Egyptian Journal of Medical Research

Print ISSN: 2682-4396 / Online ISSN: 2682-440X

Detection of Micro-RNA 511-5p in psoriasis patients and its correlation with disease severity

[1] Rehab Mohamed Naguib, [2] Mera Mohamed Galal Anan, [3]Shymaa Kamal El Din Abd El Rahman, [4] Nancy Magdy Mamdouh

[1]Assistant professor of dermatology and venerology, Faculty of Medicine, Beni-suef University

[2]Lecturer of Medical Microbiology and Immunology, Faculty of Medicine, Cairo University

[3]Lecturer of Clinical and Chemical Pathology, Faculty of Medicine, Beni- Suef University

[4]Lecturer of dermatology and venerology, Faculty of Medicine, Beni-suef University

Article Info

Corresponding Author:

Mera Mohamed Galal Anan

mira.hassan36203@postgrad.kasralainy.edu.eg

Keywords

Psoriasis

Micro RNA

rt-PCR.

Abstract

Background: A chronic inflammatory skin condition, psoriasis affects around 2-4% of people globally. The cutaneous manifestations of psoriasis are variable. MicroRNA are short, single-stranded, noncoding RNA molecules. People with psoriasis have been shown to express several miRNAs differently in their blood or psoriatic skin. **Aim:** In the current study we aimed to detect microRNA 511-5p in serum samples of psoriasis patients, correlate its presence to disease severity and compare its level between patients and control subjects. **Methods:** The research involved 30 patients who had psoriasis and 30 control individuals in Beni-Suef University hospital. Psoriasis diagnosis was confirmed according to the typical clinical presentation of psoriasis. The degree of psoriasis severity was evaluated using PASI score. Three cm peripheral blood were taken from all patients and control subjects. Levels of microRNA 511-5p in serum samples were determined using

reverse transcription PCR (rt-PCR). **Results:** A statistically significant difference was detected between miRNA 511-5p levels in cases and controls ($p < 0.001$). There is no statistically significant association was detected between miRNA 511-5p and PASI score ($p = 0.162$). the study revealed patients' ages and duration of illnesses had a positive correlation with baseline PASI. (p value = 0.04 and 0.001 respectively). **Conclusion:** we used qRT-PCR to examine Micro-RNA expression profile in psoriasis. MicroRNA 511-5p was significantly higher in psoriatic lesions, indicating that MicroRNA 511-5p might be used as a biomarker for psoriasis.

1. Introduction:

The distinctive feature of psoriasis, a persistent inflammatory skin condition, is scaly, indurated erythema. It has been identified as a systemic disease in addition to a skin condition. (1). The incidence of occurrence of psoriasis in developed countries is 1-4% (2). Psoriasis is believed to be caused by multifactor such as, lifestyle, infectious, environmental, and genetic factors.

Clinically, the most prevalent form is plaque psoriasis which presents as erythematous plaques with a silvery scale. A less typical forms include guttate, pustular, erythrodermic, and inverse psoriasis. Psoriasis is linked to number of comorbid conditions, such as depression, cancer, and cardiovascular disease (4).

The commonly used Psoriasis Area Severity Index (PASI) is a tool for figuring out the severity of psoriasis and direct the treatment options. Patients have been divided into two categories according to their PASI scores: those with mild disease and those with moderate-to-severe disease (5).

MicroRNAs (miRNAs) are defined as non-coding RNAs with a length of about 22–25 nucleotides that support the reduction in the expression of genes post-transcriptionally by repressing or damaging the messenger RNAs they target (mRNAs) (6). MiRNA-511-5p, a new miRNA for psoriasis, It has been proposed that it modulates myeloid cell activation and differentiation. (7). This study aimed to detect microRNA 511-5p in patients having psoriasis and compared its level with

healthy controls, it also helped in prediction of the disease severity.

2. Patients and methods:

We performed this cross-sectional study at the dermatology outpatient clinic, Beni-Suef University hospital, Faculty of Medicine, Beni-Suef University between January 2024 to June 2024. The research was performed in accordance with the Declaration of Helsinki and the protocol obtained clearance from the Research Ethical Committee (REC) of Beni-Suef University's Faculty of Medicine number (FMBSUREC/03102023/Anan).

Thirty psoriasis patients participated in the study and 30 matched controls from dermatology department clinic at Beni-Suef University. Informed written consent for photography and participation in the study was taken in addition to detailed patient history. Clinical assessment of the patients was done using PASI score (8). Peripheral blood samples of three cm were obtained from each participant. The obtained serum was kept at -80 °C until it was time for analysis in Microbiology and Immunology Department, Faculty of Medicine, Cairo University. Levels of microRNA 511-5p in serum samples was determined using reverse transcription PCR (rt-PCR).

