Serum and Tissue Granulysin as a Possible Key Markers to Detect the Severity of Psoriasis

Hisham Diab Gaber (Hishamdiabg@yahoo.com)
Assiut University
Radwa M. Bakr
Assiut University
Tarek Taha ElMelegy
Assiut University
Yasmin Sayed Ahmed
Assiut University
Reham M. Abdel Gaber
Assiut University

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Abstract

Background: Psoriasis is a chronic immune-mediated inflammatory skin disease, it is a disorder of both the innate and adaptive immune system. However, new updates are still emerging in its pathogenesis. Psoriasis is characterized by overexpression of antimicrobial peptides. Granulysin (GNLY) is an antimicrobial peptide that may have a role in psoriasis pathogenesis.

Objective: To detect the level of serum and tissue (GNLY) in psoriatic patients and correlate it with psoriasis severity.

Patients and Methods: The study was performed on 50 individuals, including 2 groups. The first group included 30 psoriasis vulgaris patients and the second group included 20 age and sex matched apparently healthy control individuals. Serum GNLY was determined in all individuals and tissue GNLY was determined in the skin of 8 patients (from lesional and perilesional skin) and 8 controls.

Results: serum GNLY was slightly higher in patients group compared to control group with no statistically significant difference while, the level of tissue GNLY of lesional and perilesional skin in psoriatic patients, was significantly higher when compared to control group.

Conclusion: In conclusion, granulysin is supposed to play a role in psoriasis pathogenesis and it is positively correlated with psoriasis severity

Introduction

Psoriasis is a chronic, noncontagious, inflammatory skin disorder, which most commonly manifests itself on the elbows, knees, and scalp. There is a genetic predisposition for this illness .[1] Multiple types of psoriasis are identified, with plaque-type psoriasis which is known as the most common type which usually presents with erythematous plaques that are covered with silvery-white scales.[2]

The pathogenesis of psoriasis involves interactions between different cell types and numerous cytokines in response to triggers in genetically predisposed individuals leading to activation of T cells and their migration into the skin. In addition to keratinocyte proliferation, [3] there are many potential triggers of psoriasis as infections and psychological distress .[4]

Psoriatic lesions are densely infiltrated by T cells and dendritic cells (DC). T helper (Th)1 cells secret cytokines, such as tumor necrosis factor-alpha (TNFα), interferon γ (IFN γ), and interleukin (IL)-12, Th17 cells secret IL-17 and IL-22. IL -22 stimulates epidermal proliferation, while IL-17 is responsible for the release of proinflammatory cytokines, antimicrobial peptides, and chemokines.[5] psoriasis is characterized by overexpression of antimicrobial peptides (AMPs), and this may be interpreted as a sign of activation of the innate immune system and present a link between adaptive and innate immune response. [6]

Granulysin (GNLY) is a cytolytic and proinflammatory peptide that belongs to a saposin-like family, lipid binding AMPs, and is localized in the granular compartments of cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells .[7]

GNLY is well associated with different activities of NK cells and CTL in physiological and pathological settings and could be a useful serum marker for monitoring host cell mediated immune cytotoxic responses. GNLY was found at minimal concentrations in the sera of healthy individuals.[8]

This study aims to detect the level of serum and tissue (GNLY) in psoriatic patients comparing it with healthy controls and correlating it with psoriasis severity.

Patients And Methods

Study design

A Case control study that was approved by the Institutional Ethics and Research Committee of faculty of Medicine, Assiut University, Assiut, Egypt. IRB no: 17100446 .

Clinical trial registration number is : (ClinicalTrials.gov Identifier: NCT03469219)

Patients and methods

A total of 50 individuals divided into 2 groups, the first group included 30 psoriatic patients recruited from the outpatient clinic of Dermatology and Andrology Department of Assiut University Hospital from April 2018 to October 2019 and the second group included 20 age and sex matched apparently healthy individuals were enrolled in the study as a control group. All subjects gave informed consent before being included in the study.

Inclusion criteria:

Patients with clinically typical psoriasis vulgaris lesions of different ages and sex, with different degrees of severity, were included.

