Original Research Article

Progranulin/Tumor Necrosis Factor-α ratio in Psoriasis Vulgaris

Abstract:

Background and Aim: In psoriasis, the proteins Tumor Necrosis Factor (TNF) and interleukin-6 (IL-6) are overexpressed and play important roles in the disease's progression. For treating psoriasis, biological drugs that target TNF signaling are quite successful. Embryogenesis, tissue repair, cancer, neuronal survival, host defense, and inflammation are all dependent on progranulin (PGRN). By competing with TNF binding to TNFR1/2, PGRN inhibits TNF-mediated signaling pathways and has anti-inflammatory properties in inflammatory arthritis models. The current research seeks to study the relationship between serum PGRN / TNF - α and psoriasis vulgaris activity.

Subjects & Methods: This study's subjects were categorized into 2 groups: group 1 with 35 patients with psoriasis vulgaris, and group 2 of 30 healthy subjects with matched age and sex. The present research was approved by the Ethical Committee of Tanta University, and a written informed consent was collected from all participants.

Results: We discovered a significant increase in PGRN and TNF-α in cases versus control. And we have found a significant decrease in PGRN/TNF-α ratio in cases versus control. We also detected significant differences between the Psoriasis Area Severity Index (PASI) degree and the levels of PGRN and TNF-α, as well as the PGRN/TNF-α ratio. We discovered a negative significant correlation between PASI and PGRN/TNF-α ratio.

Conclusion: Atsttrin exhibited potent anti-inflammatory properties due to its three modified granulin motifs and their associated linker regions. In the treatment of rheumatoid arthritis and contact dermatitis, PGRN and its derivative Atsttrin may be a potential therapeutic drug.

Keywords: Psoriasis Vulgaris, Progranulin, TNF-α, Atsttrin

Introduction

The main cause of psoriasis illness development and progression is uncontrolled inflammatory immune response. In the beginning of inflammatory response, cytokines are important mediators. Cytokines with 19 ligands and 35 receptors have a tumor necrosis factor (TNF). TNF-α is expressed by endothelial and immune cells. Metalloproteinase enzyme cleaves TNF-α and acts through two receptors (TNFR-1 and TNFR-2) [1]. TNF -α is a basic candidate in psoriasis vulgaris's pathogenesis as it's overexpressed in it [2].

Progranulin (PGRN) is an autocrine growth factor for cancer cells and fibroblasts. PGRN inhibits the production of neutrophil attracting chemokine. It is activated by neutrophil elastase enzyme. It has role in resolution of inflammation and wound healing [3]. TNF-α receptor ligand is PGRN. The PGRN inhibits TNF-α -media signal pathways via competition with the TNF-α binding TNFR-l and TNFR-2 [3].

The current research seeks to study the relationship between serum PGRN/ TNF-α and psoriasis vulgaris activity.

Subjects and Methods

This is a case control study in which 35 individuals with Psoriasis vulgaris who were referred to Tanta University Hospitals' Dermatology and Venereology Department were eligible to participate. A control group of 30 healthy people of same sex and age was also included in the research.

The research was authorized by the Tanta University Ethics Committee and all subjects received signed written informed consent. Samples were collected to be analyzed at the Clinical Pathology Department of Tanta University Hospitals. Approval of a New Research Protocol was taken.

The 35 patients with psoriasis included 16 females and 19 males. Their ages were between 23 and 54 years old. The 30 apparently healthy persons included 13 females and 17 males. Their ages were grouped between 28 and 54 years old. The inclusion criteria were composed of patients who were newly diagnosed as psoriasis vulgaris, who didn't receive any medications and didn't have any other systemic or skin diseases. While the exclusion criteria were formed of subjects with other dermatological diseases, other autoimmune diseases, pregnant female and female patients under any hormonal therapy or contraceptive pills, and patients with malignancy were excluded from the study.

Patients were subdivided to 2 groups: Group 1 (Patients group): 35 patients with psoriasis vulgaris. And Group 2 (Control group): 30 healthy subjects with matched age and sex.

