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ORIGINAL ARTICLE

Inflammasome NLRP3 Expression in PBMCs and Plasma Interleukin-21 in Psoriasis

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ABSTRACT

Background: *Psoriasis is a persistent, immune-related complex condition that predominantly impacts both the skin and joints, involving multiple factors.* ***Objectives:*** *Our study aimed to make a comparison between the PBMCs expression levels of NLRP3 and plasma IL-21 levels in psoriatic patients with that of healthy controls and a correlation between these levels and the severity of psoriasis.* ***Methodology:*** *This observational cross-sectional study was performed on 40 psoriatic patients and 40 healthy individuals who served as the control group. We assessed the expression levels of NLRP3 in PBMCs using real-time reverse transcription-polymerase chain reaction (RT-PCR), while plasma levels of IL-21 were determined using the enzyme-linked immunosorbent (ELISA) technique.* ***Results:*** *Psoriatic patients showed significantly higher PBMCs expression levels of NLRP3 (P< 0.001), and insignificantly different plasma IL-21 levels from those in the control group. The expression levels of NLRP3 demonstrated a significant positive correlation with the severity of psoriasis (P< 0.001). In contrast, there was no significant correlation detected between IL-21 levels and the severity of psoriasis. Additionally, no significant correlation was observed between NLRP3 and IL-21 levels.* ***Conclusions:*** *NLRP3 could be a potential diagnostic and prognostic biomarker in psoriasis diagnosis with 92.5% sensitivity and 90% specificity and a promising therapeutic target. Additional studies are warranted to examine the exact contribution of IL-21 to the pathogenesis of psoriasis.*

INTRODUCTION

Psoriasis is a chronic autoimmune genetic disease that manifests with scaly erythematous plaques on the skin, impacting approximately 2% of the global population[1]. Despite continuous research on immunopathology of psoriasis, the disease pathogenesis remains elusive [2].

The inflammasome complex is an intracellular multimeric protein complex which is regarded as a crucial element of the innate immune system. The inflammasome complex contains a sensor pattern recognition receptor (PRR), an adaptor protein and the effector enzyme "caspase-1", that catalyzes a cellular reaction designed to defend against an immediate threat by releasing cytokines and triggering cell death.[3,4]. Among the Nucleotide-binding and oligomerization domain like receptors (NLRs), The NLR protein 3 (NLRP3) inflammasome has been the most extensively researched. Despite its critical role in eliciting innate immune responses, NLRP3 has been suggested to contribute to various inflammatory diseases, including psoriasis. The NLRP3 inflammasome is recognized for its role in regulating the processing of interleukin-1β (IL-1β) caspase-1 mediation. However, studies on the underlying molecular mechanisms are deficient [5].

Peripheral blood mononuclear cells (PBMCs) represent an intact source of many prognostic and diagnostic biomarkers in a variety of diseases [6]. Immune responses are significantly higher when using isolated PBMCs than when using whole blood. NLRP3 exhibits its highest expression levels in circulating macrophages and dendritic cells [7].

Interleukin-21 (IL-21), a versatile cytokine primarily synthesized by T-helper17 (Th17) cells and T follicular helper (Tfh) cells, has drawn interest for its potent regulatory influence on a variety of immune cells. The physiological presence of the IL-21 receptor (IL-21R) on keratinocytes implies its potential involvement in inflammatory skin condition. Psoriasis is attributed to mediators within the Th17/IL-23 pathway. Contrary to IL-17 or IL-23, the precise role of IL-21 in psoriasis remains unclear, even though it is acknowledged to be involved in the pathogenesis of the condition[8]. In the same vein, previous studies showed growing evidence that NLRP3 inflammasome expression in psoriatic lesions plays an important role and correlates meaningfully with the duration of the disease and clinical severity. However, the accurate role of inflammasomes in the development of both psoriasis and psoriatic arthritis (PsA) is still undefined[9]. Additionally, IL-1β has been identified as a key player

in the pathogenesis of psoriasis. Elevated levels of both IL-1β and its activating enzyme caspase-1 are observed in psoriatic skin lesions when compared to healthy controls. IL-1β has been shown to play an important role in the pathogenesis of psoriasis. Excessive IL-1β signalling triggers a Th17 cytokine profile and induces a phenotype like psoriasis in mice. Studies using preclinical skin disease models have strongly suggested the crucial involvement of IL-21 in inflammatory and autoimmune cutaneous disorders [8]. In a human psoriasis xenograft severe combined immunodeficiency (SCID) model, the use of an IL-21 antagonist resulted in a decrease in epidermal thickness, supporting the pathogenic role of IL-21 in psoriasis [10].