Exclusion criteria:

Pregnant and lactating women, patients who received systemic medical treatment in the last one month, and patients with associated diseases reported increasing release of granulysin whether systemic e.g (infection, cancer, organ transplantation, autoimmune disease) or skin disease e.g (lichen planus, Steven Johnson syndrome, toxic epidermal necrolysis) were excluded.

All patients included in the study were subjected to :

1- Complete history taking.

2- General and local examination.

3- Grading of the disease severity according to the Psoriasis Area and Severity Index (PASI). This combines assessment of the area of involvement (%), the severity of scaling, plaque thickness, and erythema using a scale of 1-4. The PASI score varies from 0 to 72. Higher scores indicate severer conditions PASI more than 12 defines as severe, PASI 7–12 moderate and PASI less than 7 mild chronic plaque-type psoriasis.

4- Dermatological quality of life index (DLQI): The Arabic version was used to assess the affection of a patient's quality of life by psoriasis.

5- Measuring granulysin level in serum:

Blood samples were collected under aseptic conditions from 30 psoriasis vulgaris patients and 20 control subjects into Wasserman tubes. Tubes were then, centrifuged at 3000 rpm for 10 minutes to separate sera. Sera were stored at -30°C for later use to be analyzed by ELISA.

6- Measuring granulysin level in tissue extracts:

Punch skin biopsies (3mm) of both lesional and peri-lesional skin (1cm apart) were taken from 8 patients. Also, 8 skin biopsies were taken from normal skin excised from subjects undergoing plastic surgeries as control tissue samples. Skin biopsies were soaked into 1.5ml polypropylene tubes containing 50µl of Lysis Buffer AL (cat. no. 19075, Qiagen GmbH, Germany). Protein was extracted from skin biopsies by sonication using an ultrasonic cell disruptor (model soniprep150, United Kingdom), polypropylene tube was surrounded by ice then introduced into the device for 7 cycles 10 seconds each, 23Hz, amplitude 14-18 micron. Then, polypropylene tubes were centrifuged at 1400 rpm for 3 min. The supernatant was transferred into a new polypropylene tube and stored at -30°C for later use to be analyzed by ELISA.

Measurement of granulysin level in sera and tissue extracts was done using Human GNLY (Granulysin) ELISA Kit (cat. no. EH0156, Fine Test, China). This was performed according to the manufacturer's instructions in the Laboratory of Clinical Immunology, Clinical Pathology Department, Assiut University Hospital.

Data entry analysis was done using SPSS Version 24 (Armonk, NY: IBM Corporation). Data were represented as number, percentage, mean, median, range, and standard deviation. Pearson correlation, Independent samples t-test was used to compare quantitative variables between two groups. In case of non-parametric data, Mann-Whitney test was done to compare quantitative variables between two groups. The level of significance was at a p value less than 0.05.

Results

Demographic and clinical characteristics of the participants

The age of our psoriasis patients ranged from 17 to 62 years old with a mean ± standard deviation (SD) (41.9 ± 14.53) and regarding sex 30% (9 patients) were females while 70% (21 patients) were males. The control group age ranged from 17 to 56 years old with a mean ± SD (39.05 ± 11.53) and regarding the sex 40% (8 individuals) were females while 60% (12 individuals) were males.

The duration of psoriasis ranged from 1 year to 36 years with a mean ± SD (11.6 ± 9.3). Regarding psoriasis severity, PASI score ranged from 1.20 to 11.60 with a mean ± SD (5.5 ± 2.63), 21 patients (70%) were of mild severity and 9 patients (30 %) were of moderate severity and there was no severe cases. The quality of life of patients presented by DLQI ranged from 7.0 to 19.0 with a mean ± SD (11.8 ± 3.4) which means that it is greatly affected by the disease.

Serum granulysin (SG) level

Serum granulysin was slightly higher in the patients group ranging from 0.1 to 1 ng/ml with a mean ± SD (0.2± 0.2) than in the control group ranging from 0.1 to 0.4 ng/ml with a mean ± SD (0.1± 0.1), with no statistical difference between them (table 1).

Table (1): Serum granulysin level in patients and control group.

Serum Granulysin (ng/ml)	**Patients**	**Control**	P value
Mean ± SD	0.2 ± 0.2	0.1 ± 0.1	0.291
Median	0.2	0.1	
Range	0.1 – 1	0.1 - 0.4	

SG: serum granulysin SD: standard deviation

Tissue granulysin (TG)level

The level of tissue granulysin was statistically higher in lesional skin and perilesional skin of psoriatic patients compared to the control group (with p values 0.017 and 0.011, respectively), however there was no significant statistical difference between lesional and perilesional tissue granulysin levels (table 2), (figure 1).

