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Circular Ribonucleic Acid Expression in Patient with Acne

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Abstract:

Background: Acne is a common chronic skin disease with a multifactorial aetiology and pathogenesis. Recently, circular RNAs (circRNAs) have been identified as a key factor in regulating gene expression through circRNA–miRNA–mRNA networks in many biological processes and human diseases. However, the circRNAs expression in patients with acne is still unknown, **Aim and Objectives:** To evaluate circular RNA Expression in Patient with Acne and to correlate its level with acne severity, **Subjects and Methods:** this was a case control study was conducted at conducted at Dermatology outpatient clinic at Beni-Suef University hospital including 40 with acne vulgaris and 40 normal as control. The duration of the study ranged from 6 months, **Results:** The mean CIRCULAR RNA 0001073 for cases group was 0.47 (±0.3 SD) with range (0.11-1.2) and that the mean CIRCULAR RNA 0001073 for control group was 1.03 (±0.1 SD) with range (0.95-1.2), and There was high statistically significant difference between the studied groups as regard CIRCULAR RNA 0001073. **Conclusion:** This study firstly showed that circRNAs were differentially expressed in severe acne and suggested that circRNAs could be used as a potential biomarker for the drug targets of acne.

Keywords: CircRNA, Skin Diseases, Acne, Expression, Biomarker, Severity.

1. Introduction:

Acne vulgaris is a chronic inflammatory disease of the pilosebaceous unit, characterized by seborrhea, formation of comedones, erythematous papules, and pustules and less frequently by nodules, deep pustules, or pseudocysts (1). The primary and the pathognomonic lesion of acne is microcomedo, amicroscopic lesion invisible to the eye, which evolves commonly into inflammatory or noninflammatory lesions. The formation of microcomedo requires complex interplay of altered follicular keratinization, hyperplasia of sebaceous

glands, and overcolonization of sebaceous glands with Propionibacterium acnes (2).

Among adolescents, acne has prevalence over 90% (1). And persists into adulthood in approximately 12%–14% of cases with psychological and social implications (3). In some cases, acne is accompanied by scarring, a consequence of abnormal resolution or wound healing following the damage that occurs in the sebaceous follicle during acne inflammation, the scarring process can occur at any stage of acne (4).

The circRNAs are produced via pre-mRNA alternative splicing, whereby an upstream splice acceptor joins with a downstream splice donor through the back-splicing process (5).

The back-splicing results in different categories of circular RNAs, based on their sequence composition: exon-intron circRNA (EIciRNA), multi-exon circRNA, single exon circRNA and intronic circRNA. The exonic circRNA is composed of the binding of 3´ end of an exon with the 5´ end of either another upstream exon or the same exon. The net result of back-splicing is either single or multi-exon RNA. The formation of single and multi-exon RNA correlates with intronic sequence release, to produce intronic circRNAs, whereas the EIciRNAs retain introns (6).

Recent research has revealed that CircRNAs can regulate alternative splicing and transcription, and modulate the parental gene expression by acting as a miRNA sponge in various diseases (7).

2. Subjects and Methods:

Technical design:

1. Study design:

This study was a case-control study.

2. Setting:

This study was carried out at Dermatology outpatient clinic at Beni-Suef University hospital.

3. Time of the study: from June 2019 to December 2019

Topic	Period
Preparatory phase	One month
Design of examination sheet	Two months
Review of literature	One months
Collection, organization, entering of data and statistical analysis	Two months

4. Target population: Acne patients attending Beni-Suef University hospital.

Inclusion criteria:

- All patients enrolled in the study had: Different variants and degrees of severity of acne vulgaris.

Exclusion criteria:

- Any subject was excluded from the study if he/she is patients with other systemic diseases.

Sample size: 80: 40 with acne vulgaris and 40 normal as control.

Sample size was calculated based on online openepi calculator. The required sample was calculated at 95% two sided confidence interval (CI), 80% power and 1: 1 controls to cases ratio and least extreme Odds Ratio to be detected= 3.50; so the sample size will be 40 individual in each group (cases and controls). Results from OpenEpi, Version 3, open source calculator--SSCC

Sampling technique: This study was performed on Systematic random sampling.

Methods:

Each of the two groups were subjected to the following:

1. **Full history taking:**

- Including personal, Acne history as well as history of other Each skin diseases or drug intake.

2. **Full clinical examination:**

- Complete general examination.
- Complete cutaneous examination to evaluate the severity of acne, clinical diagnosis with severe acne (grade IV) was confirmed by at least two dermatologists according to the Pillsbury grading system (8).

3. **Genetic analysis of mRNA expression of Circ RNA 0001073:**

Were assessed by quantitative reverse transcriptase- polymerase chain reaction (qRT-PCR) technique (9).

Methods:

I. Sample Collection and Storage

a. Three mL peripheral venous blood samples were withdrawn from each subject by using vacutainer system. Samples were collected in serum separator tubes and allowed to clot for 15 minutes, and then centrifuged at 4000 Xg for 10 minutes. Sera were separated and stored at -80°C until the time of analysis.

b. Total RNA extraction and detection of fold change of the circHIPK3 and 124a gene using real time PCR.

