ORIGINAL ARTICLE

Assessment of Serum MicroRNA-221-3p and Tumor Necrosis Factor-α Levels in Patients with Chronic Plaque Psoriasis: A case-control Study

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ABSTRACT

Background: *Chronic plaque psoriasis (CPP) is the most prevalent subtype of psoriasis, which is a chronic inflammatory disorder. MicroRNAs (miRNAs) may contribute to psoriasis pathogenesis through regulation of cytokine production. The pro-inflammatory cytokine, tumor necrosis factor-α (TNF-α), exerts a central role in psoriasis pathogenesis.* ***Objective:*** *to compare serum levels of miR-221-3p and TNF-α among CPP patients and healthy individuals.* ***Methodology****: 45 CPP patients and 45 healthy participants were included. The Psoriasis Area and Severity Index (PASI) score was utilized to assess the disease severity. Quantitative real-time PCR and ELISA techniques were used for assessment of serum concentrations of miRNA-221-3p and TNF-α, respectively.* ***Results****: Psoriatic patients showed significantly higher serum concentrations of miR-221-3p and TNF-α than healthy controls ($P < 0.001$). Moreover, a significant direct correlation was detected between serum miR-221-3p and TNF-α concentrations, on one hand, and both PASI score and disease duration ($P < 0.001$). Furthermore, a significant direct correlation was detected between concentrations of miR-221-3p relative expression and TNF-α in the sera of psoriatic cases ($r = 0.876$, $P < 0.001$).* ***Conclusion****: The miR-221-3p/TNF-α interaction may contribute to psoriasis pathogenesis. Moreover, miR-221-3p could be utilized as a biomarker to diagnose psoriasis and to assess its severity.*

INTRODUCTION

Psoriasis is a chronic multisystem immune-mediated inflammatory disorder, which primarily affects skin, nails, and joints with various systemic comorbidities including metabolic, cardiovascular, hepatic, and gastrointestinal disorders. It affects approximately 2 to 3% of the general population. The most prevalent cutaneous variant of the disease is chronic plaque psoriasis (CPP), which manifests as well-demarcated scaly erythematous plaques that favor the extensor surface of extremities, the scalp, and the lower back. Meanwhile, pustular and erythrodermic variants are less frequent[1]. Hyper proliferation and abnormal differentiation of keratinocytes are the primary pathological features of psoriasis. It is a multifactorial disease resulting from the interplay of several pathogenetic factors, including genetic predisposition, environmental factors, immune dysregulation, and compromised skin barrier functions. It predominantly represents a T-cell-mediated immune disorder [2].

MicroRNAs (miRNAs) are small non-coding RNAs that identify and attach to the complementary sequences in the 3 untranslated regions (3.UTR) of their target messenger RNAs (mRNAs), causing translational suppression or breakdown of these mRNAs [3]. It has been found that the skin and blood of psoriatic patients exhibit disturbed expression of more than 250 miRNAs. Furthermore, serum and tissue levels of miRNAs in psoriatic patients are often correlated with disease severity; hence, they were suggested to serve as biomarkers for psoriasis diagnosis, prognosis, and treatment monitoring [4-7]. MicroRNAs may contribute to psoriasis etiology through their regulatory effects on keratinocyte proliferation and differentiation, and cytokine production. Moreover, they may control the activation of various T cell subtypes [8]. Among those miRNAs is miR-221-3p, whose role in psoriasis pathogenesis is currently being investigated [7].

Tumor necrosis factor-α (TNF-α) is a pro-inflammatory cytokine, released from several cells, including keratinocytes, dendritic cells, lymphocytes, and other immune cells. It has a crucial role in psoriasis development via stimulating the nuclear factor kappa B (NF-κB) signaling pathway, which regulates keratinocyte and lymphocyte survival and proliferation[9]. Additionally, TNF-α induces the synthesis of interleukin IL-23 that induces T-helper-17 (Th17) cells to release

various cytokines, including IL-17, which has a crucial part in psoriasis development [10]. As previously stated, miRNAs may influence psoriasis pathogenesis by modulating the release of many cytokines, including TNF-α [7].

