

NALP3 INNATE MOLECULE IS CORRELATED TO IL17A EXPRESSION IN PSORIATIC ARTHRITIS

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Key words Psoriasis, psoriatic arthritis, NALP3, IL17A.

Key points

- NALP3 expression is elevated in PsA.
- NALP3 expression is positively correlated to IL17A in PsA patients.
- NALP3 may be involved in the pathogenesis of PsA and may be a target for disease treatment in the future.

Psoriasis (PS) is an autoimmune disease that is long-lasting and is characterized by noncontagious abnormal red, or purple or sometimes darker raised areas of skin which are dry, scaly and itchy which varies in size from localized small patches to the extent of being covering the whole body. Koebner phenomenon is psoriatic skin changes that is triggered at the spot of skin injury. (Menter *et al* 2008 and Boehncke and Schön 2015) and Main types of psoriasis include plaque type, guttate type, inverse type, pustular and erythrodermic types. Plaque psoriasis or psoriasis vulgaris

occurs in 90% of cases. (Alam *et al* 2004 and James *et al* 2005).

Psoriatic arthritis (PsA) is the inflammatory arthritis that may affect people having psoriasis. Joint inflammation is usually presented by sausage-like swelling occurring in fingers and toes often with nail pitting, thickening or detachment from nailbed. Psoriatic arthritis may be oligoarticular (in less than 3 joints) in 70% of patients, this form is mild and is not affecting the same joints on both body sides, polyarticular arthritis (that affects more than or equal to five joints on both body sides) resembling

rheumatoid arthritis is the second type occurs in around 25% of patients. Spondyloarthritis type, distal interphalangeal and arthritis mutilans are the remaining types, the last type is the most severe. (Ritchlin *et al* 2017 and Janeway *et al* 2001).

PS pathogenesis is believed to be due to genetic factors, also environmental factors such as infections and stress may play a role. The innate immunity is a defense strategy in which immune system manages inflammation in response to infection or irritation, this happens through some types of inflammatory cells, their pattern recognition receptors (PRRs) and inflammatory mediators they release. Activation of PRR leads to inflammatory cell death by several mechanisms including pyroptosis, PANoptosis, and necroptosis due to cellular consequences. Innate immunity appeared to play a role in psoriasis pathogenesis. (Janeway *et al* 2001, Stvr̃tinov *et al*, 1995; Schon 2019 and Tao *et al* 2013).

The NOD-like receptor family pyrin domain containing 3 (NALP3) is a component of inflammasome and innate immunity. It is expressed mostly in macrophages, NALP3 detects damaged cells' products like extracellular ATP and uric acid, then it triggers immune response. Mutations of NALP3 gene was found to be associated with some autoimmune diseases (Lu and Wu 2015) including PS, (Seguro *et al* 2022) furthermore its gene activation showed

linkage with cardiovascular disease. (An *et al* 2019).

Interleukin 17 (IL17) is a cytokine which plays a major role in psoriasis and psoriatic arthritis pathogenesis, also targeting this cytokine family including IL17A, IL17 receptor and IL17F showed help in disease treatment. IL17 was found in several inflammatory cells among which is innate lymphoid cells of skin lesions and joints' synovial fluid. (Blauvelt and Chiricozzi 2018 and McGonagle *et al* 2019).

How is NALP3 expression in psoriatic arthritis? Is its expression related to that of IL17 that may give a clue of its possible role in psoriatic arthritis pathogenesis that needs further investigation?

Aim of the study

To study NALP3 and IL17A expression in PsA, consequently the possibility of their relevance to disease pathogenesis and/or activity.

MATERIALS AND METHODS

- The study participants were 128 (59 patients with PsA, 29 patients with skin PS only with no arthritis and 40 healthy control (HC) volunteers).
- Samples obtained were placed in EDTA tubes (5 ml) then underwent RNA isolation. GENEzol™ Reagent (GENEzol™ TriRNA) was used to

extract total RNA according to the manufacturer's instructions.

- Nanodrop spectrophotometer was used to measure quantity and purity of the extracted RNA.
- RNA was then stored at - 80°C. After that (400 ng) of RNA was converted into cDNA according to instructions of the kit used (Accupower® Rocket script™ RT PreMix, Bionner, Korea).
- Genes' Primers obtained from alpha DNA, Macrogen company, Canada were used to determine mRNA expression for studied markers (F:5'-CCAAGCCAGGGCAGCC-TTCA-3,'R:5'-GGAAG-AAGACGTACACCG-3'for NALP3 and F: 5'CTCATTGGTGTCACTGCT3', R: 5'CGGTTGTAGTAATCTGAGG3' for IL17A). (Zuo *et al* 2018 and Mansouri *et al* 2018) One µl of cDNA was mixed with 10 µl of master mix, Promega, U.S.A.
- Expression was measured using the Applied Biosystem (RT-PCR 7500) through the following steps: One cycle of GoTaq activation for 2 minutes at 95°C, denaturation for 40 cycles at 95°C (15 seconds) and 1 minute of Annealing/Extension at 56°C.
- Melting curves analysis was done for both genes.
- The data was then analyzed using the method of $\Delta\Delta CT$ (a specific

