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Original Article

Expression of miRNA-106b, miRNA-223 and miRNA-125a in Plaque Psoriasis and Their Association with Disease Severity

Dina Menesy [1*]; Emad Elgmal [2]; Sherif Ibrahim Elghannam[3]; Ibrahim Fouda[2]

[1] General Organization for Teaching Hospitals and Institutes, Damanhour Medical National Institute, Damanhour, Egypt.
[2] Department of Dermatology, Venereology and Andrology, Damietta Faculty of Medicine, Al-Azhar University, Damietta, Egypt.
[3] Department of Clinical Pathology, Damietta Faculty of Medicine, Al-Azhar University, Damietta, Egypt.

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*Corresponding author
Email: dinamenesy@yahoo.com

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ABSTRACT

Background: Psoriasis is systemic, immune-mediated skin disease. Recent advances in pathogenesis have led to the importance of microRNAs [miRNAs] detected in sera of patients which were found to be up-regulated or down-regulated and showed correlations with PASI score. Thus, miRNAs could serve as promising biomarkers for diagnosis, progression and significant target for the treatment of psoriasis.

Aim of the work: This study aimed to evaluate the role of miRNA-106b, miRNA-223, and miRNA-125a in psoriasis and to investigate the relationship between their expression and the severity of disease.

Patients and methods: This prospective case control study was carried out on 30 psoriatic patients and 20 age and gender matched volunteers. The severity of the disease was graded by PASI score. miRNA-106b, miRNA-223, and miRNA-125a were analyzed by PCR.

Results: Expression of miRNA-106b and miRNA-125a were statistically significant up-regulated in the studied patients [p=0.001 and p=0.037, respectively], and expression of miRNA-223 was statistically significant down-regulated in the studied patients [p=0.003].

Conclusion: MicroRNA-106b and miRNA-125a were up-regulated and miRNA-223 was down-regulated in psoriatic patients. Those results support the role of miRNAs in the pathogenesis of psoriasis and could open the gate for their use as biomarkers for early diagnosis, prognosis and as new emerging targeted treatments of psoriasis in future.

Keywords: miRNA-106b; miRNA-125a; miRNA-223; Psoriasis.

INTRODUCTION

Psoriasis is chronic inflammatory systemic disease characterized by erythematous scaly lesions of skin [1]. Psoriasis occurs in individuals at any age, with a prevalence ranging from 2-3% in population and affects the quality of life of the patients [2, 3]. Psoriasis could be classified into mild, moderate, or severe disease according to psoriasis Area and Severity Index [PASI] [4].

Moderate to severe plaque psoriasis, is frequently associated with several comorbidities as psoriatic arthritis, metabolic syndrome, obesity, diabetes, dyslipidemia, fatty liver disease, cardiovascular, chronic kidney disease and psychological disorders [5].

The pathogenesis of psoriasis results from the interplay between many genetic and environmental factors, especially deregulated miRNAs and associated genes which have been indicated to be causative factors of the disease [3].

MicroRNAs are non-coding small RNA which function to regulate the gene expression, and recent evidences highlight the role of miRNA in the pathogenesis of various inflammatory and immune diseases. Expression of miRNA are disclosed to be involved in the regulation of hyperproliferation of keratinocytes, their abnormal differentiation and disordered activation of immunity [6].

Recent studies have shown that miRNAs are involved in pathogenesis of psoriasis [7]. Altered expression of miRNAs was first described in psoriasis in 2007 [8], more than 250 miRNAs have been detected to be differentially expressed [9]. Circulating miRNAs were identified in the sera of patients with psoriasis showed correlations with PASI scores and could serve as promising biomarkers for diagnosis, progression of the disease, and therapy evaluation [10].

MicroRNA-106b modulates angiogenesis in the endothelial cells by affecting the signal transducer and activator of transcription 3 [STAT3] expression [11].

The transcription factor STAT3 has emerged as key player in development and pathogenesis of psoriasis [12]. MiRNA-223 may affect the pathogenesis of psoriasis and so, it is potential target for treatment of psoriasis [13].

MicroRNA-125a have been proposed as important regulators for the innate immune and inflammatory responses in many inflammatory diseases [14].

