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Original Article

Effects of acne management therapies on the levels of microRNAs and acne severity index- A randomized clinical trial

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Abstract

Background The study was aimed to identify the effects of acne treatment protocols to determine their impact on levels of miRNA and acne severity index of acne vulgaris patients after three months of treatment protocol.

Methods The outcome measures were analyzed on two parameters; levels of miRNA and acne severity index. The outcome parameters were recorded twice at baseline; before the start of the treatment session and after completion of 3 months of treatment session. Extraction of miRNA was performed from serum samples of patients using a TRIzol LS method.

Results It was estimated that isotretinoin was turned out to be effective in lowering the value of hsa-mir-223, hsa-mir-21, has-mir-155 and hsa-mir-4273 in comparison to topical clindamycin and IPL therapy whereas IPL treatment was turned out to be significantly better than clindamycin in lowering the value of hsa-mir-21.

Conclusion The study had concluded that levels of microRNAs rises among acne vulagris patients and that administration of isotretinoin significantly reduces the levels of miRNA.

Key words
Acne vulgaris; MicroRNAs; Isotretinoin; Clindamycin; Polymerase chain reaction.

Introduction

Acne is the 8th most commonly occurring skin disease that affects approximately 9.38% of all age group population across the globe.[1] According to number of studies the prevalence of disease varies with age affecting 26% of women and 12% of men in their middle adulthood.[2] Similarly in another study the prevalence of acne was estimated to around 60.7% among population in between age group of 13 to 18 years.[3] In Pakistan the prevalence as per the study conducted on undergraduate students was estimated to around 14.7% whereas in Turkey 60.7%, Malaysia 68.1% and in Saudia Arabia 14.3% of population develops acne.[4-5] Acne vulgaris (AV) is the common form of acne that significantly effects the social and psychological aspects of human life; by causing physical discomfort, permanent facial scarring, emotional stress and depressive disorders.[6] According to the recent guidelines put forwarded by American Academy of Dermatology (AAD) acne is classified into three categories that are mild, moderate and severe

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based upon its severity.[7] Mild acne according to AAD classification contains few inflammatory papules on and around forehead with post-inflammatory hyperpigmentation, moderate acne comprises of numerous inflammatory papules with limited scarring and open comedones whereas severe condition have many inflammatory papules, with both open and closed comedones and few nodules at jawline along with atrophic scarring.[7] Based upon the severity of disease the management guidelines have been designed in which benzoyl peroxide along with topical combination therapy and use of antibiotics are considered as a first line treatment for mild and moderate condition whereas in addition to same therapies as offered during mild and moderate conditions the use of oral isotretinoin has also been recommended as a first line therapy in severe conditions and also an alternative treatment approach for all those patients who developed resistance against first line therapy and in uncontrolled acne condition.[8-9] Studies have provided evidences that there are three main factors behind the pathogenesis of AV: 1) hyperseborrhea, 2) growth and proliferation of propinoibacterium acne and 3) keratinzation with formation of comedones.[10-11] Besides that evidences are also available in which the potential pathogenic impact of micro Ribonucleic acids (miRNAs) have also identified behind various skin conditions most prominently in melanoma and hence therefore in number of studies pathogenic role of miRNAs in various skin conditions like psoriasis, eczema atopic dermatitis and epidermal necrolysis has also been evident.[12-13] MicroRNAs inside human body regulates various important cellular processes such as cell growth, apoptosis (programme cell death), cell differentiation and migration besides that in extracellular spaces miRNAs are found in serum, urine and saliva and are therefore considered as an important biomarkers for diagnosis of various diseases as well.[13-14] Multiple studies were conducted by numbers of researchers in which the impact of various treatment strategies and acne management guidelines were discussed but very limited literatures are available on data search in which the impact of various treatment strategies were determined on miRNA levels. It is therefore in this study the authors are aimed to identify the efficacy of acne treatment protocols in the management of AV and to determine their impact on levels of miRNA and acne severity index of acne vulgaris patients after three months of treatment protocol.