Herein, we aimed to investigate the miR-221-3p role in the development of psoriasis by evaluating its expression in the sera of CPP patients and its relationship to serum TNF-α level.

METHODOLOGY

Study design and population

This is a case-control study including 45 CPP patients and 45 healthy volunteers as controls. The psoriasis diagnosis was determined based on clinical and dermoscopic examination; meanwhile, a skin biopsy was performed if the diagnosis was uncertain. Exclusion criteria included having pustular or erythrodermic psoriasis, having psoriatic arthritis, receiving local or systemic treatment within the previous three months, having any other skin or systemic disorders, and being a pregnant or lactating female.

Ethical consideration

The study was approved by Zagazig University-Institutional Review Board (ZU-IRB #9580/14-6-2022). All procedures were performed in compliance with the updated Declaration of Helsinki. All participants provided informed consent before enrolling in the study.

History taking and clinical evaluation

A detailed history was taken, and a comprehensive clinical examination was performed in all patients. Psoriasis severity was determined utilising the Psoriasis Area and Severity Index (PASI) score, whose values range from 0 to 72 points [11]. Then, the disease severity was classified according to PASI values into mild disease (< 7), moderate disease (7-15), and severe disease (> 15) [12].

Blood sampling and serum preparation:

Under complete aseptic conditions, 5 ml of blood was collected from every participant using a sterile, disposable plastic syringe. Then, the blood was collected into a vacutainer serum separator tube with polymer gel and clot activator for serum separation. After that, sera were separated into two aliquots; one of which was stored at -80^{o} C until measurement of the miRNA-221-3p level, and the other was stored at -20^{o} C until the TNF-α level was measured.

Measurement of serum concentration of miR-221-3p relative expression:

The miRNeasy mini kit *(QIAGEN, USA)* was used for extraction of total RNA following the manufacturer's protocol. Then, quantification of the extracted total RNA was performed using the QuantiFluor RNA system (*Promega, UK*)**.** After that, microRNA was reversibly transcribed into cDNA using reverse transcription kits *(QIAGEN, USA)*. The reverse transcription reactions were produced according to the manufacturer's protocol, including an initial incubation for 60 min at 42ºC, a subsequent incubation for 5 min at 95ºC, and immediate cooling to 4°C. Finally, Quantitative Real-Time PCR was done by miRCURY LNA miRNA PCR assays *(QIAGEN, USA)*. Reaction mixtures were prepared following the manufacturer's instructions. U6 was utilized as an internal reference. Initial heat activation for 2 min at 95 ºC was done, then the real-time cycler (*Applied Biosystems, Foster City, CA, USA*) was run for 40 cycles of each denaturation for 10 s at 95 ºC and combined annealing and extension for 60 s at 56°C . The delta-delta Ct method formula was utilized to estimate the fold changes of miR-221-3p expression.

Measurement of serum TNF-α concentration:

Serum TNF-α concentrations were measured using an ELISA kit (*Catalogue no. EH0302, Biotech Co., Ltd., Wuhan, China)* following the manufacturer's protocol, with the lower detectable TNF-α level being 15.6 pg/ml**.**

Statistical analysis

Data analysis was done using (*SPSS 26.0; SPSS Inc.)*. The following statistical tests were used when applicable: Independent sample t-test, Mann-Whitney U test, Kruskal-Wallis test, Chi-square test, and Spearman's correlation coefficient. Moreover, the ROC curve was utilized to analyze the diagnostic potential of serum miR-221-3p. *P*-values lower than 0.05 were considered statistically significant.

RESULTS

A total of 45 psoriatic patients and 45 healthy controls were included in this case-control study. No statistically significant difference was observed between the two groups regarding sex and age (**Table 1**). The mean disease duration was 7.73 ± 6.59 years and ranged from 1 to 20 years. The mean PASI score was 14.62 ± 10.54 and ranged from 2 to 33.8. According to the PASI score, psoriasis severity was mild in 11 patients (24.4%), moderate in 16 (35.6%), and severe in 18 patients (40%).