The objective of our study was to compare the PBMCs expression levels of NLRP3 and plasma IL-21 levels in psoriatic patients with those of healthy controls and to correlate between these levels and the severity of psoriasis.

METHODOLOGY

This observational cross-sectional study was conducted from November 2021 to August 2022. A total number of 40 psoriatic patients aged from16 to 65 years and 40 healthy individuals aged from 14 to 62, who attended the Outpatient Clinic of either the Dermatology Department, Cairo University Hospitals or El Hod El Marsood Hospital. Approval for this research was obtained from the Research Ethics Committee of the Institutional Review Board (code MD-330-2021), Faculty of Medicine, Cairo University. The enrolled patients were categorized based on the Psoriasis Area and Severity Index (PASI) score [11], and they refrained from topical treatment for four weeks prior to the PASI score evaluation**.**

Detection of inflammasome NLRP3 expression levels by quantitative reverse transcription-polymerase chain reaction (qRT-PCR):

Total RNA was isolated from PBMCs using the RNeasy Mini® kit **(Qiagen, Valencia, CA, USA)** following the manufacturer's guidelines. Quantitative reverse transcription real-time PCR **(One step, Clinilab, USA)** was conducted on the extracted RNA to determine the expression levels of inflammasome NLRP3. This analysis utilized a reverse transcription kit and SYBR green master mix provided by Applied Biosystems USA. The relative expression of inflammasome NLRP3 was determined using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as the house keeping gene (HKG) [12].

Purification of RNA from PBMCs:

Total RNA was extracted from each sample using RNeasy Mini® kit and protocol according to the manufacturer's instructions **(Qiagen, Valencia, CA, USA)**. Assessment of the quality and concentration of the isolated RNA was performed by Nanodrop Spectrophotometer **(ScanDrop, Delta, Germany)** which was used to measure the purity of the RNA by calculating the ratio of A260/A280. A ratio of 1.8–2.1 was acceptable.

cDNA synthesis by RT-PCR:

The total RNA (1µg) was employed for cDNA conversion using a high-capacity cDNA reverse transcription kit **(Thermo Fisher Scientific, USA)**.

Real-time qPCR of inflammasome NLRP3 gene expression using SYBR Green I:

Conducted utilizing an Applied Biosystem with software version 3.1 **(StepOne™, USA)**. The qPCR assay with the primer sets was optimized at the annealing temperature. The samples encompassed all cDNA, including those prepared earlier for inflammasome NLRP3 gene expression, an internal control (for GAPDH gene expression serving as a housekeeping gene -HKG), and a non-template control. (water to confirm the absence of DNA contamination in the reaction mixture); all were prepared in duplicate.

Detection of Interleukin-21 levels by enzyme linked immune-sorbent assay (ELISA):

Quantitative detection of plasma levels of IL-21 was executed using the Human IL-21 ELISA Kit **(Thermo Fisher Scientific, USA)** in both patients and controls following the manufacturer's instructions. The optical density (OD) readings were taken at wavelength of 450 nm using a Micro ELISA auto reader State Fax-2100 **(GMI, Germany)**.

Statistical analysis:

Statistical analysis using SPSS v28 **(IBM Corp., Armonk, NY, USA)** included presenting quantitative variables as mean and SD, compared with the unpaired Student's t-test. Qualitative variables, expressed as frequency and percentage (%), were analyzed using the Chi-square or Fisher's exact test as appropriate. Correlations between quantitative variables were assessed with the Pearson correlation coefficient. ROC curves were employed for predicting NLRP3 expression and IL-21 plasma levels, and statistical significance was defined as a two-tailed P value < 0.05 [13].

RESULTS

The cases involved in the study were 45 males and 35 females with a spectrum of ages from 14-65 years. Age and sex showed no statistically significant differences between the two groups **(Table 1).**

Table 1: Demographic data of the studied groups

Variable		Control group (n=40)	Case group (n=40)	P-value*
Age (years)		41.23±12.39	42.28±11.74	0.698
Sex	**Male**	21 (52.5%)	24 (60%)	0.499
	Female	19 (47.5%)	16 (40%)	

Data is displayed using Mean ± SD or number (%),
* Significant P value<0.05.

