's diagnosis.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Dutta, S., Chawla, S. and Kumar, S. (2018) Psoriasis: A Review of Existing Therapies and Recent Advances in Treatment. *Differentiation*, **2**, 10.

[2] Parisi, R., Symmons, D.P., Griffiths, C.E. and Ashcroft, D.M. (2013) Global Epidemiology of Psoriasis: A Systematic Review of Incidence and Prevalence. *Journal of Investigative Dermatology*, **133**, 377-385. https://doi.org/10.1038/jid.2012.339

[3] Krueger, J.G. and Bowcock, A. (2005) Psoriasis Pathophysiology: Current Concepts of Pathogenesis. *Annals of the Rheumatic Diseases*, **64**, ii30-ii36. https://doi.org/10.1136/ard.2004.031120

[4] Tsankov, N., Angelova, I. and Kazandjieva, J. (2000) Drug-Induced Psoriasis. *American Journal of Clinical Dermatology*, **1**, 159-165. https://doi.org/10.2165/00128071-200001030-00003

[5] Parsi, K.K., Brezinski, E. A., Lin, T.C., Li, C.S. and Armstrong, A.W. (2012) Are Patients with Psoriasis Being Screened for Cardiovascular Risk Factors? A Study of Screening Practices and Awareness among Primary Care Physicians and Cardiologists. *Journal of the American Academy of Dermatology*, **67**, 357-362. https://doi.org/10.1016/j.jaad.2011.09.006

[6] Kumar, S., Hanning, C.R., Brigham-Burke, M.R., Rieman, D.J., Lehr, R., Khandekar, S., *et al.* (2002) Interleukin-1F7B (IL-1H4/IL-1F7) Is Processed by Caspase-1 and Mature IL-1F7B binds to the IL-18 Receptor but Does Not Induce IFN-γ Production. *Cytokine*, **18**, 61-71. https://doi.org/10.1006/cyto.2002.0873

[7] Teng, X., Hu, Z., Wei, X., Wang, Z., Guan, T., Liu, N., *et al.* (2014) IL-37 Ameliorates the Inflammatory Process in Psoriasis by Suppressing Proinflammatory Cytokine Production. *The Journal of Immunology*, **192**, 1815-1823. https://doi.org/10.4049/jimmunol.1300047

[8] Papp, K., Gulliver, W., Lynde, C., Poulin, Y. and Ashkenas, J. (2011) Canadian Guidelines for the Management of Plaque Psoriasis: Overview. *Journal of Cutaneous Medicine and Surgery*, **15**, 210-219. https://doi.org/10.2310/7750.2011.10066

[9] Duffy, A.M., Bouchier-Hayes, D.J. and Harmey, J.H. (2004) Vascular Endothelial Growth Factor (VEGF) and Its Role in Non-Endothelial Cells: Autocrine Signalling by VEGF. *VEGF and Cancer*, **2**, 133-144. https://doi.org/10.1007/978-1-4419-9148-5_13

[10] Koch, S. and Claesson-Welsh, L. (2012) Signal Transduction by Vascular Endothelial Growth Factor Receptors. *Cold Spring Harbor Perspectives in Medicine*, **2**, a006502. https://doi.org/10.1101/cshperspect.a006502

[11] Ferrara, N., Gerber, H.P. and Le Couter, J. (2003) The Biology of VEGF and Its Receptors. *Nature Medicine*, **9**, 669-676. https://doi.org/10.1038/nm0603-669

[12] Chan, Y.H. (2003) Biostatistics 102: Quantitative Data-Parametric & Non-Parametric Tests. *Singapore Medical Journal*, **44**, 391-396.

[13] Boraschi, D., Lucchesi, D., Hainzl, S., Leitner, M., Maier, E., Mangelberger, D., *et al.* (2011) IL-37: A New Anti-Inflammatory Cytokine of the IL-1 Family. *European Cytokine Network*, **22**, 127-147. https://doi.org/10.1684/ecn.2011.0288

[14] Nast, A., Smith, C., Spuls, P. I., Avila Valle, G., Bata-Csörgö, Z., Boonen, H., *et al.* (2020) EuroGuiDerm Guideline on the Systemic Treatment of Psoriasis vulgaris–Part 1: Treatment and Monitoring Recommendations. *Journal of the European Academy of Dermatology and Venereology*, **34**, 2461-2498. https://doi.org/10.1111/jdv.16915

[15] Keermann, M., Kõks, S., Reimann, E., Abram, K., Erm, T., Silm, H. and Kingo, K.

(2015) Expression of IL-36 Family Cytokines and IL-37 but Not IL-38 Is Altered in Psoriatic Skin. *Journal of Dermatological Science*, **80**, 150-152. https://doi.org/10.1016/j.jdermsci.2015.08.002

[16] Bulau, A.M., Nold, M.F., Li, S., Nold-Petry, C.A., Fink, M., Mansell, A., *et al.* (2014) Role of Caspase-1 in Nuclear Translocation of IL-37, Release of the Cytokine, and IL-37 Inhibition of innate Immune Responses. *Proceedings of the National Academy of Sciences*, **111**, 2650-2655. https://doi.org/10.1073/pnas.1324140111

[17] Cavalli, G. and Dinarello, C.A. (2018) Suppression of Inflammation and Acquired Immunity by IL-37. *Immunological Reviews*, **281**, 179-190. https://doi.org/10.1111/imr.12605

[18] Sehat, M., Talaei, R., Dadgostar, E., Nikoueinejad, H. and Akbari, H. (2018) Evaluating Serum Levels of IL-33, IL-36, IL-37 and Gene Expression of IL-37 in Patients with Psoriasis Vulgaris. *Iranian Journal of Allergy, Asthma and Immunology*, **17**, 179-187.

[19] Pan, G., Risser, P., Mao, W., Baldwin, D.T., Zhong, A.W., Filvaroff, E., *et al.* (2001) IL-1H, an Interleukin 1-Related Protein that Binds IL-18 Receptor/IL-1Rrp. *Cytokine*, **13**, 1-7. https://doi.org/10.1006/cyto.2000.0799