Table 1: Demographic data of the studied groups

Variable	Psoriatic patients (N = 45)		Control group (N = 45)		Test value	*P*-value
Age (in years)						
Mean ±SD	38.02 ± 13.24		35.4 ± 3.89		1.2 [a]	0.235
Median (Range)	40.5 (14-62)		35 (29-45)			
	N	**%**	**N**	**%**		
Sex						
Male	16	35.6%	19	42.2%	0.42 [b]	0.516
Female	29	64.4%	26	57.8%		

[a] Independent sample t-test
[b] Chi-square test.
Note: *P*-value ≥ 0.05 was considered statistically non-significant.

Serum level of miR-221-3p relative expression

Psoriatic cases showed significantly higher serum miR-221-3p concentrations than controls ($P < 0.001$) (**Table 2, Figure 1A**). Moreover, serum miR-221-3p concentrations were significantly higher in cases with severe disease than in cases with mild or moderate disease ($P < 0.001$) (**Table 3**). Also, serum miR-221-3p level showed a statistically significant positive correlation with both PASI score ($r = 0.99$, $P < 0.001$) and psoriasis duration ($r = 0.89$, $P < 0.001$) **(Figure 1B & 1C**).

ROC curve analysis revealed that serum miR-221-3p concentration at a cutoff value of 1.72 demonstrated the greatest diagnostic performance with an area under the curve (AUC) of 0.992, specificity of 95%, and sensitivity of 95% (**Figure 2**). Hence, it could be regarded as a valid test for diagnosing psoriasis.

Table 2: Serum miR-221-3p and TNF-α levels in the studied groups

Variable	Psoriatic patients (N = 45)	Control group (N = 45)	Test value	*P*-value
Serum MiR-221-3p level				
Mean ±SD	5.57 ± 2.96	0.97 ± 0.41	6.69[a]	< 0.001*
Median (range)	4.6 (1.32-10.9)	0.9 (0.2-1.9)		
Serum TNF-α level (pg/ml)				
Mean ±SD	29.47 ± 8.91	11.34 ± 5.02	7.57[a]	< 0.001*
Median (range)	31.95 (10.63-40.9)	11 (2.32-19.4)		

[a] Mann-Whitney U test
* P-value < 0.05 was considered statistically significant.

Table 3: Comparison of serum miR-221-3p and TNF-α levels in relation to disease severity in psoriatic patients

Variable	Psoriasis severity			Test value	*P*-value
	Mild (N = 11)	Moderate (N = 16)	Severe (N = 18)		
Serum MiR-221-3p					
Mean ±SD	2.14 ± 0.53	4.29 ± 0.75	8.82 ± 1.24	34.25[a]	< 0.001*
Median	2.15	4.26	8.92		
(Range)	(1.32-2.9)	(3.09-5.87)	(7-10.9)		
Serum TNF-α (pg/ml)					
Mean ±SD	18.36 ± 7.48	30.46 ± 3.39	35.54 ± 6.33	25.03[a]	< 0.001*
Median	15.8	30.85	37.63		
(Range)	(10.63-29.8)	(21.8-35.1)	(14.1-40.9)		

[a] Kruskal Wallis Test
* P-value < 0.05 was considered statistically significant.