Within the 40 psoriatic patients included in this study, the most frequent clinical manifestations were cutaneous (95%) followed by oral (47%), nail (40%), and psoriatic arthritis (30%). Out of the patients, 9 individuals (22%) displayed a positive medical history either cardiovascular diseases or diabetes mellitus. According to PASI scoring system, patients were categorized as having mild, moderate, or severe psoriasis. The mean PASI score in the case group stood at 8.5. Based on the clinical PASI scoring system, 5 individuals (12.5%) exhibited mild psoriasis, 27 individuals (67.5%) had moderate psoriasis, and 8 individuals (20%) displayed severe psoriasis. No significant statistical difference was observed in psoriasis severity**,** assessed by mean PASI scores, between males and females (P > 0.05) **(Table 2)**.

Table 2: Distribution of clinical features in psoriatic patients

Variable		**Psoriatic patients (total =40)**	
Cutaneous manifestations		38 (95%)	
Oral manifestations		19 (47%)	
Nail manifestations		16 (40%)	
Psoriatic arthritis		12 (30%)	
Positive medical history (CVS or DM)		9 (22%)	
Categories of PASI score guided by scores observed among the case group			
	PASI scoring system	**PASI score findings**	**No. (%)**
Mild	0-5	4.16 ± 0.75	5 (12.5%)
Moderate	6-10	7.44 ± 1.22	27 (67.5%)
Severe	>10	17.3 ± 10.5	8 (20%)
Categories of PASI score considering sex			
	Male (n=24)	**Female (n=16)**	**value- P**
Mild (n=5)	2	3	0.372
Moderate (n=27)	16	11	0.890
Severe (n=8)	6	2	0.572

Data are presented by Mean ± SD or number (%). DM: Diabetes mellites. PASI: Psoriasis Area and Severity Index.

Among the laboratory tests carried out for both the case and the control groups, only erythrocyte sedimentation rate (ESR) displayed a statistically significant difference. The case group exhibited a significant increase in ESR when compared to the control group (P value<0.001). However, no statistically significant differences existed in Hb, RBS, WBCs, and platelet levels between the two groups. NLRP3 protein expression was significantly higher in the case group (mean value± SD =5.3 ±2.7) than the control group (mean ± SD = 1.16± 0.22) (P value<0.001). IL-21 plasma level exhibited a minor rise in psoriasis patients in comparison with healthy controls, but the difference lacked statistical significance (P value = 0.231) **(Table 3).**

Table 3: Laboratory results, NLRP3 protein expression and IL-21 plasma levels among the studied groups

	Case group	**Control group**	**P value**
ESR (mm/hr.)	50.1 ± 24.2	11.98 ± 5.02	**<0.001***
Hb (gm/dl)	11.86 ± 2.21	12.77 ± 2.13	0.065
RBS (mg/dl)	142.8 ± 54	122.53 ± 3.16	0.059
WBCs (10^3 /cm)	6.8 ± 5.63	7.54 ± 2.05	0.088
Platelets(10^3/cm)	265.84 ± 82	248.68 ± 60	0.357
NLRP3 protein expression	5.3 ± 2.7	1.16 ± 0.22	**<0.001**
IL-21 plasma levels	0.38 ± 0.07	0.36 ± 0.08	0.231

Data is expressed by Mean ± SD or number (%). *(Significant P value <0.05)

No correlation was observed between NLRP3 protein expression and age within the case group (r=0.176, P value= 0.119). The current study revealed a positive correlation between NLRP3 expression and severity of psoriasis measured by PASI score (r=0.960 and P value<0.001). There was no correlation between NLRP3 expression levels and IL-21 plasma levels (r=0.013, P value= 0.912) among individuals within the case group. This study observed no correlation between IL-21 plasma levels and age (r=0.022, P-value = 0.849) within the case group. This study displayed no correlation between IL-21 and severity of psoriasis, as measured by PASI score (r=-0.155, P-value = 0.341). Only NLRP3 expression demonstrated a significant positive correlation with Erythrocyte Sedimentation Rate (ESR) (r=0.503, P value=0.009). Nevertheless,

there was no statistically significant correlation observed between the levels of NLRP3 protein or plasma IL-21 and the laboratory parameters among individuals with psoriasis **(Table 4)**.