All subjects were asked to present personal history with name, age, occupation, residency and special habits. A report on past history with diseases, drug therapy or allergy was also presented. Moreover, general clinical and dermatological examinations were conducted to search for any types of psoriasis and to calculate the Psoriasis Area Severity Index (PASI) score. Furthermore, we have conducted routine laboratory investigations and other specific investigations including detecting PGRN and TNF-α levels using the Enzyme-linked Immunosorbent Assay (ELISA).

For calculating the PASI score, the patient's body was split into four parts (head (H) (10% of a person's skin), arms (A) (20%), trunk (T) (30%), and legs (L) (40 percent). Each of these categories is assessed separately, and the four scores are then added together to get the total PASI. The percentage of skin area affected in each region is calculated and then converted into a grade from 0 to 6: (0: 0% of involved area, 1: < 10% of involved area, 2: 10–29% of involved area, 3: 30–49% of involved area, 4: 50–69% of involved area, 5: 70–89% of involved area, 6: 90–100% of involved area)

Three clinical symptoms are used to determine the severity: erythema (redness), induration (thickness), and desquamation (scaling). Severity parameters are rated on a scale of 0 to 4, with 0 being the least severe and 4 being the most severe. For each part of skin, the total of all three severity criteria is computed, multiplied by the area score for that region, and multiplied by the weight of that section (0.1 for head, 0.2 for arms, 0.3 for body and 0.4 for legs).

a) PGRN Assay procedure:

All reagents brought to room temperature before use (20-25°C). The original standard solution reagent was diluted according to the instructions (400-200-100-50-25 ng/ml). I added prepared samples and standards, as well as antibodies labeled with enzymes, reacting a total of 60 minutes at 37℃ in the plate. Then i washed plate 5 times then I added chromogen A, B. The plate was left for 10 minutes at 37°C. Then i added stop solution, and afterwards, i measured the optical density (OD) value within 10 minutes. The OD of samples and standards was measured at 450 nm against blank. On graph paper a standard curve was built by graphing OD obtained in each vertical (Y) axis standard against the horizontal axis of its ng/ml concentration. The appropriate concentration shown at the standard curve was

determined using the OD value for every sample. Samples with high concentrations were diluted and measured using spectrophotometric plate reader.

b) TNF-α Assay procedure:

All reagents brought to room temperature before use (20-25°C). The original standard solution reagent was diluted according to the instructions (640-320-160-80-40 ng/ml). I added prepared samples and standards, in addition to antibodies labeled with enzymes, reacting a total of 60 minutes at 37°C in the plate. Then i washed Plate 5 times then I added the chromogen A, B. The plate was left for 10 minutes at 37°C. Afterwards, i added stop solution, and then I measured the OD value within 10 minutes.

The OD of samples and standards was measured at 450 nm against blank. A standard graphic paper curve was created by tracing the OD of the vertical axis (Y) with its concentration in ng-ml on the horizontal axis from every standard. For each sample the OD value was utilised to calculate the appropriate standard curve concentration. Samples with high concentrations were diluted and measured using spectrophotometric plate reader.

Statistical Analysis

The information was presented as a standard mean deviation (SD). For statistical analysis, the SPSS software (version 22) was utilized. In Mann-Whitney or Student's t-test, variations were examined. In Spearman rank correlation, the relationships between PGRN and other continuous variables were analyzed. At a $P < 005$ level, statistical significance was determined.

Results

Table 1 shows Distribution of studied patients based on in patients' group

Table 1: Distribution of studied patients based on in patients' group

Type of psoriasis	No. (=35)	%
Plaque psoriasis	26	74.3
Erythroderma	3	8.6
Flexural (inverse) psoriasis	1	2.9
Palmoplantar pustulosis	5	14.3

Table 2 shows a significant rise in PGRN and TNF-α levels and a significant decline in PGRN/TNF-α ratio in patients versus control (p value <0.001).