This objective was achieved across 4consecutive steps:

1.Total RNA purification, including miRNA.

2.Quantitation of purified RNA, including miRNA.

3.miRNA reverse transcription into complementary DNA (cDNA).

4.Amplification and qRT-PCR analysis of the targeted miRNAs (miRNA 511-5p) and U6 as an internal control using SYBR Green detection.

Serum total RNA purification, including miRNAs

This step was performed by the usage of miRNeasy mini kit and purification methods of total RNA, including miRNA (Qiagen, Germany. Cat. No. 217004).

Principles

The miRNeasy Mini Kit purifies total RNA using silica membranes and lyses samples using phenol/guanidine. In order to promote tissue lysis, inhibit RNases, and remove the majority of the cellular proteins and DNA from the lysate through organic extraction, the kit included QIAzol Lysis Reagent, a phenol and guanidine thiocyanate monophasic solution.

Cells or tissue samples are standardized in QIAzol Lysis Reagent. Centrifugation is used to separate the homogenate into aqueous and organic phases following the addition of chloroform. Proteins part to the lower, organic phase or the interphase, whereas

RNA parts to the upper, aqueous phase and DNA to the interphase.

After extracting the uppermost aqueous phase, ethanol is added to establish the ideal conditions for binding for all RNA molecules equal or more than 18 nucleotides. The sample is subsequently transported to the RNeasy Mini spin column, where phenol and other impurities are successfully eliminated and total RNA binds to the membrane. High quality RNA is then eluted in RNase-free water.

Purified RNA quantitation, including miRNA

A purity assessment and RNA quantification were performed on the extracted RNA by the usage of NanoDrop® (ND)-1000 spectrophotometer (NanoDrop technologies, Inc. Wilmington, USA).

MiRNA reverse transcription into complementary DNA (cDNA)

This procedure carried out as a component of the miScript PCR system, which uses whole RNA, utilizing the miScript® II RT kit and protocol for RNA reverse transcription into cDNA (Qiagen, Germany. Cat. No. 218161). From a single cDNA synthesis, this technique enables the identification of several miRNAs. The kit miScript® II RT is supplied with two buffers, miScript HiSpec buffer and miScript HiFlex to satisfy the specific needs of qRT-PCR-based miRNA quantification investigations.

Mature miRNAs can be selectively converted into cDNA using the distinct formulation that is pending a patent of miScript HiSpec buffer. This cDNA can be utilized for miRNA quantification using miScript Primer Assays or miScript miRNA PCR Arrays, whereas all RNA species (mRNA, noncoding RNA, precursor miRNA, and mature miRNA) are converted into cDNA by the miScript HiFlex buffer, which can subsequently be utilized in qRT-PCR to measure each RNA species using the proper primer assays.

The main objective of this step is selective reverse transcription of mature miRNA only, miScript HiSpec buffer-based protocol is applied.

Detection of mature miRNA using qRT-PCR

This step was carried out as part of the miScript PCR system, which uses the miScript II RT kit with miScript HiSpec buffer produced cDNA as beginning material, the miScript SYBR® Green PCR kit and methodology for mature miRNA quantitative analysis (Qiagen, Germany. Cat. No. 218073) are used. This procedure made use of the target-specific miScript Primer Assay (forward primer) for miRNA-511-5p.

Principles:

Utilizing its reverse-transcribed cDNA, this methodology enables real-time PCR measurement of mature miRNA (by miScript HiSpec Buffer) as template for qRT-PCR analysis target miRNA-specific miScript Primer Assays (forward primers) and the miScript Universal Primer (reverse primer) are used in the miScript SYBR Green PCR Kit, which also contains the QuantiTect SYBR Green PCR Master Mix. The qRT-PCR cycler has been set up to run under the following cycling conditions:

Table 1: The qRT-PCR cycler cycling conditions

Step	**Time**	**Temperature**
Initial activation step	15 min	95 ^{0}C
3 steps cycling		
Denaturation	15 s	94 ^{0}C
Annealing	30 s	55 ^{0}C
Extension	30 s	70 ^{0}C
Cycle numbers	40 cycles	

Statistical methods:

The data was coded and entered using IBM Corp.'s statistical software for the social sciences (SPSS) version 28 (Armonk, NY, USA). The data was summarized using the mean and standard deviation for quantitative variables and the frequencies (number of cases) and relative frequencies (percentages) for categorical variables. When comparing two groups, the unpaired t test was used for comparisons, and when comparing more than two groups, the analysis of variance (ANOVA) with multiple comparisons post hoc test was used (9). Using the Chi square (χ^2) test, categorical data were compared. When the anticipated frequency was less than 5 (10), the exact test was employed instead. Statistical significance was determined as P-values below 0.05.