Table 4: Correlation between NLRP3 protein expression levels or plasma IL -21 levels and other variables within the case group

NLRP3 expression		
	r*	**P value***
Age	0.176	0.119
PASI score (clinically)	0.960	**<0.001**
IL-21 plasma level	0.013	0.912
ESR (mm/hr.)	0.503	0.009
Hb (gm/dl)	-0.088	0.591
RBS (mg/dl)	-0.033	0.841
WBCs (10^3 /cm)	0.124	0.444
Platelets (10^3 /cm)	-0.008	0.962
IL-21 level		
	r*	**P value***
Age (years)	0.022	0.849
PASI score (clinically)	-0.155	0.341
ESR (mm/hr.)	-0.186	0.251
Hb (gm/dl)	0.181	0.264
RBS (mg/dl)	0.303	0.057
WBCs (10^3 /cm)	0.011	0.948
Platelets (10^3 /cm)	0.213	0.188

r: correlation coefficient, PASI: Psoriasis Area and Severity Index, *(Significant P value <0.05).

In males, NLRP3 protein expression ranged from 3.52 to 13.25, while in females, it ranged from 2.03 to 4.22. NLRP3 protein expression was significantly higher among males compared to females (P <0.001) within the case group. IL-21 plasma levels ranged from 0.31 to 0.46 in males, while in females it ranged from 0.3 to 0.76. Regarding plasma IL-21 levels, no statistical significance was found in different sexes **(Table 5)**.

Table 5: Relation between NLRP3 protein expression, IL-21 plasma levels and gender within the case group

Variable	Male (n=24)	Female (n=16)	P-value*
NLRP3 protein expression	6.67±2.67	3.24±0.7	**<0.001**
IL-21 plasma levels	0.37±0.04	0.39±0.1	0.114

Data is represented as Mean ± SD, *(Significant P value <0.05).

No statistically significant difference was detected in the mean expression levels of NLRP3 protein and IL-21 plasma levels between psoriatic patients with and without other clinical parameters **(Table 6).**

Table 6: Relation between expression levels of NLRP3 and plasma IL-21 levels with the clinical parameters of psoriatic patients:

Variable	Psoriatic patients (total =40)	NLRP3 expression	P-value*	IL-21 plasma level	P-value*
Cutaneous manifestations	38 (95%)	5.41 ± 2.73	0.272	0.38 ± 0.07	0.940
Oral manifestations	19 (47%)	5.08 ± 2.35	0.638	0.39 ± 0.1	0.117
Nail psoriasis	16 (40%)	4.29 ± 1.59	0.053	0.41 ± 0.1	0.033
Arthritis	12 (30%)	5.83 ± 3	0.055	0.38 ± 0.03	0.640
Positive medical history (cardiovascular or diabetes)	9 (22%)	4.76 ± 1.77	0.503	0.42 ± 0.13	0.04

Data are presented by Mean ± SD, *(Significant P value <0.05).

The Area Under the Curve (AUC) for NLRP3 expression was 0.965, signifying a strong discriminatory capacity. At a cutoff expression value greater than 1.594, the sensitivity was 92.5%, and the specificity was 90%, with a significant P value <0.001. Regarding IL-21 plasma levels the calculated AUC for this differentiation was 0.694. Notably, when evaluating IL-21 plasma levels with a cutoff expression value exceeding 0.363, the sensitivity was 60%, while the specificity was 80%. These outcomes led to the generation of a statistically significant P value of 0.001 **(Figure 1)**.