Methods

A total number of n=75 acne vulgaris patients including both male and female population were recruited and allocated into three groups A, B and C based on envelope method of randomization technique. Study was performed in the outpatient department of Isra University Hospital, Hyderabad, Sindh, Pakistan. Each group comprises of n=25 participants to whom three different acne treatments were provided. Participants in group A were given isotretinoin 20mg/day orally for three month, for group B patients topical clindamycin was recommended twice a day for 3 months and for patients in group C intense pulse light (IPL) therapy session twice a month for three months was given; making a total of six session of IPL treatment for patients in group C. The outcome measures were analyzed on two parameters; levels of miRNA including two inflammatory microRNAs; miRNA223 and miRNA21 and two fibroids based microRNAs; miRNA155 and miRNA4273 and acne severity index. The outcome parameters were recorded twice at baseline; before the start of the treatment session and after completion of 3 months of treatment session. Extraction of miRNA was performed from serum samples of patients using a TRIzol LS method.[15] A Unites States based TRIzol LS

reagent (patent#5, 346,994) was used for the extraction of RNA and to keep integrity of RNA during homogenization and lysis step. Real Time Polymerized Chain Reaction (RT-PCR) for the purpose of quantification of miRNA was performed using a Bio-Rad CFX 96 real time system.[16] Cycle threshold (Ct) was determined for relative expression analysis of miRNAs. Inclusion criteria includes all those patients whom were diagnosed with acne and acne scarring of age greater than and equal to11 years whereas all those patients with secondary acne, major systemic diseases and undergoing hormonal therapy were excluded from the study. Study had completely followed the guidelines provided by Helsinki declaration for human subjects and the requirements of participant's autonomy, beneficence and non-malafescience was maintained throughout during the course of study. Consent from every participant recruited in the study was taken prior to their enrollment and all necessary information received from study participants' were kept with utmost secrecy.

Results

A total number of n=75 participants were equally divided into three groups n=25 participants in each group. The demographic description revealed that the mean age and standard deviation of the participants in group A was estimated to around 26.50±3.2years, in group B it was 27.12±1.2 years and in group C the age was 26.21±2.5 years. The demographic descriptions, numbers and percentages of male and female participants in each group were shown in **Table 1**.

Subjects in all the three groups were given intervention for acne management and the results of the interventional strategies were recorded on levels of four different micro RNAs that were hsa-mir-223, hsas-mir-21, has-mir-155 and hsa-mir-4273. Further the effects of acne management strategies were also determined on acne severity index.

The analysis of the findings had revealed that for within the group analysis all the treatment strategies were turned out to be effective in reducing the levels of micro RNAs after three months of intervention. For the purpose of determining the within group analysis paired t-test at 95% of confidence interval was applied and the results obtained were shown in **Table 2**.

Further for determining the effectiveness of interventional strategies one way ANOVA was applied and it was found that isotretinoin found to be effective over topical clindamycin and intense pulse light laser therapies in reducing the values of microRNAs among patients with acne vulgaris. It was estimated that isotretinoin was turned out to be effective in lowering the value of hsa-mir-223, has-mir-21, has-mir-155 and has-mir-4273 in comparison to topical clindamycin and IPL therapy whereas IPL treatment was turned out to be significantly better than clindamycin in lowering the value of has-mir-21 and also found to be effective though not statistical significantly effective than clindamycin in plummeting the levels of has-mir-155 and has-mir-4273. The descriptive analysis of the findings were provided in **Table 3**; **Figure 1,2**.

Further the effects of the treatment strategies were also monitored on acne severity index and it was found that all three interventional strategies were found to be significantly effective $p<0.05$ in improving acne severity

Table 1 Demographic description of participants in each of three groups.

Variables	*Mean age in years ± SD*	*Frequency of*	
		Male (%)	*Females (%)*
Group A	26.50±3.2	7 (28%)	18 (72%)
Group B	27.12±1.2	8 (32%)	17 (68%)
Group C	26.21±2.5	7 (28%)	18 (72%)

Table 2 Levels of MicroRNA before and after intervention.