3. Results:

Clinical features of the patients:

1. Clinical data of psoriasis group:

-Age and sex:

In cases group, mean age was 36.93 (±12.59), 33.3% were females and 66.7% were males **(Table 3)**.

- Duration of illness:

In cases group mean disease onset was 4.69 (±4.79) and range from 2 months to 20 years. 43.3% had Progressive disease course and 56.6% had Stationary disease course. Mean disease duration was 6.66 (±5.7) and median 5.5 with range from 5 month to 22 years **(Table 2).**

Table (2): Disease onset in cases group.

	Count	
Onset (Year) **- Mean ± SD** **- Range**	**4.69 ± 4.79** **2 months - 20 years**	
	No.	**%**
Course **- Stationary** **- progressive**	 **17** **13**	 **56.6** **43.3**
Duration (months) **- Mean ± SD** **- Range**	**6.66 (±5.7)** **5-22 years**	

-Treatment regimens

Treatment regimens in case group was as follows; topical treatment with steroids or calcinurin inhibitors 56.7%, systemic treatment with acitretin or methotrexate 36.7%, Biological treatment with humira 6.7% (Figure 1).

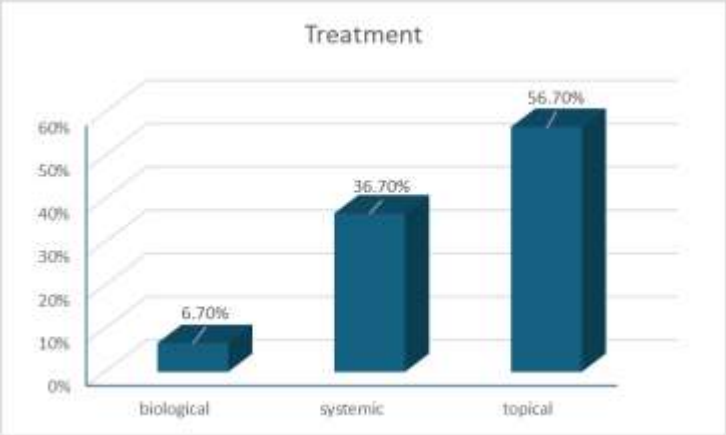

Figure (1): Treatment regimens in case group

- Disease history

Fifteen (15) patients had associated diseases (Hypertension and Diabetes mellitus) (50%)

Seven (7) patients had positive family history of psoriasis (23.3%).

2. Clinical data of control group:

Age and sex:

In control group, mean age was 37.9 ± SD 14.14 years, 33.3% were females and 66.7% were males **(Table 3).**

3. Comparing the clinical data of the cases and the controls.

- Age and sex in both groups:

In cases group, mean age was (36.93 ± SD 12.59 years), 66.7% were males and 33.3% were females. In Control group, mean age was (37.9 ± SD 14.14 years), 33.3% were females and 66.7% were males. No statistically significant differences was found between both groups regarding age (p = 0.781) and sex (p =1) **(Table 3, Figure 2).**

Table (3): demographics in cases and control groups.

	Cases		**Controls**		**P value**
Age (years) - Mean ± SD	36.93 ± 12.59 years		37.9 ± 14.14 years		0.781
	No.	**%**	**No.**	**%**	
Sex - Male - Female	20 10	66.7 33.3	20 10	66.7 33.3	1

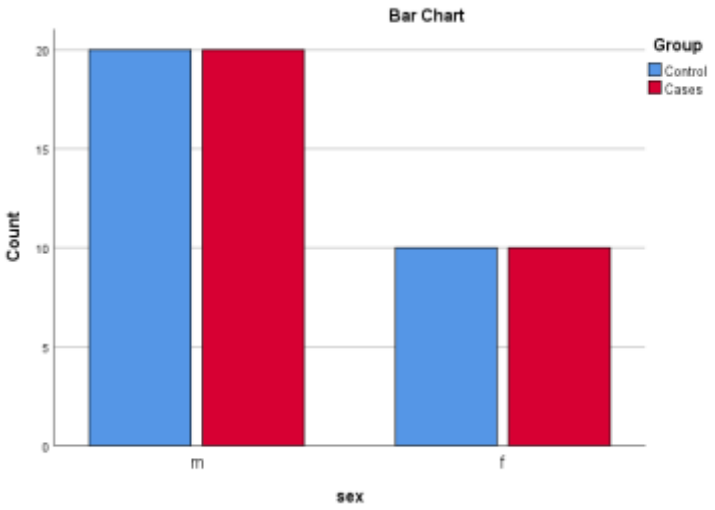

Figure (2): Gender distribution in both groups.