THE AIM OF THE WORK

The aim of this work was to study the role of miRNA-106b, miRNA-223, and miRNA-125a in psoriasis and to investigate the relationship between their expression and severity of the disease using psoriasis area severity index [PASI] score.

PATIENTS AND METHODS

The prospective case control study will be conducted on 30 psoriasis patients and 20 age and gender matched normal healthy individuals as control, patients and control will be collected from the outpatient clinic of Dermatology, Andrology and STDs Department, Damietta faculty of medicine, Al-Azhar University Hospital between October 2021 and January 2023.

Methods:

This study was approved by Damietta faculty of medicine, Al-Azhar University ethics committee and was conducted in accordance with Declaration of Helsinki. All participants provided written informed consent after the procedure had been fully explained. Patients which are included in this study suffered from plaque psoriasis and above 18 years old. Patients having psoriasis other than plaque type [Erythrodermic psoriasis, pustular psoriasis patients with any systemic diseases [as liver failure, coronary artery disease, renal failure and malignancy], and patients who were pregnant or breastfeeding were excluded. Every patient was subjected to full history taking, general and dermatological examinations. Psoriasis area severity index [PASI] score was done for all patients to assess severity of the disease.

Blood samples: Three cc of blood was collected in the EDTA tubes. All blood samples were sent to the Clinical Pathology Department, Damietta Faculty of Medicine, Al-Azhar University and Centrifugation of blood sample for 10 minutes at 1500 × g was done to obtain EDTA plasma, which was separated and stored at – 80 °C till further processing.

Molecular analysis: Using the miRNeasy Mini Kit [TaqMan® Small RNA Assays], mi-RNA was isolated in accordance with the manufacturer's recommended protocol. Then, microRNA RT Kit [TaqMan®] was used to convert obtained RNAs into a computational DNA [cDNA]. Levels of miRNA expressions were determined by StepOne™ Real Time PCR also manufactured by Applied Biosystems™. miRNA-106b, miRNA-223, and miRNA-125a as internal control were analyzed.

Statistical analysis:

Data analysis was performed by the SPSS software, version 18 [SPSS Inc., PASW statistics for windows version 18. Chicago: SPSS Inc.]. Quantitative data were described using the arithmetic mean [X], standard deviation [SD], percentage [%] and the median for the normally distributed data after testing normality using the Kolmogrov-Smirnov test / Shapiro Wilk test. Results were considered statically significant for $P \leq 0.05$. Chi-Square [X^2- test], Fischer exact test, Monte Carlo tests were used to compare the qualitative data between groups as appropriate. Mann Whitney U and Kurskal Wallis test were used to compare between the two studied groups and more than two studied groups, respectively for non-normally distributed data**.** The student t test was used to compare two independent groups for normally distributed data. Spearman's rank order correlation is used to detect the strength and direction of linear relationship between two non-normally distributed continuous variables and ordinal variables**.** The receiver operating characteristics curve [ROC curve] was used to calculate sensitivity and specificity [validity] of variables with calculation of the best cut off point. Predictive values and the accuracy are determined using cross tabulation.

RESULTS

Socio-demographic and anthropometric characteristics of the studied groups: Clinical criteria of psoriatic patients were demonstrated in Table 1. There were no significant differences between patients and controls regarding demographic and clinical data except for smoking habits, which was more frequent among patients' group.

Distribution of studied cases according to family history and clinical examination: The median disease duration was 9.5 years ranging from 1 to 30 years and 100% of the studied cases have skin affection and pruritus, 30% Koebner, 23.3% scalp and 6% nail affection [Table 2].

Distribution of studied cases according to PASI score: Median PASI score was 6.6 ranging from 2.4 to 19, 70% moderate disease and 30% severe disease [Table 3].

Comparison of miRNA-106b, miRNA-125a and miRNA-223 between studied groups: There was a statistically significant higher median miRNA-106b among patients than control group [0.775 versus 0.27, respectively] P=0.001. Expression of miRNA-106b was statistically significant up-regulated in the studied patients when compared with control group [p=0.001] [Table 4, Figures 1,2]. There was statistically significant higher median miRNA-125a among patients than among control group [0.775 versus 0.27] P=0.013. Expression of miRNA-125a was statistically significant up-regulated in the studied patients when compared with control group [p=0.037]. There was statistically significant lower median miRNA-223 among patients than control group [0.465 versus 0.990] P=0.001. Expression of miRNA-223 was statistically significant down-regulated in the studied patients when compared with control group [p=0.003].