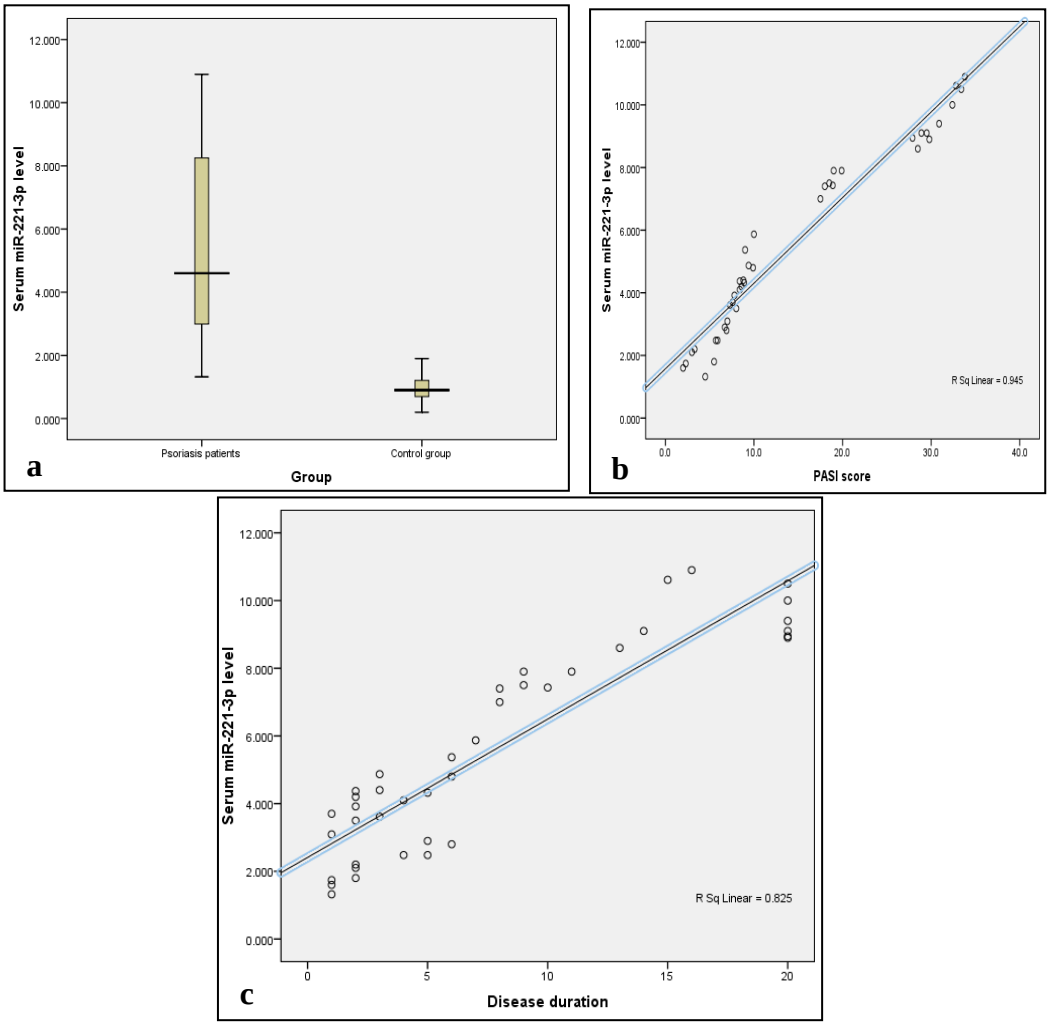

Fig. 1: **(a)** Box whisker plot illustrating the distribution of serum miR-221-3p level in psoriatic patients and healthy controls. **(b)** Scatter diagram illustrating the positive correlation between serum miR-221-3p level and Psoriasis Area and Severity Index (PASI) score in psoriatic patients. (**c**) Scatter diagram illustrating the positive correlation between serum miR-221-3p level and disease duration in psoriatic patients.

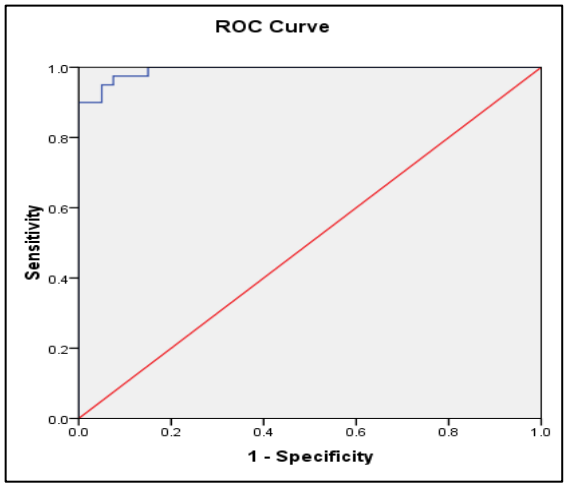

Fig. 2: ROC curve illustrating the validity of serum miR-221-3p level in psoriasis diagnosis.