4- Correlation between PASI score and patients variables

Patients' ages and duration of illness had a positive correlation with baseline PASI (p value = 0.04 and 0.001 respectively) (Figure 3,4).

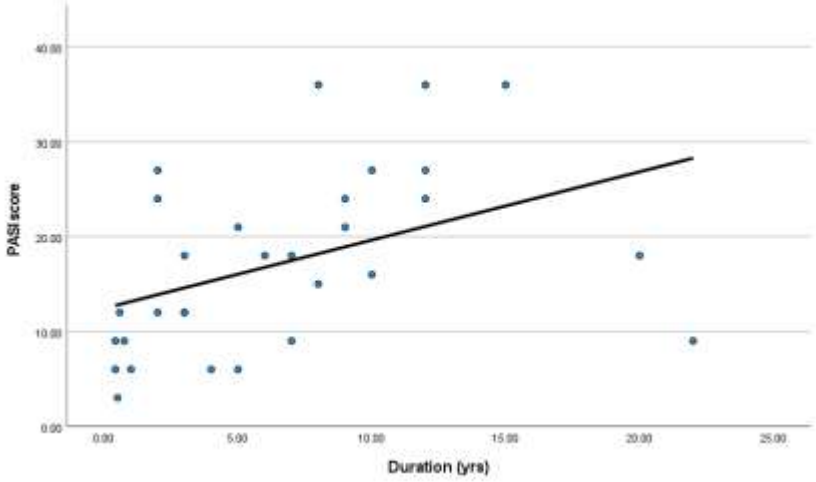

Figure (3): Correlation between PASI score and patients duration of illness

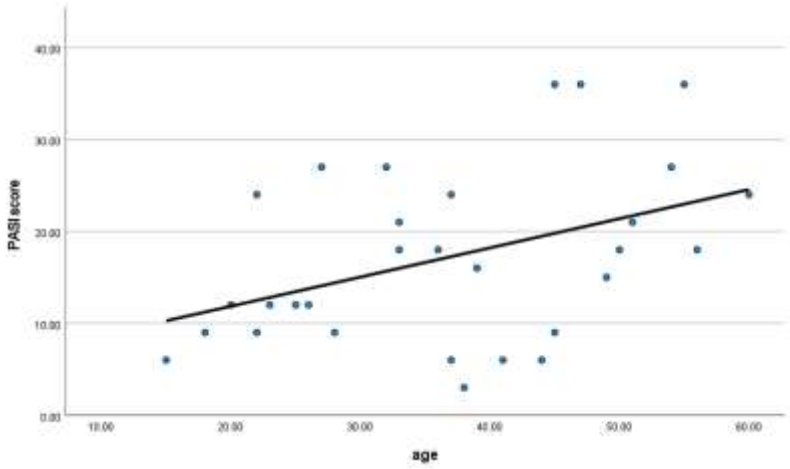

Figure (4): Correlation between PASI score and patients age

PASI score was higher in patients receiving biological treatments compared to those receiving systemic or topical treatments (p value = <0.001) (Figure 5).

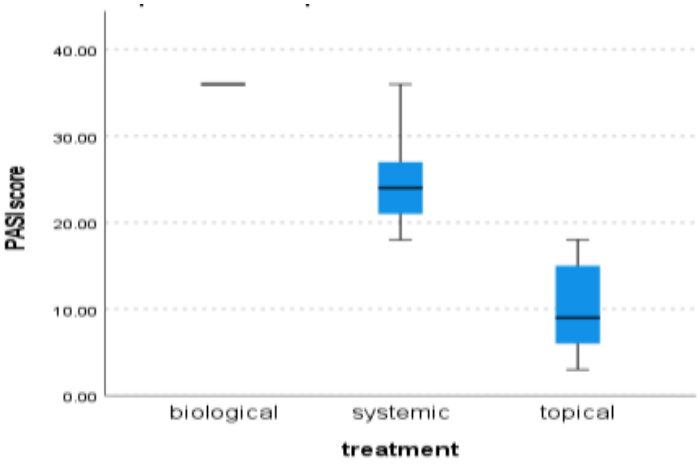

Figure (5): Correlation between PASI score and treatment options

The PASI score did not significantly correlate with the patient's sex or family history (Table 4).