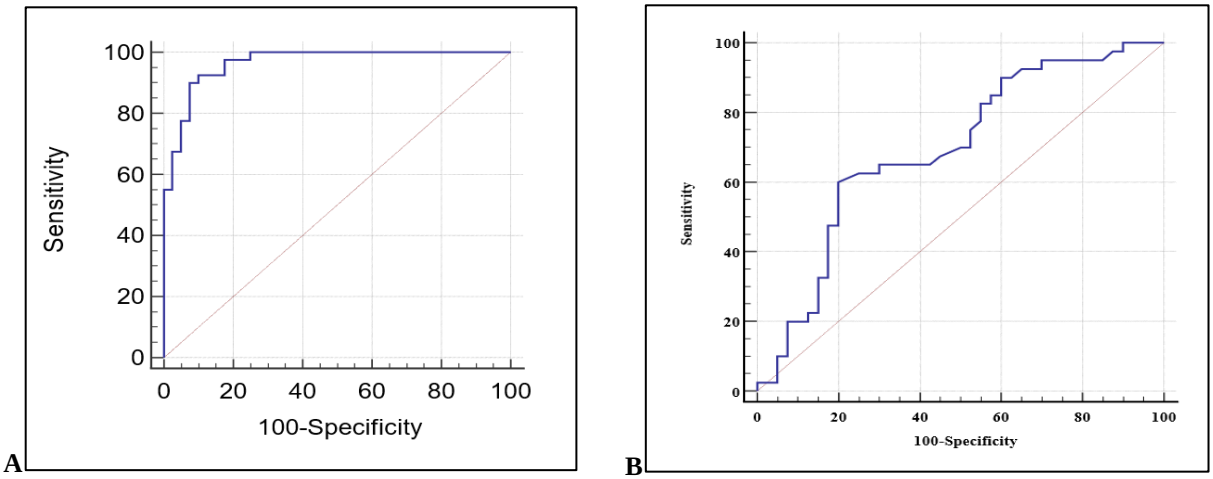

Fig. 1: ROC curve showing sensitivity and specificity of A) NLRP3 expression and B) IL-21 plasma levels

DISCUSSION

Psoriasis is a persistent inflammatory skin disorder that impacts approximately 3% of the worldwide population. Its characteristic red, scaly plaques and papules recur frequently [14].

A significant positive correlation was established between the severity of PASI scoring and the ESR, as patients with higher ESR levels generally present more severe forms of psoriasis. In correlation analysis performed by Kim et al. [15] on psoriasis patients, PASI correlated positively with ESR being a strong predictor of PsA in psoriatic patients.

The psoriatic patients showed a significantly higher NLRP3 protein expression compared with the control group. These findings align with the results reported by Verma et al.[16], indicating a significantly enhanced expression of NLRP3 in the CD14+ monocyte, CD16+ neutrophil, and CD4+ lymphocyte subsets of fresh whole blood from patients with psoriasis compared with those from the healthy controls. Moreover Zhang et al.[17] found that the genetic mutations in NLRP3 are associated with psoriasis susceptibility through three pathways: NF-κB upregulation, caspase activation, and plasma IL-1β pathways.

However, Li et al. [18] reported insignificant NLRP3 levels in serum in a group of psoriatic patients. They attributed that to the need of inflammasomes for assembly and activation of following its translation.

This study is the first to explore the relation of NLRP3 protein expression with age and gender of psoriatic patients. Our results showed no correlation between NLRP3 protein expression and age, although significantly higher NLRP3 protein expression was observed in males. A systematic review conducted by Iskandar et al.[19] suggested that it presents slightly earlier in females than in males. Meanwhile, our study confirmed a significant positive correlation between NLRP3 expression and PASI score. To our knowledge, this is the first study employing Receiver Operating Characteristic (ROC) curve analysis to assess the role of NLRP3 expression levels as a diagnostic biomarker in diagnosis of psoriasis. ROC curve analysis revealed that NLRP3 expression levels could distinguish between psoriatic patients and healthy controls with sensitivity 92.5% and specificity 90%. This aligns with the results of Yu et al [20] found that NLRP3 expression levels are significantly associated with psoriasis severity evaluated using PASI score.

Our data showed a slight increase in IL-21 plasma levels in psoriatic patients in contrast to healthy controls, yet without any statistically significant difference. Also, no significant association was detected between IL-21 plasma levels and age or gender in psoriatic patients. In agreement with our results, Oliveira et al. [21] revealed increased IL-21 serum levels in psoriatic patients than healthy controls, but with no statistically significant difference. On the other hand, Wang et al. [22] found significant higher levels of IL-21 in psoriatic lesions compared to healthy controls and significantly correlated with PASI score. It may be attributed to some reasons like that IL-21 levels are more obviously detected in skin biopsy samples rather than plasma samples or may be due to relatively low range of disease activity or duration included in our study.