mathematical approach which is used to analyze the data resulting, it calculates the expression relative fold change between the two groups samples).as follow:

$$\Delta CT_{(PsA)} = \Delta CT_{(Target\ gene)} - \Delta CT_{(Housekeeping\ gene)}, \Delta CT_{(HC)} = \Delta CT_{(Target\ gene)} - \Delta CT_{(Housekeeping\ gene)},$$

$$\Delta\Delta CT = \Delta CT_{(PsA)} - \Delta CT_{(HC)}, \text{ Then Gene Expression} = 2^{-\Delta\Delta CT}$$

$$\text{Fold change (FC)} = \text{Gene Exp. PsA} / \text{Gene Exp. HC.}$$

Statistics

The data was statistically analyzed by normality tests then by Mann-Whitney U test (non-parametric) used to compare differences between groups in (MINITAB) programs. Parametric tests used were: Two sample T-Test to compare the means of the two groups, and confidence interval to assess the precision and statistical significance.

RESULTS DISCUSSION

Results showed no significant differences in the age among groups of participants (35.67±12.35 years for PsA ,30.44±16.94 years for PS and 37.31±18.02 years for HC, p values are shown on the supplementary table. Females' percentage was 52.5% of all patients while males' one was 47.5%. Family history was positive in 15.7% in patients with PS and 11.24% in patients

with PsA.

Disease activity in PsA group was measured using DAS28 and patients were divided into three categories: high disease activity (6.07 ± 0.034 score: 6.12 ± 0.71 in 16 female patients and 5.72 ± 0.35 in 4 male patients), moderate disease activity (4.16 ± 0.06 score: 4.31 ± 0.07 in two female patients and 4.21 ± 0.63 in 8 male patients) and low disease activity (2.88 ± 0.13 score in two female patients). While patients involved in the study who were having only skin psoriasis were classified according to psoriasis type (plaque psoriasis: 53 patients (89.8%), three patients with erythrodermic psoriasis (5.1%), pustular psoriasis in two patients (3.4%) and one patient with guttate psoriasis (1.7%)) and according to psoriasis severity using PASI score into mild (PASI < 3) which was present 5.13% of patients, moderate (PASI 3-10) which was present in 30.76% of patients and severe (PASI > 10) which was present in 64.10% of patients. (Nicola *et al* 2022)

IL-17A qRT-PCR expression results in psoriasis patients was 2.13 folds that of healthy control, while in PsA was 0.84 that of normal control and 0.39 that of psoriasis with nonsignificant difference $p = 0.219$ (fig. 1). NALP3 expression in psoriasis patients was 7.48 folds that of healthy control, while in PsA was 3.0535 folds of that of normal control and 0.41 that of psoriasis with nonsignificant difference $p = 0.844$ (figs 1 and 2).

Spearman tests showed that NALP3 expression was having a

significant positive correlation with IL-17A expression in PsA patients ($r = 0.172$) (fig. 3).

PsA is a destructive disease to joints which may affect patients' functional ability and cause many morbidities. (Menter *et al* 2008) Pathogenesis of the disease is still unknown but disordered immune system where both innate and adaptive immunity play important roles and are related to genetic factors. (Janeway *et al* 2001) Knowledge of new facts in disease pathogenesis may help finding new effective treatments.

The study results showed increased IL 17A expression in PS patients agreeing with many studies on the other hand, current results did not show an increase in PsA patients although many studies showed increased expression of this cytokine in PsA and its role in joint destruction, enthesitis and bone erosion. (Blauvelt and Chiricozzi 2018; McGonagle *et al* 2019; Shih *et al* 2023 and Wang *et al* 2017) Usage of anti-IL 17 and IL 17 A antibody showed improvement of the disease skin and joint manifestations. (Iznardo and Puig (2021).