Relation between sociodemographic characteristics, clinical characters and miRNA-106b, miRNA-125a and miRNA-223 of the studied cases: There was no statistically significant relation between miRNA-106b, miRNA-223 and all other demographic and clinical characteristics. There was a statistically significant negative correlation between miRNA-125a and PASI score [r=-0.403 and P=0.027] [Table 5, Figure 3].

Table [1]: Socio-demographic and anthropometric characteristics of the studied groups

		Patients [n=30]	Control [n=20]	Test	p
Age/years	mean±SD	45.87±15.17	40.60±9.66	1.38	0.175
Sex [n, %]	Male	15[50]	10[50]	0.001	1.00
	Female	15[50]	10[50]		
Special habits [n, %]	non smoker	11[36.7]	16[80]	9.07	0.002
	Smoker	19[63.3]	4[20]		
Weight [**kg**]	mean±SD	91.83±14.87	86.6±13.28	1.27	0.210
Height [**cm**]	mean±SD	169.10±9.92	164.0±8.55	1.88	0.07
BMI[Kg/m^2]	mean±SD	32.13±4.76	32.25±4.61	0.083	0.935

Table [2]: Distribution of the studied cases according to family history and clinical examination

		n=30	%
Disease duration[years]	Median [min-max]	9.5[1-30]	
Family history	Negative	27	90.0
	Positive	3	10.0
Clinical examination	Skin	30	100.0
	Pruritis	30	100.0
	Koebner	9	30
	Scalp	7	23.3
	Nail	3	6.0

Table [3]: Distribution of the studied cases according to PASI score

		n=30	%
Disease severity [n,%]	**Moderate**	21	70.0
	Severe	9	30.0
PASI score	**Median [range]**	6.6[2.4-19]	

Table [4]: Comparison of miRNA-106b, miRNA-125a and miRNA-223 between studied groups**.**

		Patients N=30	Control N=20	Test	Test of significance
miRNA-106b	Median [range]	0.775[0.12-8.78]	0.27[0.10-3.8]	3.62	0.001*
	Down-regulated	5[16.7]	5[25]	14.55	<0.001*
	Normal	3[10]	1[5.0]		
	Upregulation	22[73.3]	14[70]		
miRNA-125a	Median [range]	1.27[0.03-63.76]	0.24[0.05-17.32]	2.49	0.013*
	Down-regulated	12[40]	14[70]	6.59	0.037*
	Normal	5[16.7]	4[20]		
	Upregulation	13[43.3]	2[10]		
miRNA-223	Median [range]	0.465[0.14-0.85]	0.990[0.25-6.25]	3.29	0.001*
	Down-regulated	15[50]	3[15]	11.81	0.003*
	Normal	15[50]	12[60]		
	Upregulation	0	5[25]		

Table [5]: Relation between sociodemographic characteristics, clinical characters and miRNA-106b, miRNA-125a and miRNA-223 of the studied cases.