Table (2): Tissue Granulysin in patients and control groups:

Tissue Granulysin (ng/ml)	**Lesional (TG patient)**	**Perilesional (PTG)**	**Control (TG control)**	**p-value**
Mean ± SD	0.68 ± 0.24	0.70 ± 0.24	0.43 ± 0.1	P1= 0.838
Range	0.31 - 1.05	0.39 - 1.14	0.24 - 0.54	P2= 0.017*
Median	0.65	0.70	0.44	P3= 0.011*

SD: Standard Deviation

independent samples t test *significant p value <0.05

P1: p value between lesional and perilesional tissue granulysin

P2: p value between lesional tissue garnulysin and control

P3: p value between perilesional tissue granulysin and control

There was a strong positive correlation between lesional tissue granulysin and PASI score, there was no significant correlation between PASI score and serum granulysin, perilesional tissue granulysin ,or DLQI (table 3), (figure 2).

Table (3): Showing the correlations between PASI and other variables.

Variables	**PASI**	
	r-value	**p-value**
Serum Granulysin	-0.037	0.845
Lesional tissue granulysin	0.898**	0.002*
Perilesional tissue granulysin	0.456	0.256
DLQI	-0.014	0.941

PASI: Psoriasis Area And Severity Index

pearson correlation test *significant p value <0.05

DLQI: Dermatology Life Quality Index

*significant p value <0.05

There was no significant correlation between serum granulysin and tissue granulysin.

There was a significant statistically negative relationship between lesional tissue granulysin and Disease duration.

Discussion

Psoriasis is a chronic, immune-mediated inflammatory skin disease .[8] Despite the presence of many studies on the aetiopathogenesis of psoriasis, it is not clear yet. Until now, many indicators that may bring more clarity to the progress of the disease have been studied but none are directly related to it. Many indicators of the disease severity have been studied but until now none have been directly related to it.

GNLY is an antimicrobial peptide that acts as a chemoattractant and proinflammatory activator.[10] It is expressed in activated CD4 + and CD8 + T cells and NK cells as a cytolytic granule protein.[9] Few studies analyzed the GNLY level which is thought to be an indicator of cytotoxicity in psoriasis.

In the present study, the level of Serum GNLY was measured using ELISA technique in 30 patients and 20 control individuals where it was slightly higher in the patients group when compared to the control group, however, there was no statistical difference between them (p = 0.291). Ayvaz & Baysak [11] detected similar results when they measured serum GNLY level in 40 psoriatic patients using ELISA where there was no significant difference between psoriasis patients and the control group (p = 0.243). However, Vičić et al. [12] detected a significant increase in the mean frequency of GNLY expressing peripheral blood lymphocytes, in patients with severe psoriasis compared to mild disease and healthy individuals, when they used direct immunofluorescence staining followed by flow cytometry analysis. The difference in this result could be attributed to the different techniques used in the two studies or may be due to the difference in disease severity of the studied group as half of them had severe psoriasis according to PASI score. Also, Massari et al. [13] observed a significant increase in the mean fluorescence intensity of GNLY in peripheral blood lymphocytes in psoriatic arthritis patients when compared to healthy control, it is well known that psoriatic arthritis is considered a severe form of psoriasis.

Regarding tissue GNLY our study revealed that the level of tissue GNLY of lesional and perilesional skin in psoriatic patients, was significantly higher when compared to the control group (p value 0.017 and 0.011, respectively). This was consistent with a study done by Mahgoub et al. [7] which measured GNLY by ELISA technique and showed a significant elevation of its level in lesional psoriatic tissue when compared to the control group (P = 0.001), also consistent with Elgarhy et al. [6] and Vičić et al. [12] studies which detected a highly significant difference in GNLY expression in lesional tissue in psoriasis patients when compared to the control group.