Our results revealed that there was no significant correlation between IL-21 plasma level and the severity of psoriasis measured by PASI score although the ROC curve analysis revealed that IL-21 plasma level could distinguish between psoriatic patients and healthy controls, exhibiting a sensitivity of 60% and specificity of 80%. In support with our findings, Nakajima et al. [23] noted the absence of a significant correlation between plasma IL-21 and psoriasis severity measured by PASI score. However, El-Boghdady et al.[24] confirmed a positive significant correlation between IL-21 and

severity of psoriasis measured by PASI score, supporting its role as a biomarker in psoriasis pathogenesis by the ROC curve. The correlation they observed may stem from the wider range of PASI values in their patients (4.8 to 64.2), exceeding our study's range (3 to 41). Additionally, their study had a higher male proportion (80%) compared to ours. The present study demonstrated no significant correlation between NLRP3 protein expression and plasma IL-21 levels. The lack of correlation between NLRP3 expression levels and plasma IL-21 levels suggests that the role of NLRP3 in psoriasis pathogenesis may be mediated by different pathways [25].

In our study, higher expression levels of NLRP3 protein were significantly linked with PsA. Regarding other clinical manifestations of psoriatic patients, NLRP3 protein expression showed no significant statistical association. In agreement with our results, Juneblad et al. [26] confirmed a significant association between NLRP3 inflammasome expression and PsA, indicating a possible involvement in the pathogenesis of the PsA disease.

Regarding the laboratory parameters of psoriatic patients in our study, only ESR showed a significant positive correlation with higher levels of NLRP3 expression. In support with our results, Christophers and van de Kerkhof[27] revealed a significant positive correlation between NLRP3 expression and ESR in psoriatic patients, indicating that psoriasis is a systemic inflammatory disease. An explanation may be that elevated cytokines from NLRP3 activation stimulate liver to produce various proteins, including fibrinogen which affect the aggregation of red blood cells, leading to an elevated ESR [28]. In contrast to our findings, Yin et al.[29] documented that higher NLRP3 expression in psoriasis patients correlated with elevated peripheral blood leukocyte and neutrophil count which considered a measure of systemic inflammation in psoriasis. The variation in results may be clarified through the heterogeneity of the patient population, cyclic nature of psoriasis, variations in treatment regimens, and the specific assays used to measure laboratory parameters[30].

The current study showed that higher plasma levels of IL-21 were significantly linked with both nail manifestations, and positive medical history of diabetes or cardiovascular diseases. Meanwhile, no statistically significant difference was noted among psoriatic patients with and without other clinical parameters regarding IL-21 plasma levels. Michalak-Stoma et al. [31] supported our results.

In our study, there was no significant correlation between plasma IL-21 levels with laboratory parameters among psoriatic patients. In line with our results, Mease and Armstrong [32] revealed insignificant correlation between plasma IL-21 levels and peripheral blood leukocyte count. On the contrary, Gökalp [33] and Bertesi et al. [30] found higher plasma IL-21 levels positively correlated with elevated ESR. The partial variance between our findings and those of earlier studies could be attributed to variations in patient demographics, diverse sample sizes, and discrepancies in disease duration [34].

Recommendations:

Our study recommended using larger sample size, studies investigating different inflammasomes and interleukins, to validate the main mediator in inflammasome activation. NLRP3 expression levels can be used as potential biomarkers for diagnosis of psoriasis and potential therapeutic targets.

CONCLUSIONS

NLRP3 could be a potential diagnostic and prognostic biomarker in psoriasis diagnosis and a promising therapeutic target. Further studies are necessary to investigate the role of IL-21 in the pathogenesis of psoriasis.

Conflict of Interest:

This manuscript is original, not previously published, and not under consideration elsewhere in a similar form. I have contributed significantly to the project to be qualified as an author. To the best of my knowledge, there are no conflicts of interest. All authors participated in the project's conception, design, data analysis and interpretation, manuscript drafting and revision, and have given approval for its submission.

Author Contributions:

Alaa M.I.S. Aboutaleb: Carried out the experiments, performed the analysis, discussed the results, and wrote the final manuscript with input from all authors.

Essraa A. Hegazy: Conceived the original idea, planned and supervised the experiments, discussed the results, revised the work, and approved the final version to be published.