		miRNA-106b	P value	miRNA-125a	P value	miRNA-223	P value
Age/years		r=-0.186	0.325	r=-0.225	0.233	r=-0.316	0.089
BMI		r=-0.284	0.128	r=-0.343	0.063	r=0.103	0.589
Disease duration		r=-0.311	0.094	r=-0.277	0.139	r=-0.008	0.968
PASI		r=-0.006	0.974	**r=-0.403**	**0.027***	r=0.284	0.128
Disease severity	**Moderate** **Severe**	0.77[0.12-8.78] 1.08[0.181.74]	0.248	1.29[0.05-63.76] 0.77[0.03-10.25]	0.483	0.43[0.14-0.85] [0.56-0.30-0.74]	0.603
Sex n [%]	Male Female	0.775[0.18-2.06] 0.815[0.12-8.78]	0.803	1.66[0.03-33.23] 0.765[0.05-63.76]	0.190	0.34[0.14-0.74] 0.55[0.15-0.85]	0.145
Occupation	Not working Manual worker Employee Student Housewife	0.785[0.18-1.74] 0.745[0.12-2.06] 0.84[0.7-3.24] 0.78[0.77-0.99] 0.63[0.49-8.78]	0.894	0.80[0.03-16.85] 0.615[0.05-12.86] 5.6[0.25-63.76] 17.93[1.46-33.23] 0.24[0.13-11.43]	0.172	0.45[0.14-0.74] 0.395[0.30-0.72] 0.32[0.22-0.64] 0.56[0.21-0.64] 0.77[0.15-0.85]	0.351
Marital status	Single married	0.780[0.70-0.99] 0.77[0.12-8.78]	0.824	5.6[0.25-33.23] 1.19[0.03-63.76]	0.148	0.520[0.21-0.64] 0.43[0.14-0.85]	0.867
Special habits	non smoker Smoker	0.72[0.18-8.78] 0.93[0.12-3.24]	0.175	1.46[0.13-33.23] 0.46[0.03-63.76]	0.272	0.56[0.14-0.85] 0.33[0.24-0.74]	0.204
Hypertension	-ve +ve	0.86[0.32-8.78] 0.63[0.12-1.75]	0.230	5.6[0.03-63.76] 0.46[0.12-2.06]	0.07	0.43[0.15-0.85] 0.50[0.14-0.83]	0.964
DM	-ve +ve	0.78[0.18-8.78] 0.72[0.12-1.25]	0.738	1.29[0.05-63.76] 0.24[0.03-5.6]	0.133	0.50[0.14-0.83] 0.33[0.29-0.85]	0.889
Family history	**-ve** **+ve**	0.77[0.12-8.78] 0.93[0.58-0.99]	0.917	1.19[0.03-63.76] 11.43[1.46-16.85]	0.120	0.43[0.14-0.85] 0.56[0.25-0.63]	0.972
Kobner	**-ve** **+ve**	0.78[0.52-8.78] 0.70[0.12-1.74]	0.357	5.6[0.05-63.76] 0.41[0.03-9.58]	0.027*	0.35[0.21-0.85] 0.54[0.14-0.83]	0.451
Scalp	**-ve** **+ve**	0.77[0.12-8.78] 0.91[0.49-3.24]	0.303	1.25[0.03-33.23] 1.29[0.13-63.76]	0.713	0.50[0.14-0.85] 0.36[0.22-0.83]	0.883
Nail	-ve +ve	0.77[0.12-8.78] 0.96[0.86-3.24]	0.178	1.25[0.03-33.23] 9.61[0.05-63.76]	0.557	0.52[0.14-0.85] 0.33[0.24-0.43]	0.299

Parameters described as median [min-max]