Variables		*Pre mean±SD*	*Post mean±SD*	*MD±SD*	*t-stats*	*p-value*
Group A	hsa-mir- 223	161.34±6.73	132.32±6.83	-29.02±9.6	-15.03	<0.001
	hsa-mir- 21	228.14±10.81	200.7±13.56	-27.48±17.03	-8.05	<0.001
	hsa-mir- 155	28.10±0.81	15.04±0.79	-13.06±6.35	-10.28	<0.001
	hsa-mir- 4273	77.35±2.38	59.81±8.21	-17.53±7.98	-10.98	<0.001
Group B	hsa-mir- 223	168.5±9.43	165.06±9.24	-3.29±3.39	-4.85	0.001
	hsa-mir- 21	228.11±14.35	224.12±2.33	-3.99±6.19	-3.22	0.036
	hsa-mir- 155	24.27±3.48	22.51±2.77	-1.76±2.28	-3.84	0.0008
	hsa-mir- 4273	75.56±8.51	74.18±8.36	-1.38±1.25	-5.51	<0.001
Group C	hsa-mir- 223	153.77±9.65	150.77±11.99	-3.002±7.14	-2.1	0.04
	hsa-mir- 21	204.14±23.43	202.41±23.49	-1.72±1.58	-5.4	<0.001
	hsa-mir- 155	28.10±4.08	21.45±4.71	-6.64±7.52	-4.41	0.002
	hsa-mir- 4273	74.72±5.26	73.78±5.16	-0.94±0.7	-6.73	<0.001

Table 3 Analysis of variance for the purpose of determining the effectiveness of treatment strategies.

Variables	*Group*	*Mean±SD*	*F-ratio*	*p-value*	*Factors*	*p-value (Factor Difference)*
hsa-mir- 223	A	132.32±6.83	73.25	<0.001	B	<0.05
					C	<0.05
	B	165.06±9.24			A	<0.05
					C	<0.05
	C	150.77±11.99			B	<0.05
					A	<0.05
hsa-mir- 21	A	200.7±13.56	14.65	<0.001	B	<0.05
					C	>0.05
	B	224.11±11.68			A	<0.05
					C	<0.05
	C	202.41±23.49			B	<0.05
					A	>0.05
hsa-mir- 155	A	15.04±3.96	26.88	<0.001	B	<0.05
					C	<0.05
	B	22.51±2.77			A	<0.05
					C	>0.05
	C	21.45±4.71			B	<0.05
					A	>0.05
hsa-mir- 4273	A	59.81±8.21	30.59	<0.001	B	<0.05
					C	<0.05
	B	74.18±8.36			A	<0.05
					C	>0.05
	C	73.78±5.16			B	<0.05
					A	>0.05

index of patients with acne severity index the analysis of the finding was provided in **Table 4**. Further in term of effectiveness the application of one-way analysis of variance test revealed that isotretinoin were turned out to be significantly effective $p<0.05$ than topical clindamycin and IPL as shown in **Table 5**.

Discussion

The findings of this study had revealed that treatment based on oral isotretinoin found to be significantly effective in reducing the values of microRNAs and acne severity index of patients diagnosed with acne vulgaris. It was shown from the results that not only treatment based on isotretinoin was found to be effective but also turned out to be efficacious in comparison to topical clindamycin and intense pulsed light therapy protocols. Moreover both topical clindamycin and IPL therapy based treatment

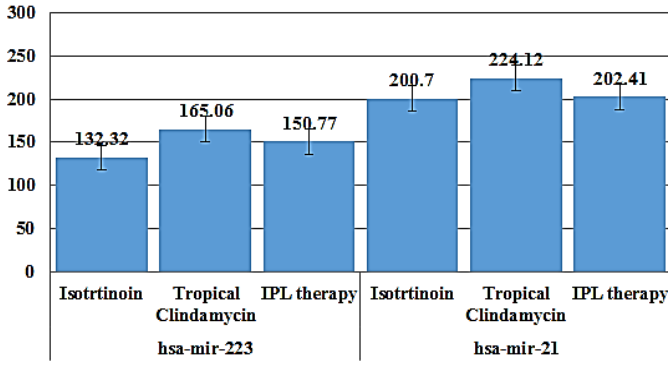

Figure 1 Levels of has-mir-233 and has-mir-21.