Table (4): Correlation between PASI score and patients sex, family history and treatment methods:

		PASI score					
		Mean	**SD**	**Median**	**Minimum**	**Maximum**	**P value**
Sex	**Male**	17	8.27	18	6	36	0.948
	Female	17.70	12.04	12	3	36	
Family history	**Positive**	18.43	7.63	18	9	27	0.501
	Negative	16.87	10.10	16	3	36	
Treatment	**Biological**	36	0.00	36	36	36	<0.001
	Systemic	23.73	6.07	24	12	36	
	Topical	10.82	4.81	9	3	18	

P value < 0.05 is significant

5- Results of laboratory investigations

Differential miRNA 511-5p in psoriasis patients and controls

The miRNA 511-5p expression was more prevalent in patient's sera compared to controls, with a mean 2.21± SD 0.44 in cases and a mean 1± SD 0.01 in controls.

Statistically significant difference was detected between miRNA 511-5p levels in cases and controls ($p < 0.001$) (Table 5).

Table (5): Differential miRNA 511-5p expression in patients and controls

		Group		P value
		Cases	**Controls**	
Micro-RNA 511-5p	Mean ± SD	2.21± 0.44	1 ± 0.1	< 0.001
	Minimum	1.56	0.98	
	Maximum	3.08	1.02	

P value < 0.05 is significant

Correlating the serum levels of miRNA 511-5p in patients with other variables

There were no statistically significant relationships found between serum expression of miRNA 511-5p and the patient's sex (p = 0.775), age (p = 0.781), duration (p = 0.28), receiving treatment (p = 0.261), positive family history (p = 0.828) (Table 6,7).

No statistically significant association foundbetween miRNA 511-5p and disease severity or PASI score (p = 0.162) (Table 7).

Table (6): Correlation between serum expression of miRNA 511-5p in patients with other variables

Cases		Micro RNA 511-5p					
		Mean	SD	Median	Minimum	Maximum	P value
Sex	Male	2.14	0.46	1.96	1.56	3.04	0.242
	Female	2.34	0.40	2.35	1.76	3.08	
Family history	Positive	2.24	0.55	2.08	1.56	3.08	0.828
	Negative	2.20	0.42	2.09	1.56	3.04	
Treatment	Topical	1.98	0.30	1.95	1.56	2.43	0.158
	Systemic	2.35	0.45	2.09	1.72	3.08	
	Biological	2.13	0.46	2.08	1.56	3.02	

Table (7): Correlation between serum expression of miRNA 511-5p in patients with disease severity and PASI score

Cases		Micro RNA 511-5p
Age	Correlation Coefficient	0.255
	P value	0.174
	N	30
PASI score	Correlation Coefficient	0.262
	P value	0.162
	N	30
Duration (yrs)	Correlation Coefficient	0.204
	P value	0.280
	N	30

4. Discussion:

As a clinically distinctive chronic skin condition, psoriasis manifests in different forms such as plaque, flexural, guttate, pustular or erythrodermic (11). Among the treatments for psoriasis are topical medications (corticosteroids and vitamin D analogs), phototherapy (narrowband ultraviolet B radiation, or NB-UVB), systemic medications (methotrexate, cyclosporin, and acitretin), small molecule inhibitors (dimethyl fumarate and apremilast), biologic drugs (tumor necrosis factor, or TNF), or inhibitors of interleukin (IL)-17 and IL-23 (11). As our knowledge of its pathophysiology has grown, highly effective and targeted treatments have been developed.

MicroRNAs (miRNAs) known as small, highly conserved, noncoding RNAs with a length of 21–24 nucleotides and function as critical post-transcriptional regulators (12). Growing investigation has revealed the significant miRNAs' role in the development of psoriasis by controlling keratinocyte hyperproliferation and aberrant differentiation, as well as atypical immune activation and inflammation reactions of the epidermis (13).

In the current study, thirty patients with psoriasis and thirty healthy individuals who were matched for age and sex were compared. The psoriasis group had 66.7% males and 33.3% femalesThe male preponderance was similar to research findings from Malaysia, Nepal, and the Maghreb (53.7%, 55.7%, and 56.6%, respectively) (14, 15, 16). On the other hand, Anani et al. (17) found that among Egyptian patients, psoriasis was more prevalent in women (70%) than in men. In many western counties, a female majority has also been noted, including Minnesota and Denmark (53.2%) (18, 19). Racial differences among the different populations examined were cited by several academics as the cause of the variation.

In the psoriasis group, the mean duration of illness in the psoriasis group was 6.66 ± SD 5.7years. Ali et al. (20), found that the duration of the condition ranged from one to 35 years (mean of 10.59 years). Agreed with Genc et al. (21) who reported disease duration in psoriasis patients ranged from one to forty years (mean: 13.8 ±12.0 years).