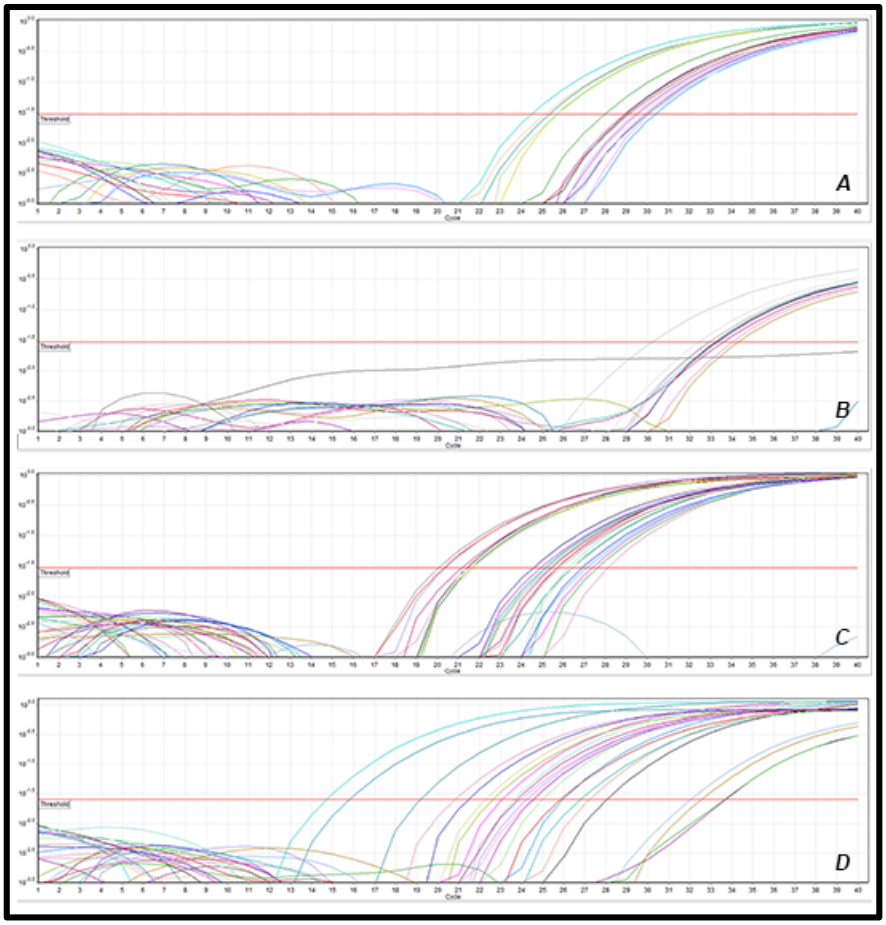

Figure [1]: Amplification curve of A: miRNA-106b, B: miRNA-125a, C: miRNA-223 and D: miRNA16 between studied groups