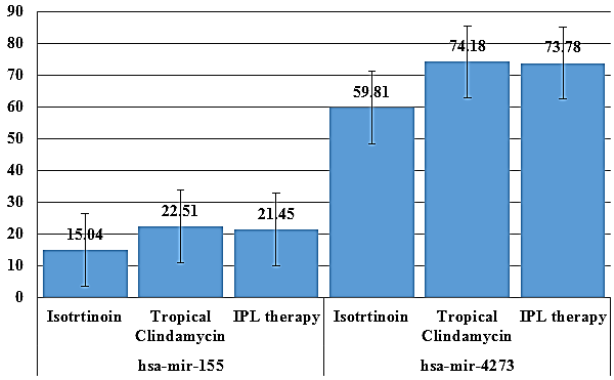

Figure 2 Levels of has-mir-155 and has-mir-4273.

Table 4 Within group analysis of treatment strategies on acne severity index (paired t-test).

Variables	*Pre mean±SD*	*Post mean±SD*	*MD±SD*	*p-value*
Group A	3.64±0.95	1.88±0.78	-1.76±0.83	<0.001
Group B	3.84± 1.06	2.8±0.91	-1.04±0.53	<0.001
Group C	3.8±0.86	2.88±0.78	-0.92±0.64	<0.001

protocol were also found to be effective in improving acne severity index and reducing miRNA levels significantly but both the treatment protocol have shown no such significant difference in comparison to each other and hence considered as equally effective and can be used as an alternative to each other. The findings of this study was according to the findings of another study in which the effects of isotretinoin was determined on inflammatory biomarkers like levels of platelets, platelets to lymphocyte ratio (PLR) and neutrophil lymphocyte levels (NLR) and the results had shown evidences that isotretinoin had significantly p<0.05 lowered the levels inflammatory markers after three months of intervention[17]. Similarly in another study conducted on acne vulgaris patients it was observed that treatment based on isotretinoin of 0.5 to 1 mg/kg/day for three months had significantly lowered p<0.05 the values of neutrophils, neutrophils to lymphocyte ratio (NLR), platelets to lymphocyte ratio (PLR) and over all systemic inflammation response index (SII).[18] In a Randomized Clinical Trial conducted to determine the effect of herbal gel versus clindamycin on total acne lesions and acne severity index it was revealed that although both the therapies were found to be effective but the effects of herbal therapy on the outcome measures that were total acne lesions and acne severity index was found to be better than topical clindamycin.[19]

A study that was conducted on thirty Iranian females to determine the effects benzoyl peroxide with intense pulse light therapy versus adapalene with IPL, it was found that both treatment strategies were found to be equally effective and combining IPL therapy with any of benzoyl peroxide and adapelene produces no such significant difference.[20]

Table 5 One way analysis of variance determining the effectiveness of treatment strategies on acne severity index.

Groups	*Mean±SD*	*F-ratio*	*p-value*	*Factors*	*p-value (Factor Difference)*
A	1.88	11.27	<0.001	B	<0.05
				C	<0.05
B	2.8			A	<0.05
				C	>0.05
C	2.88			A	<0.05
				B	>0.05

Similarly in another such study the combination of low level light therapy (LLT) combine with low dose oral isotretinoin was given to thirty six patients with mild to moderate levels of acne. Light therapy was given for 2 session per week for 2 weeks whereas dose of isotretinoin was 10-20 mg/day. Outcome measures after the 2 weeks of treatment revealed a significant effects where number of comedones, pustules, papules, melanin and erythema index were significantly reduced $p<0.05$.[21] It was thus observed after discussing the findings from various literatures that isotretinoin had much better results and that clindamycin and IPL approach can be a good adjunct therapy for acne vulgaris patients. However, literatures determining the effects of acne vulagris treatment strategies on levels of miRNA were scarce and hence it requires further collection of robust evidences.

Conclusion

The study had concluded that levels of microRNAs rises among acne vulagris patients and that administration of isotretinoin significantly reduces the levels of miRNA in comparison to clindamycin and IPL therapy. Further acne severity index also improves significantly with the use oral isotretinoin in comparison to clindamycin and IPL. Thus in term of efficacy it was concluded that isotretinoin was effective than clindamycin and IPL in the management of acne vulagris.

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