A positive family history is one of the risk factors for the development of psoriasis, which was present in seven cases (23.3%) in our study. Lower results were found by El-Komy et al. (22) who found that 10.5% of their patients reported disease history in their families. Our findings closely matched the

percentages found in research from China, Malaysia, and the Maghreb, who reported 28.6%, 23.1%, and 23.1 % of their patients reported psoriasis in their families, respectively (23, 24, 25).

These findings would be attributed to cultural and social variables, such as patients' denials that their family has a history of illness. For more precise data, greater investigation into the rate of family history is needed, ideally by family doctors.

In recent years, a variety of studies have examined the connection between cutaneous psoriasis and other illnesses. Evidence suggested that Psoriasis patients have a higher chance than the general population to have cardiovascular disease, obesity, diabetes, hypertension, dyslipidemia, metabolic syndrome, nonalcoholic fatty liver disease and cancer (26, 27). 50% of our patients had at least one of these comorbidities, with diabetes and hypertension being the most prevalent.

The mean PASI score among the study patients was 17.23 ± SD 9.48, it was high compared to study done by El-Komy et al., (22) whose score was (8.7 ± 0.09), this probably due to decreased patients′ compliance to the treatment. We observed significantly higher PASI score among older patients (p value = 0.04) because comorbidities and side effects from psoriasis treatment are more likely to occur in these patients. in agreement with our results, Fernandez-Torres et al., (28) found PASI score was significantly higher in older patients.

PASI score increased significantly with longer disease duration (p value = 0.001), this could be explained by the chronicity of the disease and the development of comorbidities Consistent with our research, El-Komy et al, (22) reported that the patient's age and the duration of illness both increased their PASI score ($P < 0.001$).

In the present study the miRNA 511-5p was higher in patient's sera compared to controls which was statistically significant ($p < 0.001$). A study done by Solvin et al., (7) reported that miRNA-511-5p, a novel miRNA for psoriasis has been proposed to impact myeloid cell activation and differentiation, and is also impacted by endogenous glucocorticoid levels.

On the other hand, a study done by Pierouli et al., (29) stated that TNF resistance results from miR-511-5p's targeting of the p55 TNF receptor mRNA, in cases where there is increased expression of this miRNA due to elevated levels of endogenous glucocorticoids. Another study done by Puimège et al., (30) stated that

overexpression of miR- 511 suppresses tumor necrosis factor receptor-1 (TNFR-1) and guards against TNF, whereas anti-miR-511 increases TNFR-1 and makes individuals more sensitive to TNF.

In the present research, the PASI score and disease severity did not significantly correlate with miRNA 511-5p (p = 0.162). these results agreed with the study done by Alatas et al., (31) who stated that no statistically relevant relation between miRNAs and disease severity.

In conclusion, we used qRT-PCR to search for the Micro-RNA expression profile in psoriasis. MicroRNA 511-5p was significantly higher in psoriatic lesions, indicating that MicroRNA 511-5p might be a good biomarker for psoriasis.

This study had limitations that should be considered. Firstly, number of clinical samples was limited. Secondly, future studies are needed to screen more differentially expressed Micro-RNAs and lastly, to correlate the levels of miRNA511-5p with levels of other Micro-RNAs.

5. References:

1- Kamata M, Tada Y. Efficacy and Safety of Biologics for Psoriasis and Psoriatic Arthritis and Their Impact on Comorbidities: A Literature Review. Int J Mol Sci. 2020 Mar 1;21(5):1690. doi: 10.3390/ijms21051690. PMID: 32121574; PMCID: PMC7084606.

2- Banaszczyk K. Risankizumab in the treatment of psoriasis - literature review. Reumatologia. 2019;57(3):158-162. doi: 10.5114/reum.2019.86426. Epub 2019 Jun 28. PMID: 31462831; PMCID: PMC6710847.

3- Giampiero Girolomoni, Paolo Gisondi and Anna Chiara Fostini (2017): psoriasis and metabolic syndrome, J Eur Acad Dermatol Venereol ;36 (1),21-28.

4- Okubo Y, Takahashi H, Hino R, Endo K, Kikuchi S, Ozeki Y. et al., Efficacy and Safety of Apremilast in the Treatment of Patients with Mild-to-Moderate Psoriasis in Japan: Results from PROMINENT, A Phase 3b, Open-Label, Single-Arm Study. Dermatol Ther (Heidelb). 2022 Jun;12(6):1469-1480. doi: 10.1007/s13555-022-00747-5. Epub 2022 Jun 11. PMID: 35689737; PMCID: PMC9209617.

5- Llamas-Velasco M, de la Cueva P, Notario J, Martínez-Pilar L, Martorell A, Moreno-Ramírez D. Moderate Psoriasis: A Proposed Definition. Actas Dermosifiliogr. 2017 Dec;108(10):911-917. English, Spanish. doi: 10.1016/j.ad.2017.07.002. Epub 2017 Aug 17. PMID: 28823420.