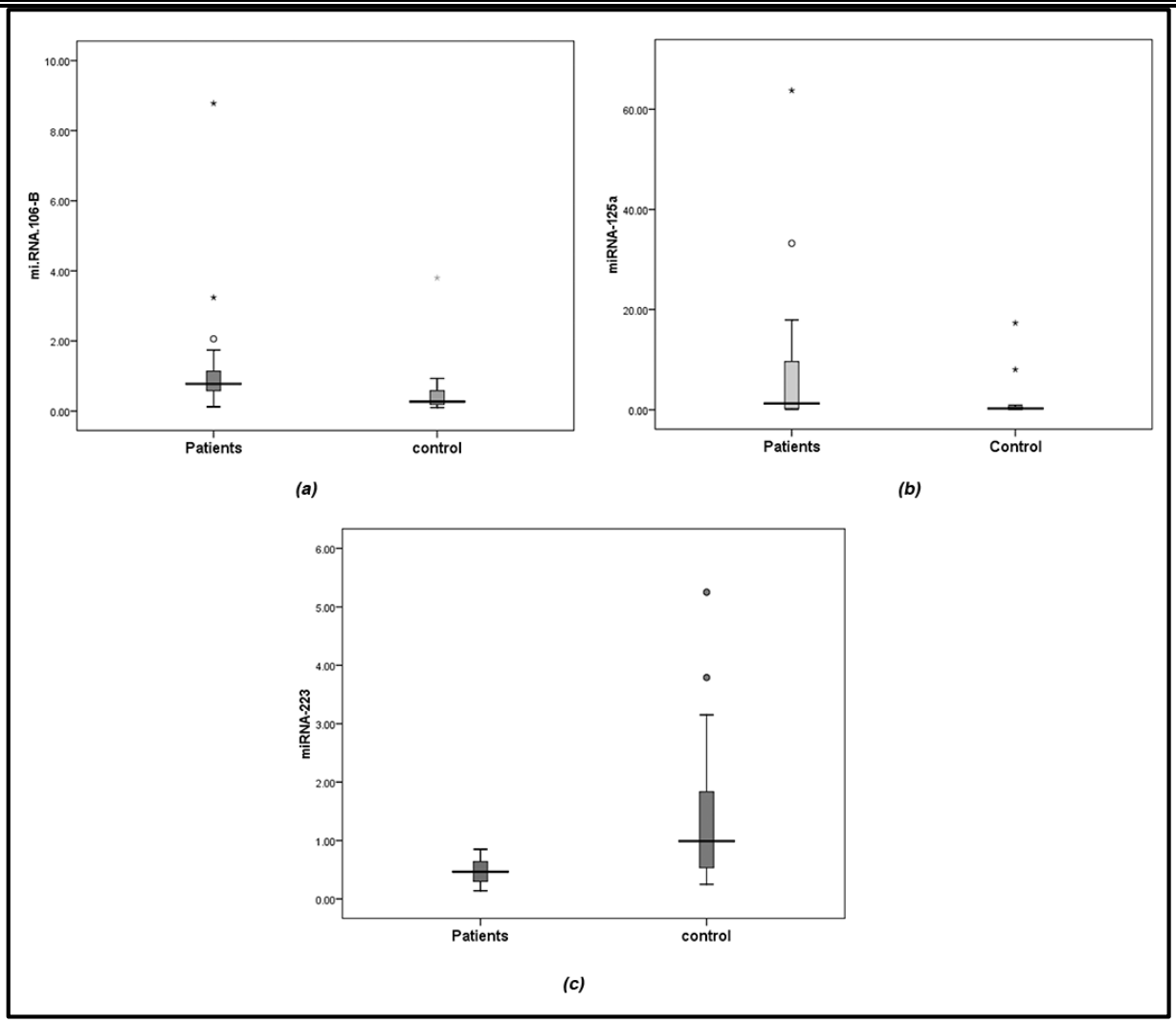

Figure [2]: [a] Box whisker plot showing median miRNA 106-b, [b] miRNA 125-a, and [c] miRNA-223 between patients and control group

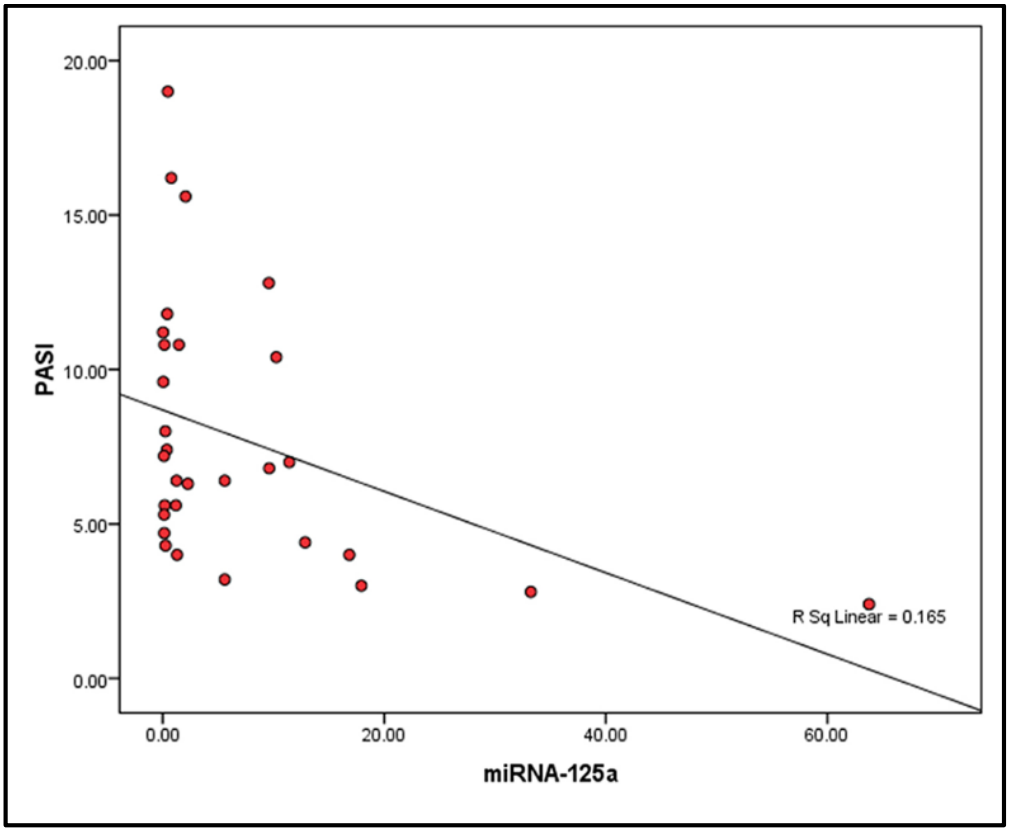

Figure [3]: Correlation between miRNA-125a and PASI score

DISCUSSION

Psoriasis is genetic and immune mediated skin disorder manifesting in the skin, joints or both. The etiology of psoriasis is related to several factors, as infection, medications, genetics and trauma [15].

Genomic studies have established psoriasis as Th-17/IL-23 dominated disease and guided the development of novel treatments. Although, the exact pathophysiological mechanisms are still unknown, and the regulatory mechanisms of psoriasis genetics have not been fully explained yet [16].