GNLY has become a point of interest more for its antimicrobial activity in several chronic inflammatory skin diseases. Raychaudhuri et al. [14] measured the level of GNLY in skin biopsies of psoriatic patients, normal control individuals, patients with atopic dermatitis (AD), and eczema. They observed an increased number of GNLY + T cells in psoriasis, where secondary bacterial infection is extremely rare, very few

GNLY + T cells were observed in AD and nummular eczema, two conditions frequently associated with gram positive bacterial infections.

Our study showed no statistically significant correlation between serum granulysin and tissue GNLY levels in either lesional or perilesional tissue (p value was 0.319 and 0.464, respectively).

There was a significant positive correlation between lesional GNLY level and PASI score (p = 0.002). This was in agreement with Mahgoub et al. [7] and Elgarhy et al. [6] studies.

In the present study, there was no significant correlation between serum GNLY and PASI score (p = 0.845). In agreement with our study Ayvaz & Baysak [11], didn't detect any significant correlation between serum GNLY level (using ELISA) of psoriasis patients and PASI score. However, Vičić et al. [12] detected a significant positive correlation between GNLY expression (using flow cytometry) and PASI score ($p < 0.01$), the difference in the result could be attributed to the elevated level of granulysin expression in peripheral blood of psoriatic patients detected by Vičić et al. [12]. Our study detected a significant negative correlation between lesional tissue GNLY and disease duration (p = 0.048). However, Mahgoub et al. [7] didn't find a statistically significant correlation between the granulysin levels in the skin biopsies of the patients and disease duration (P = 0.330).

Serum GNLY levels and immunohistochemical GNLY expression were analyzed in patients with skin diseases other than psoriasis as alopecia areata (AA) as in a study done by Ono et al. [15] and as a result, the serum GNLY levels were stated to be a cytotoxicity indicator that can demonstrate the disease activity and prognosis in acute illness. The cytotoxic effect of GNLY has also been implicated in basal keratinocytes apoptosis in lichen planus lesions, increases in GNLY expression in tissue and blood also reported in various clinical conditions such as acute rejection and steroid resistance in human renal transplantation and graft-versus-host reaction [12]. So serum GNLY may be a promising marker of cytotoxicity in various diseases, however, more experiments on its cytotoxic effect on psoriasis patients are needed for better insight into the role of GNLY in the immunologic events in psoriasis.

Conclusion

In conclusion, granulysin is suggested to play a role in psoriasis pathogenesis when measured in tissue and it is positively correlated to disease severity. However, its measurement in serum need further studies on larger population with different disease severity to be assessed.

Declarations

Financial support: None.

Ethical Approval: This study was approved by the Ethical Committee, Faculty of Medicine, Assiut University. IRB no: 17100446 .

Disclosure of interest: The authors report no conflict of interest.

Patient consent: All participants gave informed consent before being included in the study

Data availability statement: The data supporting the results of analyses presented in the paper is available with the corresponding author whenever needed.