For NALP3 expression, it was increased in both PS and PsA, but the increase was much evident in PS without arthritis, these results agree with results of other studies measuring NALP3 expression in PS. ⁽²¹⁾ NLP3 is thought to be contributing in PS pathogenesis since skin damage and trauma trigger PS through release of intracellular ATP from

damaged cells, which in turn activates the inflammasome, leading to cascade of inflammatory responses involved in the pathogenesis of the disease. (Ferrari *et al* 2021)

The study results showed that NALP3 expression is weakly linked IL17 A expression, the positive correlation between both was shown between both genes in pyroptosis, where Interleukin-17 appeared to induce osteoblasts pyroptosis in vitro through NLRP3 inflammasome pathway, which may contribute in bone metabolism and remodeling. There was an evidence that pyroptosis; the lytic programmed cell death; is involved in the pathogenesis of autoimmune disorders including Ps, this relation should be tested in further studies. (Lei *et al* 2021; Zhang *et al* 2022 and Song *et al* 2022) It is important to mention that the relatively nonlarge sample size and the difficulty of excluding all confounding factors that may affect inflammation and IL17A and NALP3 expression were limitations in the study design.

Abbreviations

PS: psoriasis.

PsA: psoriatic arthritis.

HC: healthy control.

IL17A: interleukin 17 A.

NALP3: NLR family pyrin domain containing 3.

DAS28: disease activity score 28.

PASI: Psoriasis Area Severity Index.

PRRs: pattern recognition receptors.

RNA: ribonucleic acid.

cDNA: complementary deoxyribonucleic acid.

DNA: deoxyribonucleic acid.

Conclusion

NALP3 may have a role in PsA pathogenesis due to what was shown about its positive correlation with IL17A expression, that cytokine which is known to cause various sequelae in inflammatory arthritis. Confirming NALP3 role in Ps and/or PsA pathogenesis may help developing new treatments for those diseases by targeting NALP3.

Ethical approval: Patients were recruited from Al-Sadr Teaching Hospital, and Basrah Teaching Hospital, Rheumatology Unit, Biological Therapy Center. Prior conducting the study ethical approval was received after being reviewed by Ministry of Higher Education and Scientific Research, University of Basrah, College of Medicine, Basrah, Iraq. ethical approval was also obtained from ethical committee, Faculty of Medicine, Alexandria University, Alexandria, Egypt, FWA number: 00018699, serial number: 0305820.

Availability of Data: available on request.

Conflict of Interest: no conflict of interest.

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Consent for publication: Not applicable, the manuscript does not contain patients' details, images or videos.

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Authors contributions:

First Author ZSA: obtaining samples and clinical data, measuring genes expressions, sharing in methods and results writing

Second author NHA: Idea of studying different innate molecules in psoriatic arthritis, supervision of clinical work, a corresponding author.

Third author KIA: referral of patients who participated in the study, supervision of clinical work.

Fourth author SSH: choosing the scope of manuscript from the given data, writing the manuscript, sharing in writing of methods and results, a corresponding author.

SUMMARY

Objective Psoriatic arthritis (PsA) is an erosive type of autoimmune arthritis that may associate psoriasis (PS) disease. Pathogenesis of the disease includes genetic factors. To understand more about

disease pathogenesis and to develop relevant therapies, different genes expression should be tested. Here NOD-like receptor family pyrin domain containing 3 (NALP3) and interleukin 17A (IL17A) expression was measured in PsA patients.

Methods Blood samples were obtained from the participating 128 subjects including 59 PsA patients, 29 skin PS only without arthritis group and 40 healthy control volunteers' group, to undergo RNA extraction, reverse transcription, amplification and reading target genes (NALP3 and IL17A) expression.

Results NALP3 gene expression was increased in PsA patients and was positively correlated to IL17A gene expression in the disease.

Conclusions NALP3 which is considered one of innate molecules may have a role in psoriatic arthritis pathogenesis due to what was shown about its positive; though weak; correlation with IL17A expression and this needs further investigation in future studies.

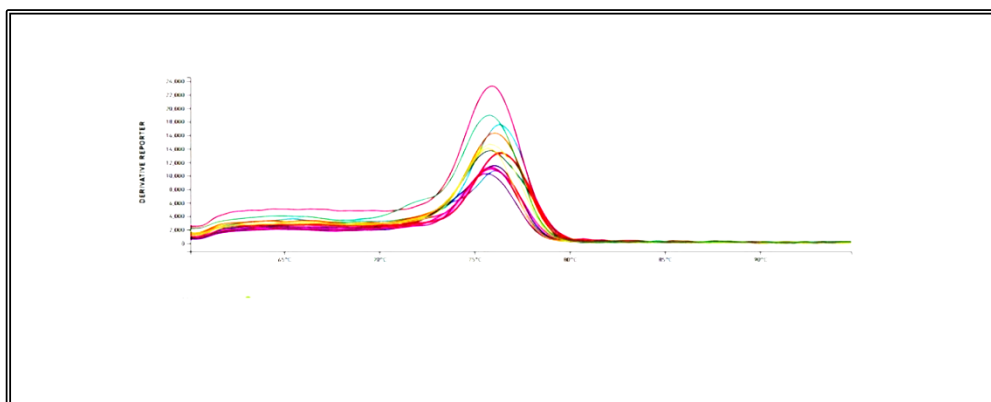
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.Fig. (1): Melting curve for IL-17A gene using SYBR green chemistry qRT-PCR.