6- Masalha M, Sidi Y, Avni D. The contribution of feedback loops between

miRNAs, cytokines and growth factors to the pathogenesis of psoriasis. Exp Dermatol. 2018 Jun;27(6):603-610. doi: 10.1111/exd.13520. Epub 2018 Apr 10. PMID: 29479749.

7- Solvin ÅØ, Chawla K, Olsen LC, Hegre SA, Danielsen K, Jenssen M, Furberg AS, Saunes M, Hveem K, Saetrom P, Løset M. MicroRNA profiling of psoriatic skin identifies 11 miRNAs associated with disease severity. Exp Dermatol. 2022 Apr;31(4):535-547. doi: 10.1111/exd.14497. Epub 2021 Nov 17. PMID: 34748247.

8- Fredriksson T, Pettersson U. Severe psoriasis--oral therapy with a new retinoid. Dermatologica. 1978;157(4):238-44. doi: 10.1159/000250839. PMID: 357213.

9- Chan YH. Biostatistics102: Quantitative Data – Parametric & Non-parametric Tests. Singapore Medical Journal 2003a;44(8): 391-396.

10- Chan YH. Biostatistics 103: Qualitative Data –Tests of Independence. Singapore Medical Journal 2003b;44(10): 498-503.

11- Raharja A, Mahil SK, Barker JN. Psoriasis: a brief overview. Clin Med (Lond). 2021 May;21(3):170-173. doi: 10.7861/clinmed.2021-0257. PMID: 34001566; PMCID: PMC8140694.

12- Ambros, V. The functions of animal microRNAs. Nature 431, 350–355 (2004).

13- Jiang NJ, Yin YN, Lin J, Li WY, Long DR, Mei L. MicroRNA-21 in gynecological cancers: From molecular pathogenesis to clinical significance. Pathol Res Pract. 2023 Aug;248:154630. doi: 10.1016/j.prp.2023.154630. Epub 2023 Jun 21. PMID: 37393665.

14- Mikrani, J. A., & Shrestha, A. Clinical and epidemiological features of psoriasis in patients visiting Lumbini Medical College. Journal of Lumbini Medical College. 2014; 2(1), 1-3.

15- Mohd Affandi A, Khan I, Ngah Saaya N. Epidemiology and Clinical Features of Adult Patients with Psoriasis in Malaysia: 10-Year Review from the Malaysian Psoriasis Registry (2007-2016). Dermatol Res Pract. 2018 Apr 23;2018:4371471. doi: 10.1155/2018/4371471. PMID: 29849578; PMCID: PMC5937568.

16- Shalom G, Zisman D, Babaev M, Horev A, Tiosano S, Schonmann Y, et al. Psoriasis in Israel: demographic, epidemiology, and healthcare services utilization. Int J Dermatol. (2018) 57:1068–74. doi: 10.1111/ijd. 14130.

17- Anani HAA, Tawfeik AM, Maklad SS, Kamel AM, El-Said EE, Farag AS. Circulating Cell-Free DNA as Inflammatory Marker in Egyptian Psoriasis Patients. Psoriasis (Auckl). 2020 May 21;10:13-21.

doi: 10.2147/PTT.S241750. PMID: 32607312; PMCID: PMC7293414.

18- Bilgili ME, Yildiz H, Sarici G. Prevalence of skin diseases in a dermatology outpatient clinic in Turkey. A cross-sectional, retrospective study. J Dermatol Case Rep. 2013 Dec 30;7(4):108-12. doi: 10.3315/jdcr.2013.1156. PMID: 24421863; PMCID: PMC3888779..

19- Egeberg A, Sørensen JA, Gislason GH, Knop FK, Skov L. Incidence and Prognosis of Psoriasis and Psoriatic Arthritis in Patients Undergoing Bariatric Surgery. JAMA Surg. 2017 Apr 1;152(4):344-349. doi: 10.1001/jamasurg.2016.4610. Erratum in: JAMA Surg. 2018 Jul 1;153(7):692. doi: 10.1001/jamasurg.2018.0253. PMID: 28002543.

20- Ali G, Elsayed AK, Nandakumar M, Bashir M, Younis I, Abu Aqel Y, Memon B, Temanni R, Abubaker F, Taheri S, Abdelalim EM. Keratinocytes Derived from Patient-Specific Induced Pluripotent Stem Cells Recapitulate the Genetic Signature of Psoriasis Disease. Stem Cells Dev. 2020 Apr 1;29(7):383-400. doi: 10.1089/scd.2019.0150. Epub 2020 Mar 2. PMID: 31996098; PMCID: PMC7153648.