Table 2: Comparing between the two studied groups regarding progranulin, TNF-α levels and Progranulin/Tumor Necrosis Factor-α Ratio

Parameter		Patients (n = 35)	Control (n = 30)	t	p
PGRN level (ng/ml)	Range	40.0 – 89.0	20.0 – 47.0	10.861*	<0.001*
	Mean ± SD.	63.83 ± 15.02	33.73 ± 6.07		
TNF-α levels (pg/ml)	Range	8.0 – 20.0	2.40 – 4.20	14.046*	<0.001*
	Mean ± SD.	13.63 ± 4.24	3.44 ± 0.58		
PGRN/TNF-α ratio	Range	3.70 – 6.80	4.70 – 16.70	U= 993.0*	<0.001*
	Mean ± SD.	4.78 ± 0.63	10.13 ± 2.75		

*significant as P value <0.05, Progranulin (PGRN), Tumor Necrosis Factor-Alpha (TNF-α), T: Student's T test, U: Mann-Whitney test

Table 3 shows a positive significant correlation among PASI score and the levels of PGRN and TNF-α and a negative significant correlation between PASI score and PGRN/TNF-α Ratio.

Table 3: Relation between PASI score and the levels of PGRN and TNF-α in patients' group

Parameter		PASI			Test	p
		Mild (n = 14)	Moderate (n = 11)	Severe (n = 10)		
PGRN level (ng/ml)	Range	40.0 – 55.0	60.0 – 75.0	76.0 – 89.0	150.329*	<0.001*
	Mean ± SD.	48.36 ± 5.31	66.55 ± 3.70	82.60 ± 5.13		
	Sig. bet. Grps	p_1<0.001*	p_2<0.001*	p_3<0.001*		
TNF-α level (pg/ml)	Range	8.0 – 11.0	12.0 – 17.0	18.0 – 20.0	226.17*	<0.001*
	Mean ± SD.	9.50 ± 0.94	13.64 ± 1.57	19.40 ± 0.70		
PGRN/TNF-α Ratio	Range	4.50 – 6.80	3.80 – 5.50	3.80 – 4.70	14.226*	0.001*
	Mean ± SD.	5.09 ± 0.59	4.89 ± 0.54	4.22 ± 0.34		
	Sig. bet. Grps	p_1=0.653	p_2<0.001*	p_3=0.003*		

*significant as P value <0.05. Progranulin (PGRN), Tumor Necrosis Factor-Alpha (TNF-α)

Table 4 and Figure 1 show negative significant correlation among PASI score and PGRN / TNF-α Ratio.

Table 4: Correlation between PASI score and PGRN/TNF-α Ratio in patients' group

	PASI	
	r_s	P
TNF-α level (pg/ml)	0.889	<0.001*
PGRN level (ng/ml)	0.867	<0.001*
PGRN/TNF-α Ratio	-0.472	0.004*

* Progranulin (PGRN), Tumor Necrosis Factor-Alpha (TNF-α), Psoriasis Area Severity Index (PASI)

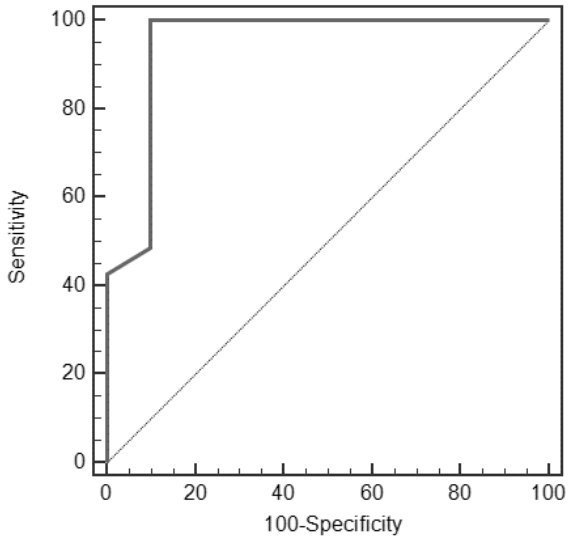

Figure 1: Receiver Operating Characteristic (ROC) Curve for PGRN/TNG-α Ratio

Table 5 shows a sum of different parameters on the levels of PGRN/TNG-α Ratio, PGRN, and TNG-α with sensitivity, specificity as well as PPV8 and NPPV.