First miRNAs associated with the psoriasis were published in 2007. With increasing sample sizes, new technologies and analytical methods, several miRNAs have been detected in psoriasis. Several miRNAs have been shown to have central roles in the pathophysiology of psoriasis. Specific miRNAs show promise as biomarkers for the prognosis, disease activity, and treatment response, as well as targets for new epigenetic modifying therapies [16].

To the best of our knowledge, there are scarce number of studies that aimed to investigate the role of miRNA-106b, miRNA-223, and miRNA-125a in psoriasis and to investigate the relationship between their expression and severity of the disease using psoriasis area severity index [PASI] score. Expression of miRNA-106b was statistically significant up-regulated in the studied patients when compared with control group [p=0.001].

Although, **Alatas *et al.*** [11] found that the expression of 106b-5p was significantly down-regulated in the group of psoriasis when compared with the control group.

MicroRNA-106b modulates angiogenesis in endothelial cells by affecting STAT3 expression [11] which play a key role in development and pathogenesis of psoriasis. STAT3 hyperactivity has been reported to mediate the signaling of most cytokines, including interleukin IL-23/IL-17 [12].

Expression of miRNA-125a was statistically significant up-regulated in the studied patients when compared with control group [p=0.037].

Although, **Pei *et al.*** [6] found that miRNA-125a expression was decreased in psoriatic patients compared with healthy control.

MicroRNA-125a is reported to decrease proliferation of various cells, including adipocytes, endothelial cells and granulosa cells among others, suggesting that it could play inhibitory role in cell proliferation [17]. Also, there are evidences suggesting that miRNA-125a decreases secretion of inflammatory cytokines including TNF-a [18].

Expression of miRNA-223 was statistically significant down-regulated in the studied patients when compared with control group [p=0.003].

Also, **Alatas *et al.*** [11] found that expression of hsa-miR-223-3p was significantly down-regulated in psoriasis group when compared with the control group.

Although**, García-Rodríguez *et al.*** [19] found that Plasma miRNA-223 were increased in Psoriasis samples versus control.

MicroRNA-223 may influence pathogenesis of psoriasis and thus is potential target for psoriasis treatment [13].

MiRNA-223 increased the proliferation and inhibited apoptosis of IL-22 stimulated keratinocytes. Expression levels of miRNA-223 has been elevated in the peripheral blood mononuclear cells of patients with psoriasis and miRNA-223 have been increased in the plasma samples of patients [20].

It is known that IL23/17 pathway plays a major role in the pathogenesis of psoriasis and treatments which target this pathway, such as anti-IL17 [secukinumab], are effective in treatment of psoriasis [21].

There was no statistically significant relation between miRNA-106b and all other demographic and clinical characteristics [p>0.05].

Also, **Alatas *et al.*** [11] found that no significant association was observed between miRNA expressions and PASI score. There was statistically significant negative correlation between miRNA-125a and PASI score [r=-0.403]. Median miRNA-125a is lower among cases with associated disease than cases without associated disease [7.59 versus 0.435]. Median miRNA-125a is lower among cases with positive Koebner sign than cases with negative Koebner [0.41 versus 5.6] with statistically significant relationship between them.

Also, **Pei *et al.*** [6] there was a negative association between miR-125a expression with PASI score. MicroRNA-125a may be associated with inflammation and severity in psoriatic patients.

As, miRNA-125a may suppress the phagocytic and bactericidal activities through suppressing macrophage M1 functionality, and psoriatic patients had elevated levels of inflammatory cytokines [22]. MicroRNA-125a is of good predictive value for risk of psoriasis, which might also serve as potential biomarker for severity of the disease and treatment response [14].

In the current study, there was no statistically significant association between miRNA-223 and all other demographic and clinical characteristics [p>0.05]. Also**, Alatas *et al.*** [11] found that no significant association was observed between miRNA expressions and PASI score.

Although, **Løvendorf *et al.*** [23] and **Ghumra *et al.*** [24] found that expression of miRNA-223 in peripheral blood mononuclear cells correlates positively with PASI score.

Conclusion:

MicroRNA-106b and miRNA-125a were up-regulated in psoriatic patients and miRNA-223 was down-regulated in psoriatic patients. Those results support the role of miRNAs in the pathogenesis of psoriasis and may open the gate for their use as biomarkers for early diagnosis of psoriasis, to predict prognosis and can be used as new emerging targeted treatments of psoriasis in future.

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