21- Genc M, Can M, Guven B, Cinar S, Buyukuysal C, Acikgoz B, Mungan AG, Acikgoz S. Evaluation of Serum Fetuin-A and Osteoprotegerin Levels in Patients with Psoriasis. Indian J Clin Biochem. 2017 Mar;32(1):90-94. doi: 10.1007/s12291-016-0570-0. Epub 2016 May 6. PMID: 28149018; PMCID: PMC5247368.

22- El-Komy MHM, Mashaly H, Sayed KS, Hafez V, El-Mesidy MS, Said ER, Amer MA, AlOrbani AM, Saadi DG, El-Kalioby M, Eid RO, Azzazi Y, El Sayed H, Samir N, Salem MR, El Desouky ED, Zaher HAE, Rasheed H. Clinical and epidemiologic features of psoriasis patients in an Egyptian medical center. JAAD Int. 2020 Jul 21;1(2):81-90. doi: 10.1016/j.jdin.2020.06.002. PMID: 34409325; PMCID: PMC8362248.

23- Altobelli E, Petrocelli R, Marziliano C, Fargnoli MC, Maccarone M, Chimenti S, Peris K. Family history of psoriasis and age at disease onset in Italian patients with psoriasis. Br J Dermatol. 2007 Jun;156(6):1400-1. doi: 10.1111/j.1365-2133.2007.07906.x. PMID: 17535245.

24- Ammar-Khodja A, Benkaidali I, Bouadjar B, Serradj A, Titi A, Benchikhi H, Amal S, Hassam B, Sekkat A, Mernissi FZ, Mokhtar I, Dahoui R, Denguezli M, Doss N, Turki H. EPIMAG: International Cross-Sectional Epidemiological Psoriasis Study in the Maghreb. Dermatology. 2015;231(2):134-44. doi:

10.1159/000382123. Epub 2015 Jun 19. PMID: 26111474.

25- Chen K, Wang G, Jin H, Xu J, Zhu X, Zheng M, Gu H. Clinic characteristics of psoriasis in China: a nationwide survey in over 12000 patients. Oncotarget. 2017 Jul 11;8(28):46381-46389. doi: 10.18632/oncotarget.18453. PMID: 28637026; PMCID: PMC5542274.

26- Ni C, Chiu MW. Psoriasis and comorbidities: links and risks. Clin Cosmet Investig Dermatol. 2014 Apr 17;7:119-32. doi: 10.2147/CCID.S44843. PMID: 24790463; PMCID: PMC4000177.

27- Oliveira Mde F, Rocha Bde O, Duarte GV. Psoriasis: classical and emerging comorbidities. An Bras Dermatol. 2015 Jan-Feb;90(1):9-20. doi: 10.1590/abd1806-4841.20153038. PMID: 25672294; PMCID: PMC4323693.

28- Fernandez-Torres RM, Paradela S, Fonseca E. Psoriasis in patients older than 65 years. A comparative study with younger adult psoriatic patients. J Nutr Health Aging. 2012;16(6):586-91. doi: 10.1007/s12603-012-0009-0. PMID: 22660002.

29- Pierouli K, Papageorgiou L, Mitsis T, Papakonstantinou E, Diakou I, Leptidis S, Sigala M, Dragoumani K, Spandidos DA, Bacopoulou F, Chrousos GP, Goulielmos GN, Eliopoulos E, Vlachakis D. Role of microRNAs and long non- coding RNAs in glucocorticoid signaling (Review). Int J Mol Med. 2022 Dec;50(6):147. doi: 10.3892/ijmm.2022.5203. Epub 2022 Nov 11. PMID: 36367164; PMCID: PMC9662139.

30- Puimège L, Van Hauwermeiren F, Steeland S, Van Ryckeghem S, Vandewalle J, Lodens S, Dejager L, Vandevyver S, Staelens J, Timmermans S, Vandenbroucke RE, Libert C. Glucocorticoid-induced microRNA-511 protects against TNF by down-regulating TNFR1. EMBO Mol Med. 2015 Aug;7(8):1004-17. doi: 10.15252/emmm.201405010. PMID: 25995337; PMCID: PMC4551340.

31- Alatas ET, Kara M, Dogan G, Akın Belli A. Blood microRNA expressions in patients with mild to moderate psoriasis and the relationship between microRNAs and psoriasis activity. An Bras Dermatol. 2020 Nov-Dec;95(6):702-707. doi: 10.1016/j.abd.2020.07.001. Epub 2020 Aug 5. PMID: 32811699; PMCID: PMC7672403.