Table 5: Agreement (sensitivity, specificity) for PGRN/TNF-α Ratio

	Cutoff	**Sensitivity**	**Specificity**	**PPV**	**NPV**
PGRN/TNF-α Ratio	**≤6.8**	100.0	90.0	92.1	100.0
TNF-α level (pg/ml)	**>4.2**	100.0	100.0	100.0	100.0
PGRN level (ng/ml)	**>40**	94.29	93.33	94.3	93.3

Discussion

Psoriasis is reported to afflict about 3% of world's population as chronic inflammatory skin condition mediated by the immune system. The presence of red scaly plaques with many dense immune cell infiltration that may generate several cytokines and inflammatory chemicals is known as psoriasis in the clinical context [4].

Increased cytokines and chemokines pro-inflammatory were characteristic of psoriasis lesions. Incredible cytokines are said to be produced by psoriasis keratinocytes (e.g., IL-1, IL-6, IL-8, IL-1β, IFN-β, and T NF-α). Therefore, keratinocytes are often utilised to investigate in vitro psoriasis inflammatory mechanisms. Prograanulin (PGRN) is a glycoprotein of 576 human amino acids, commonly known as proepithelin, granuline/epitheline precursor [5].

Our findings go with Huang et al. [6] as founded also that two groups were matched regarding age and sex. No significant differences among psoriatic patients and gender and age control individuals were found [7]. Our findings agree with others who discovered no statistically significant variations among patients and controls as regards gender, age, waist circumference and Body mass index (BMI) [8].

Our findings on PGRN levels are consistent with those published by Huang et al [6], who used ELISA to measure blood levels of PGRN in 34 patients suffering psoriasis vulgaris and 20 normal subjects. Serum PGRN levels in psoriasis vulgaris patients were substantially greater than in healthy controls (p value < 0·01).

Other studies looked examined PGRN in lesional skin and discovered that it was substantially higher in lesional skin from psoriasis patients than in non-lesional skin from patients suffering psoriasis and biopsies from normal subjects [5].

In another study they found that serum PGRN level in psoriasis patients was significantly higher than in controls (p value <0.033) [8]. These results were in accordance with that reported in this work.

Regarding TNF-α results, our findings were consistent with Huang et al. [6] who revealed that the levels of TNF-α in psoriasis vulgaris patients were up-regulated significantly compared to levels in the healthy controls (p value < 0·05). And Aadil et al., 2018 also showed that TNF-α serum level in psoriasis patients were significantly higher (225±22.82 pg/ml) in comparison to controls (69.80±10.02 pg/ml). Statistically significant differences were observed after data analysis (P =0.02).

Also, our findings agree with Kyriakou et al., 2014 that showed that the median serum levels of TNF-α were significantly elevated in psoriatic patients than in healthy controls.

Another study found that TNF-α serum levels in psoriatic patients were extremely significantly high (3.98 ± 0.65) and (6.85 ± 1.12) pg/ml, respectively (P = 0.001) [9].

However, there was no differences found in TNF-α levels between patients and healthy controls. This disagreement may be attributed to the variety of the inclusion criterion and research participants. Yet On the other hand, the variation in severity level (PASI) was shown to be substantially associated with serum TNF- level, suggesting that these cytokines may play a role in illness severity [10].

Regarding PGRN/TNF-α ratio the present research discovered a significant decline in patients versus control (p value< 0.001). These were consistent with Huang et al., 2015 [6].

Concerning disease activity and PASI score, our results agreed with El Ashry et al., 2014 who discovered a positive significant correlation among PGRN level and PASI score.

Other researchers showed that serum PGRN level in psoriasis patients was significantly elevated in patients suffering severe psoriasis than patients having mild and moderate psoriasis (P<0.001). They also discovered that the concentrations of serum PGRN are at positive correlation with PASI score [8].

However, some studies showed that serum PGRN levels was high in psoriasis vulgaris patients prior to treatment, then the level was down-regulated after treatment (p value < 0·005). Furthermore, a positive correlation was detected among the serum PGRN and TNF-α levels in the pre-treatment of patients suffering from psoriasis vulgaris [6].