Serum TNF-α level

Psoriatic patients demonstrated significantly higher serum TNF-α concentrations than controls ($P < 0.001$) (**Table 2, Figure 3A**). Moreover, cases with severe psoriasis showed significantly higher serum TNF-α concentrations than cases with mild or moderate psoriasis ($P < 0.001$) (**Table 3**). Also, in psoriatic patients, the serum TNF-α level showed a statistically significant direct correlation with both PASI score (r = 0.87, $P < 0.001$) and psoriasis duration (r = 0.75, $P < 0.001$) (**Figures 3B & 3C).**

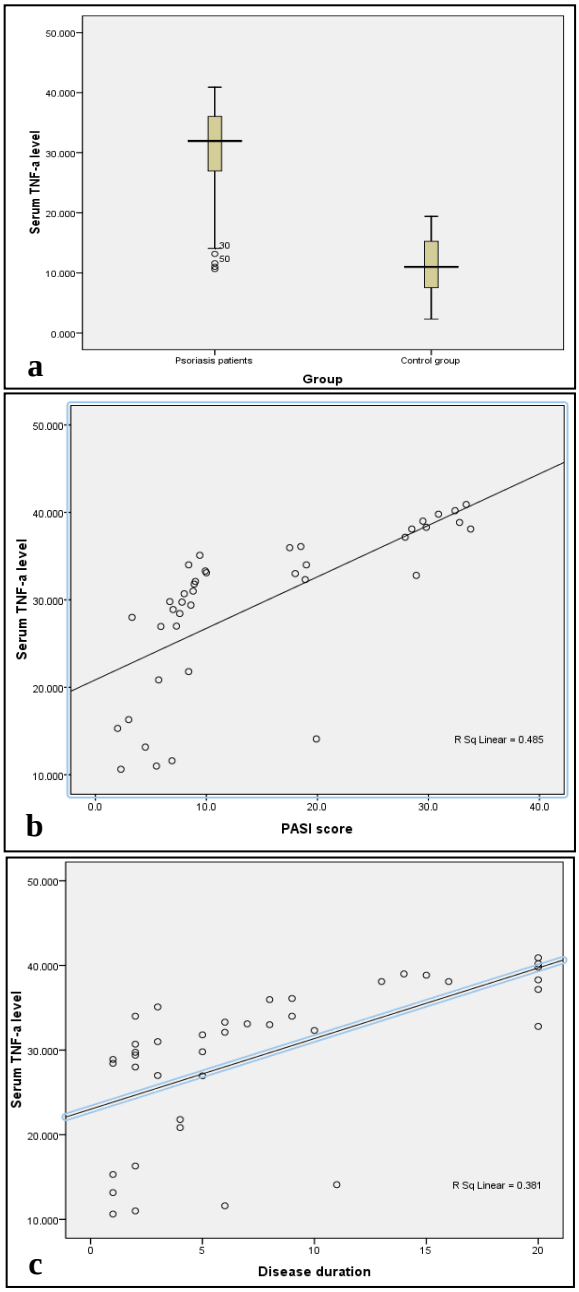

Fig. 3: (**A**) Box whisker plot illustrating the distribution of serum TNF-α level in psoriatic patients and healthy controls. (**B**) Scatter diagram illustrating the positive correlation between serum TNF-α level and Psoriasis Area and Severity Index (PASI) score in psoriatic patients. (**C**) Scatter diagram illustrating the positive correlation between serum TNF-α level and disease duration in psoriatic patients.

Relationship between serum concentrations of mi-R-221-3p and TNF-α

Psoriatic cases showed a statistically significant direct correlation between serum concentrations of miR-221-3p relative expression and TNF-α ($r = 0.876$, $P < 0.001$) (**Figure 4**).

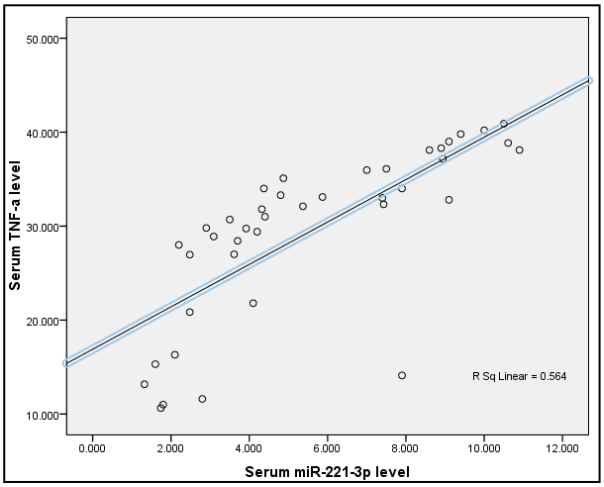

Fig. 4: Scatter diagram illustrating the positive correlation between serum levels of miR-221-3p and TNF-α in psoriatic patients.