Amal A.A. Elshimy: Supervised the findings of this work, revised the work critically for important intellectual content, discussed the results, and approved the final version to be published.

Marwa A.Amer: Supervised the findings of this work, revised the work for important intellectual content, and approved the final version to be published.

Maha M. Kotb: Conceived the original idea, planned and supervised the experiments, discussed the results, revised the work, and approved the final version to be published.

REFERENCES

1. Olejnik M, Adamski Z, Dorocka-Bobkowska B. Psoriasis: From antiquity to the present. IJDVL. 2023;44:1-5.
2. Chen C, Yi X, Liu P, Li J, Yan B, Zhang D, et al. CD147 facilitates the pathogenesis of psoriasis through glycolysis and h3k9me3 modification in keratinocytes. Research. 2023;10:12-417.
3. Amin J, Boche D, Rakic S. What do we know about the inflammasome in humans? Brain Pathol. 2017;27:192-204.
4. Boucher D, Monteleone M, Coll RC, Chen KW, Ross CM, Teo JL, et al. Caspase-1 self-cleavage is an intrinsic mechanism to terminate inflammasome activity. J Exp Med. 2018;215:827-40.
5. Tang L, Zhou F. Inflammasomes in common immune-related skin diseases. Frontiers in immunology. 2020;11:882.
6. Mosallaei M, Ehtesham N, Rahimirad S, Saghi M, Vatandoost N, Khosravi S. PBMCs: A new source of diagnostic and prognostic biomarkers. Arch Physiol Biochem. 2022;128:1081-7.
7. Betsou F, Gaignaux A, Ammerlaan W, Norris PJ, Stone M. Biospecimen science of blood for peripheral blood mononuclear cell (PBMC) functional applications. Curr Pathobiol Rep. 2019;7:17-27.
8. Mesas-Fernández A, Bodner E, Hilke FJ, Meier K, Ghoreschi K, Solimani F. Interleukin-21 in autoimmune and inflammatory skin diseases. Eur J Immunol. 2023;53:2250075.
9. Ciążyńska M, Olejniczak-Staruch I, Sobolewska-Sztychny D, Narbutt J, Skibińska M, Lesiak A. The Role of NLRP1, NLRP3, and AIM2 inflammasomes in psoriasis: review. Int J Mol Sci. 2021;22:125-47.
10. Gensous N, Charrier M, Duluc D, Contin-Bordes C, Truchetet M-E, Lazaro E, et al. T follicular helper cells in autoimmune disorders. Front immunol. 2018;9:16-37.
11. Naldi L. Scoring and monitoring the severity of psoriasis. What is the preferred method? What is the ideal method? Is PASI passé? facts and controversies. Clinics in Dermatology. 2010;28:67-72.
12. Tang Q, Huang K, Liu J, Wu S, Shen D, Dai P, et al. Fine particulate matter from pig house induced immune response by activating TLR4/MAPK/NF-κB pathway and NLRP3 inflammasome in alveolar macrophages. Chemosphere. 2019;236:124373.
13. Chan Y. Biostatistics 101: data presentation. Singapore medical journal. 2003;44:280-5.
14. Dainichi T, Kitoh A, Otsuka A, Nakajima S, Nomura T, Kaplan DH, et al. The epithelial immune microenvironment (EIME) in atopic dermatitis and psoriasis. Nat Immunol. 2018;19:1286-98.
15. Kim DS, Shin D, Lee MS, Kim HJ, Kim DY, Kim SM, et al. Assessments of neutrophil to lymphocyte ratio and platelet to lymphocyte ratio in Korean patients with psoriasis vulgaris and psoriatic arthritis. J Dermatol. 2016;43:305-10.
16. Verma D, Fekri SZ, Sigurdardottir G, Bivik Eding C, Sandin C, Enerbäck C. Enhanced inflammasome activity in patients with psoriasis promotes systemic inflammation. J Invest Dermatol. 2021;141:586-95.
17. Zhang Y, Yang W, Li W, Zhao Y. NLRP3 Inflammasome: Checkpoint Connecting Innate and Adaptive Immunity in Autoimmune Diseases. Front Immunol. 2021;12:732933.
18. Li S, Zhu X, Zhang N, Cao R, Zhao L, Li X, et al. LncRNA NORAD engages in psoriasis by binding to miR-26a to regulate keratinocyte proliferation. Autoimmunity. 2021;54:129-37.
19. Iskandar IYK, Parisi R, Griffiths CEM, Ashcroft DM. Systematic review examining changes over time and variation in the incidence and prevalence of psoriasis by age and gender. Br J Dermatol. 2021;184:243-58.
20. Yu P, Hao S, Zheng H, Zhao X, Li Y. Association of NLRP1 and NLRP3 polymorphisms with psoriasis vulgaris risk in the Chinese Han population. BioMed Research International. 2018;2018.
21. de Oliveira PS, Cardoso PR, Lima EV, Pereira MC, Duarte AL, Pitta Ida R, et al. IL-17A, IL-22, IL-6, and IL-21 serum levels in plaque-type psoriasis in brazilian patients. Mediators Inflamm. 2015;2015:819-149.
22. Wang Y, Wang LL, Yang HY, Wang FF, Zhang XX, Bai YP. Interleukin-21 is associated with the severity of psoriasis vulgaris through promoting CD4+ T cells to differentiate into Th17 cells. Am J Transl Res. 2016;8:318-96.
23. Nakajima H, Nakajima K, Tarutani M, Morishige R, Sano S. Kinetics of circulating Th17 cytokines and adipokines in psoriasis patients. Arch Dermatol Res. 2011;303:451-5.
24. El-Boghdady, N. A., Ismail, M. F., Abd-Alhameed, M. F., Ahmed, A. S., & Ahmed, H. H. (2018). Bidirectional association between psoriasis and obesity: benefits and risks. Journal of Interferon & Cytokine Research, 38(1), 12-19.