Regarding clinical picture of the patients, PASI score, our results were in accordance with that found by Elfarargy et al., 2014 who showed a positive significant correlation among the TNF-α serum levels and disease severity. This, also in consistent with Aadil et al., 2018 as revealed significant correlation among the TNF-α serum level and disease severity [11].

These results may indicate the PGRN role in psoriasis pathogenesis. This finding may reveal that PGRN stands as a protective and anti-inflammatory factor in psoriasis development.

Regarding the negative significant correlation amongst PASI and PGRN/TNF-α ratio, we discovered the same as Huang et al., 2015 who showed that the serum PGRN/TNF-α ratio had negatively correlated with PASI scores.

Furthermore, injection of recombinant human PGRN or a recombinant PGRN derivative known as (Atsttrin) showed significant anti-inflammatory effects. PGRN and its derivative Atsttrin may hold promise as a therapy for rheumatoid arthritis and contact dermatitis [12].

Conclusion

This study gave more lights on levels of PGRN and TNF-α in psoriasis that may aid in understanding the disease's pathogenesis. Also, the ratio of PGRN and TNF-α declared the association of clinical severity of the disease with its pathogenesis. Recombinant PGRN and its derivative Atsttrin may be a potential therapeutic agent for therapy of RA and contact dermatitis.

References

1. Bathon JM, Martin RW, Fleischmann RM, Tesser JR, Schiff MH, Keystone EC, et al. A comparison of etanercept and methotrexate in patients with early rheumatoid arthritis. N Engl J Med. 2000;343:1586-93.

2. Jian J, Li G, Hettinghouse A, Liu C. Progranulin: a key player in autoimmune diseases. J Cytokine. 2016;101:48-55.

3. Tang W, Lu Y, Tian QY, Zhang Y, Guo FJ, Liu GY, et al. The growth factor progranulin binds to TNF receptors and is therapeutic against inflammatory arthritis in mice. Science. 2011;332:478-84.

4. Rendon A, Schäkel K. Psoriasis Pathogenesis and Treatment. Int J Mol Sci. 2019;20.

5. Tian R, Li Y, Yao X. PGRN Suppresses Inflammation and Promotes Autophagy in Keratinocytes Through the Wnt/β-Catenin Signaling Pathway. Inflammation. 2016;39:1387-94.

6. Huang K, Chen A, Zhang X, Song Z, Xu H, Cao J, et al. Progranulin is preferentially expressed in patients with psoriasis vulgaris and protects mice from psoriasis-like skin inflammation. Immunology. 2015;145:279-87.

7. Kyriakou A, Patsatsi A, Vyzantiadis TA, Sotiriadis D. Serum levels of TNF-α, IL-12/23p40, and IL-17 in plaque psoriasis and their correlation with disease severity. J Immunol Res. 2014;2014:467541.

8. Gaber RMA, Hefny RM, El Din TMK, Sotohy RS, Moez SIA. Serum level of progranulin in patients with psoriasis and its relation to disease severity. Al-Azhar Assiut Med J. 2020;18:359.

9. Elfarargy S, Farag AG, Azmy RM, Abdullah MS. Study of tumor necrosis factor-α-308 promoter polymorphism in psoriasis vulgaris. Journal of the Egyptian Women's Dermatologic Society. 2014;11:117-22.

10. Jacob SE, Nassiri M, Kerdel FA, Vincek V. Simultaneous measurement of multiple Th1 and Th2 serum cytokines in psoriasis and correlation with disease severity. Mediators Inflamm. 2003;12:309-13.
11. Aadil W, Ganai BA, Akhtar T, Narang T, Kaur R. Association of proinflammatory cytokine tumor necrosis factor-α gene promoter-308 and-238 polymorphism with psoriasis in North Indian population. Egyptian Journal of Dermatology and Venerology. 2018;38:12.
12. Wang BC, Liu H, Talwar A, Jian J. New discovery rarely runs smooth: an update on progranulin/TNFR interactions. Protein & cell. 2015;6:792-803.