DISCUSSION

Psoriasis is a chronic immune-mediated disorder that often develops in genetically predisposed individuals upon exposure to various environmental triggers. Understanding psoriasis pathogenesis may result in the creation of highly effective treatments [9]. Several studies have detected abnormal expression of several miRNAs in psoriatic patients compared to healthy individuals. In patients with psoriasis, some types of miRNAs were stated to be increased, while others were reduced [13-16]. Therefore, several miRNAs may have a role in the pathogenesis of psoriasis and hence represent crucial targets for psoriasis treatments [17].

In the current work, the relationship between miR-221-3p and TNF-α in psoriasis development was evaluated by measuring their serum concentrations in CPP patients in comparison to healthy controls. In psoriatic patients, we detected significantly higher serum miR-221-3p levels compared to controls. This is concordant with Meng et al. [7] and Wade et al. [18], who observed a significantly higher serum miR-221-3p level in cases with plaque psoriasis and psoriatic arthropathy, respectively, compared to healthy individuals. Similarly, Zibert et al. [19] observed a significantly higher tissue expression of miR-221 and miR-222 in psoriatic lesions in comparison to healthy skin. These findings may imply a possible role for miR-221-3p in psoriasis evolution.

On the contrary, Alatas et al. [20] detected a lower miR-221-3p level in serum of psoriatic cases than healthy controls, but this was not statistically significant (P=0.125). The fact that all cases in their study had mild to moderate disease may be the cause for the variation between the two studies.

In our work, the serum miR-221-3p level showed a significant direct correlation with PASI score in

psoriatic cases. Additionally, patients with severe psoriasis showed significantly higher serum levels of miR-221-3p than cases with mild or moderate disease, suggesting a potential involvement of miR-221-3p in psoriasis severity. Similar findings were observed by Meng et al. [7]. Furthermore, our research revealed a significant positive correlation between serum miR-221-3p level and disease duration, suggesting the possible implication of miR-221-3p in disease chronicity.

Conversely, Alatas et al. [20] found no significant relation between circulating levels of various miRNAs, including miR-221, and psoriasis severity (measured by PASI score). This discrepancy may be attributed to the fact that all cases in their study had mild to moderate disease.

There is an increasing interest in the study of miRNAs as potential biomarkers in psoriasis. In this work, we utilized a ROC curve to evaluate the possibility of using serum miR-221-3p level as a diagnostic tool for psoriasis. The cutoff value of serum miR-221-3p concentration at 1.72 showed the greatest diagnostic performance with an AUC of 0.992 and high specificity and sensitivity. Similarly, Meng et al. [7] stated that the best diagnostic performance was demonstrated by a serum miR-221-3p level at a cutoff value of 1.091, with an AUC of 0.992 and high specificity and sensitivity. These findings imply that the serum level of miR-221-3p relative expression could be utilized as a biomarker for diagnosing psoriasis.

Collectively, our findings show that miR-221-3p may contribute to psoriasis pathogenesis and might be an optimistic biomarker for diagnosing psoriasis and evaluating the disease severity. The main mechanism through which miR-221-3p may contribute to psoriasis development is still obscure. One potential mechanism may be through control of cell apoptosis and proliferation. It has been found that downregulating miR-221-3p in vitro significantly inhibits cellular proliferation. Therefore, it was mentioned that the overexpression of miR-221-3p may promote keratinocyte proliferation, a hallmark of psoriasis pathology [7]. Another potential mechanism may be through controlling the production of several cytokines, including TNF-α [21].