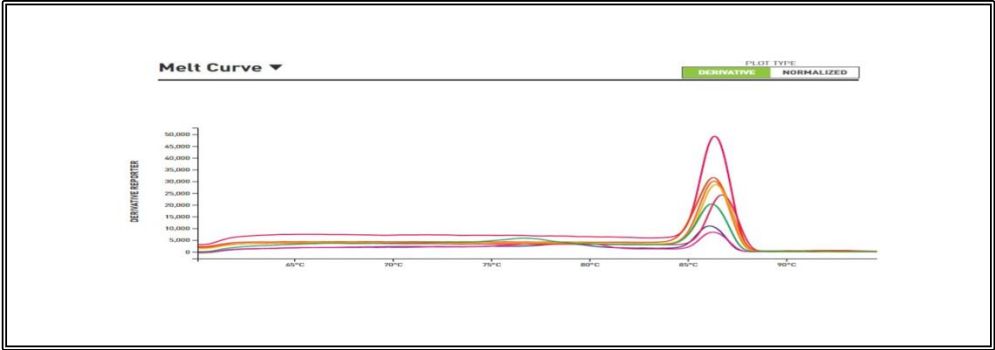


Fig. (2): Melting curve for NALP3 gene using SYBR green chemistry qRT-PCR.

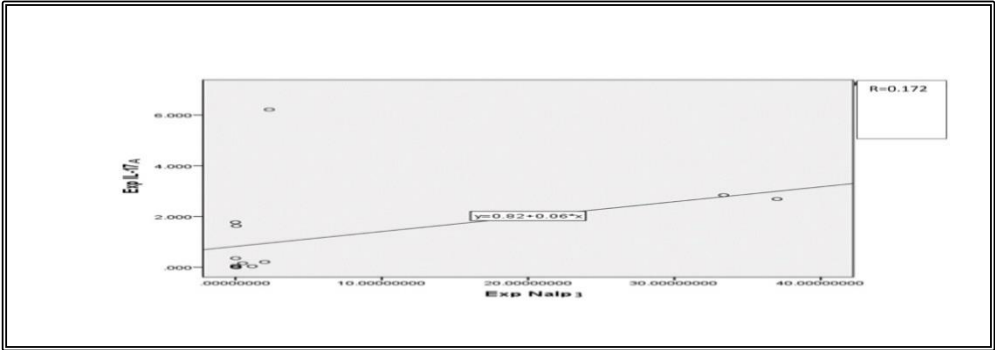


Fig. (3): Correlation between NALP3 and IL17A genes expression in PsA patients.

Supplementary table showing age and sex differences between groups.

Age groups			Sex		Total	Sig.	
			Male	Female			
Fifteen years or younger	Ps/PsA	Ps	6 75.0%	8 72.7%	14 73.7%	0.333*	
		PsA	0 0.0%	2 18.2%	2 10.5%		
		Control	2 25.0%	1 9.1%	3 15.8%		
		Total	8 100.0%	11 100.0%	19 100.0%		
	From 16 to 49 years	Ps/PsA	Ps	13 41.9%	15 34.9%	28 37.8%	0.839* *
			PsA	8 25.8%	12 27.9%	20 27.0%	
			Control	10 32.3%	16 37.2%	26 35.1%	
Total			31 100.0%	43 100.0%	74 100.0%		
From 50 to 65 years	Ps/PsA	Ps	4 30.8%	6 60.0%	10 43.5%	0.372*	
		PsA	2 15.4%	1 10.0%	3 13.0%		
		Control	7 53.8%	3 30.0%	10 43.5%		
		Total	13 100.0%	10 100.0%	23 100.0%		
	Sixty six or older	Ps/PsA	Ps	1 9.1%	4 57.1%	5 27.8%	0.078* *
			PsA	4 36.4%	0 0.0%	4 22.2%	
Control			6 54.5%	3 42.9%	9 50.0%		
Total			11 100.0%	7 100.0%	18 100.0%		
Total		Ps/PsA	Ps	24 38.1%	33 46.5%	57 42.5%	0.586* *
			PsA	14 22.2%	15 21.1%	29 21.6%	
	Control		25 39.7%	23 32.4%	48 35.8%		
	Total		63 100.0%	71 100.0%	134 100.0%		

* Chi² Test
** Fisher's Exact Test