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Figures

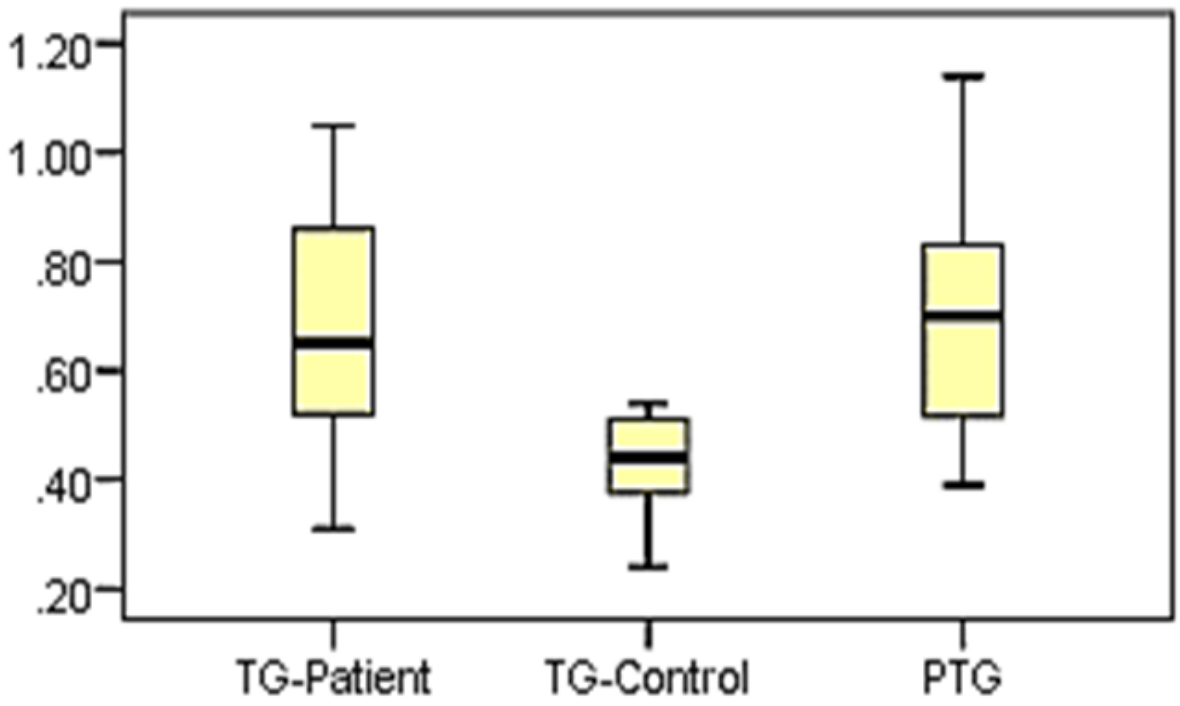

Figure 1

Tissue granulysin level patients and control groups.

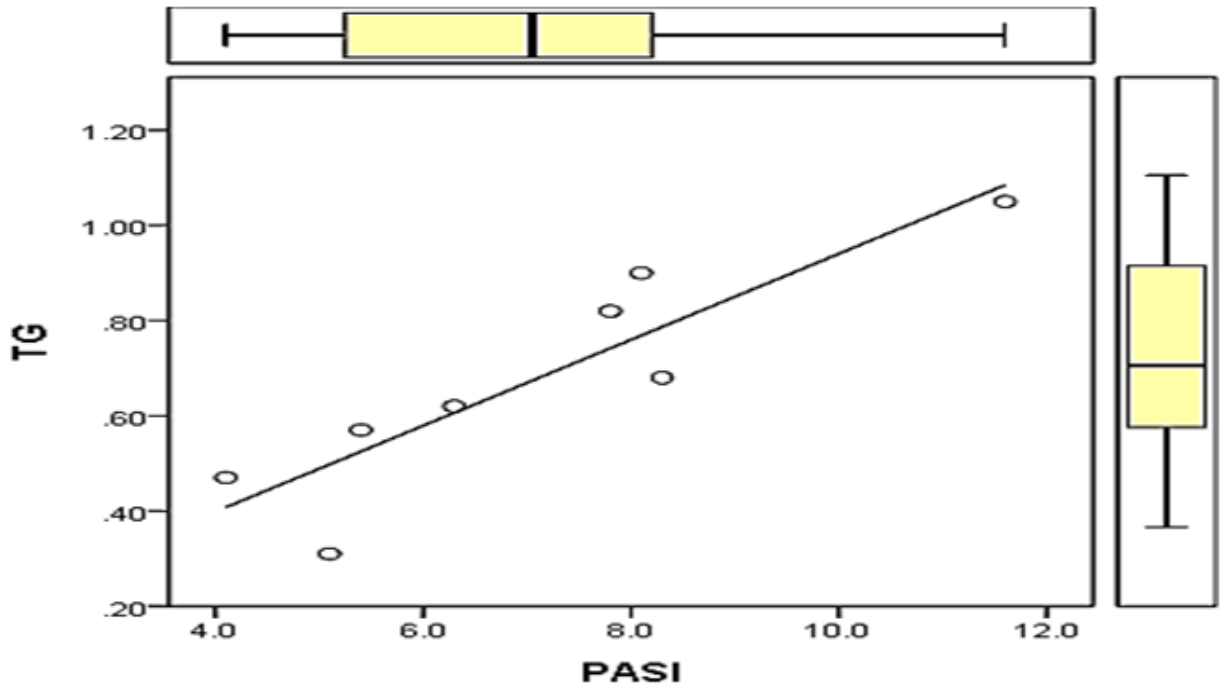

Figure 2

Correlation between PASI and lesional tissue Granulysin (TG).