25. Li Z, Guo J, Bi L. Role of the NLRP3 inflammasome in autoimmune diseases. Biomed Pharmacother. 2020;130:110-542.
26. Juneblad, K., Kastbom, A., Johansson, L., Rantapää-Dahlqvist, S., Söderkvist, P., & Alenius, G. M. (2021). Association between inflammasome-related polymorphisms and psoriatic arthritis. Scandinavian Journal of Rheumatology, 50 .212 - 206 ,(3) https://doi.org/10.1080/03009742.2020.1834611
27. Christophers, E., & van de Kerkhof, P. C. M. (2019). Severity, heterogeneity and systemic inflammation in psoriasis. *Journal of the European Academy of Dermatology and Venereology*, *33*(4), 643-647.
28. Hayran, Y., Allı, N., Akpınar, Ü., Öktem, A., Yücel, Ç., Fırat Oguz, E., & Turhan, T .(2021) . Serum galectin-3 levels in patients with psoriasis. International journal of clinical practice, 75(10), e14545.
29. Yin, L., Xu, J.-L., Hu, Y.-Y., Johnston, A., & Yin, Z.-Q. (2016). Systemic abnormalities of psoriatic patients: a retrospective study. Clinical, cosmetic and investigational dermatology, 443-449.
30. Bertesi, M., Fantini, S., Alecci, C., Lotti, R., Martello, A., Parenti, S., Carretta, C., Marconi, A., Grande, A., & Pincelli, C. (2021). Promoter methylation leads to decreased ZFP36 expression and deregulated NLRP3 inflammasome activation in psoriatic fibroblasts. Frontiers in Medicine, 7, 579383.
31. Michalak-Stoma, A., Bartosińska, J., Raczkiewicz, D., Kowal, M., Kozak, J., Gujski, M., Krasowska, D., & Chodorowska, G. (2022). Multiple cytokine analysis of Th1/Th2/Th9/Th17/Th22/Treg cytokine pathway for individual immune profile assessment in patients with psoriasis. Medical Science Monitor: International Medical Journal of Experimental and Clinical Research, 28 ,e938277-938271.
32. Mease ,P. J., & Armstrong, A. W. (2014). Managing patients with psoriatic disease: the diagnosis and pharmacologic treatment of psoriatic arthritis in patients with psoriasis. Drugs, 74, 423-441.
33. Gökalp, H. (2018). Effect of psoriasis severity on inflammation parameters: Controlled study. Turk J Dermatol, 52.
34. Garzorz-Stark, N & ,.Eyerich, K. (2019). Psoriasis pathogenesis: keratinocytes are back in the spotlight. Journal of Investigative Dermatology, 139(5), 995-996.