In the present study, psoriatic patients demonstrated significantly higher serum concentrations of TNF-α than the control group. This observation is consistent with previous studies [7, 22-26] and supports the crucial role of TNF-α in psoriasis development.

Conversely, Jacob et al. [27] detected no significant difference between psoriatic patients and controls regarding serum TNF-α concentration. This disparity could be due to the smaller sample size of their research (12 psoriatic cases and 5 controls) and the different method used for measuring serum TNF-α concentration.

We detected a significant direct relationship between serum TNF-α level and psoriasis severity, measured by the PASI score. Furthermore, serum TNF-α levels were significantly higher in cases with severe psoriasis compared to cases with mild or moderate psoriasis. Our results were in agreement with those reported by Alobaidi et al.[26]

On the contrary, Ovcina-Kurtovic and Kasumagic-Halilovic [22], Abdel-Hamid et al. [24], and Kyriakou et al. [25] did not detect any significant correlation between PASI score and serum TNF-α concentration. Most of their patients had mild to moderate psoriasis, which could explain this discrepancy. Furthermore, serum concentrations of many cytokines, including TNF-α, may be affected by several in vivo processes like tissue synthesis, deposition, elimination, or degradation [26].

In the present work, psoriatic patients demonstrated a significant direct correlation between serum TNF-α level and disease duration, supporting the potential role of TNF-α in psoriasis chronicity. Our findings were in agreement with those reported by Wu et al. [28].

Collectively, our findings are consistent with earlier studies that highlight the crucial role of TNF-α in psoriasis development. TNF-α activates several innate immune cells and stimulates their trafficking to the skin, resulting in increased keratinocyte proliferation. Also, TNF-α induces keratinocytes to produce IL-8 and intercellular adhesion molecule-1 (ICAM-1), which enhance the chemotaxis of neutrophils and lymphocytes. Moreover, it stimulates the release of various proinflammatory mediators, including IL-1 and IL-6, form keratinocytes [2, 9]. Furthermore, TNF-α may mediate the action of several miRNAs, including miR-221-3p, in psoriasis development [16].

In our research, a significant direct correlation was found between serum levels of miR-221-3p and TNF-α in psoriasis cases, which was in agreement with the results reported by Meng et al. [7]. Moreover, in their study, they found that reducing miR-221-3p expression in vitro caused a significant decrease in TNF-α release, implying that TNF-α may mediate the mechanism through which miR-221-3p contributes to psoriasis development.

The interaction between miR-221-3p and TNF-α may be mediated by the Tissue Inhibitor of Metalloproteinase-3 (TIMP3) protein that takes part in TNF-α degradation. The expression of miR-221-3p may downregulate TIMP3 protein leading to accumulation of TNF-α and the consequent development of psoriatic lesions. In consistency with this theory, Zibert et al. [19] observed significantly lower levels of TIMP3 mRNA associated with significantly higher levels of miR-221 expression in psoriatic skin compared to healthy skin. Moreover, in vitro overexpression of miR-221 has resulted in significant reduction of TIMP3 mRNA levels, showing that the *TIMP3* gene could be a potential target for miR-221 that causes degradation of TIMP3 mRNA and subsequent downregulation of TIMP3 protein, which consequently

causes upregulation of TNF-α levels and ultimately psoriasis development [19].

Limitations of our work include the small sample size and not including patients with psoriatic arthritis and other variants of cutaneous psoriasis. Also, the relationship between miR-221-3p and other inflammatory mediators contributing to psoriasis pathogenesis was not investigated due to lack of financial support.

CONCLUSION

There could be an interaction between miR-221-3p and TNF-α in the pathogenesis of psoriasis. Moreover, miR-221-3p could be utilized as a biomarker to diagnose psoriasis and to assess its severity. Future research is still required to investigate the miR-221-3p/TNF-α interaction in the pathogenesis of psoriasis through investigating their relationship in other psoriasis variants and the role of TIMP3 in this interaction. Additional studies that investigate the effect of various psoriasis treatments on miR-221-3p expression and its ability to monitor clinical outcomes are also needed, paving the way for developing new treatments that specifically target miR-221-3p expression.

Declarations:

Availability of data and material: Data are available upon request.

Competing interests: The authors declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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