II. Estimation of Circ RNA 0001073 expression levels in serum:

a) RNA extraction:

This step was performed using miRNeasy mini kit and protocol for purification of serum total RNA, including miRNA and circRNA (Direct-zol™ RNA MiniPrep Catalog No. #K0731).

Principle:

The Direct-zol™ RNA MiniPrep provides a streamlined method for the purification of up to 50 µg (per prep) of high-quality RNA directly from samples in TRI Reagent®.

Total RNA, including small RNAs (17-200 nt), is effectively isolated from a variety of sample sources (cells, tissues, serum, plasma, blood, biological liquids, etc.).

Isolation of RNA by conventional phase separation was shown to selectively enrich for some species of miRNA, leading to bias in

downstream analysis. The Direct-zol™ method assures unbiased recovery of small RNAs including CircRNA.

The procedure is easy. Simply a prepared sample is applied in TRI Reagent® directly to the Zymo-Spin™ IICR Column and then spinning washing, and elution of the RNA is done. No phase separation precipitation, or post-purification steps are necessary. The eluted RNA is high quality and suitable for subsequent molecular manipulation and analysis (including RT-PCR, transcription profiling, hybridization, sequencing etc.)

Buffer Preparation

- 10 ml ethanol (100%) was added to the 40 ml Direct-zol™ RNA PreWash concentrate, respectively.
- 48 ml 100% ethanol was added to the 12 ml RNA Wash Buffer concentrate.

Protocol

This protocol consists of two parts: (I) Sample Preparation and (II) RNA Purification.

(I) Sample Preparation

- 3 volumes TRI Reagent® was added to each liquid sample (300: 100) and mixed thoroughly for 5 minutes.
- To remove particulate debris, the supernatant was centrifuged and transferred into an RNase-free tube.
- RNA Purification is proceeded. (below).

(II) RNA Purification

All steps were performed at room temperature and centrifugation at 10,000 -16,000 x g for 30 seconds.

1. An equal volume ethanol (100%) was added to a sample lysed in TRI Reagent® and mixed thoroughly.
2. The mixture was transferred into a Zymo-Spin™ IICR Column2 in a Collection Tube and centrifuged. The column was transferred into a new collection tube and the flow wash discarded through.
3. 400 µl Direct-zol™ RNA PreWash5 was added to the column and centrifuged. The flow was discarded through and this step was repeated.
4. 700 µl RNA Wash Buffer5 was added to the column and centrifuged for 2 minutes to ensure complete removal of the wash buffer. The column was transferred carefully into an RNase-free tube.
5. To elute RNA, 50 µl of DNase/RNase-Free Water was added directly to the column matrix and centrifuged.

RNA was stored frozen at ≤ -70°C.

a) Quantitation and assessment of RNA purity:

RNA samples were subjected to RNA quantitation and purity assessment using the NanoDrop® (ND)-1000 spectrophotometer (NanoDrop Technologies, Inc. Wilmington, USA).

Blanking and Absorbance Calculations

1. The NanoDrop ND-1000 Spectrophotometer was ‘‘blanked’’ with 1 µL RNase free water, a spectrum was taken from it as a reference material (blank) and stored in memory as an array of light intensities by wavelength.
2. Measurement of a sample was taken where the intensity of light that has transmitted through the sample is recorded.

b) Reverse transcription of Circ RNA into complementary DNAs (cDNAs):

Reverse transcription (RT) was carried out on total RNA using COSMO cDNA synthesis Kit (willowfort- 1020500x – UK) as follows:

1. Reagents were allowed to thaw and place on ice. All solutions were mixed well before preparing the master mix& Checked for any signs of precipitation.
2. **Table (1)** was used as a guide to prepare the cDNA Master Mix. It was mixed well and briefly centrifuged.

Table (1): cDNA reagent per sample

Reagents per reaction tube	Volume
cDNA reaction buffer (5x)	4 □l
Nuclease- free water	To 20 □l
Rt Enzyme Mix	1 □l
Template RNA (4,0pg-400ng)	Volume varies

3. Master mix was Aliquoted into 0.2mL PCR tubes, before adding the template RNA.
4. Gently samples were mixed using a vortex and briefly centrifuged.
5. **Table (2)** was used as a guide to set up the thermocycler for cDNA synthesis.
6. Once the reaction is complete, the newly synthesized cDNA can be used for downstream applications. Using 5□l of the cDNA from the reaction in a PCR or qPCR application is recommended.

c) Quantitative Real-time PCR (qPCR) for Detection of Mature CircRNA:

This step was carried out using HERA SYBR® Green qPCR Kit (WF1030400X) (Willowfort, UK) and protocol for mature miRNAs quantitative detection.

Stages

1. HERA Enzyme Activation
2. Cycle denaturation
3. Primer annealing and extension

Table (2): Temperatures and timings for cDNA protocol

Stage	Temperature	Time
Stage1; primer annealing	25 °C	*5 mins
Stage2; Extension	**45°C	15mins
Stage3; Inactivation(optional)	80-85°C	5mins

Protocol for HERA SYBR® Green qPCR kit;

1. All reagents thawn and stored on ice. All reagents were mixed and centrifuged well

before preparing the master mix. Precipitation were Checked for.

2. All reagents were combined together in a nuclease free eppendorf, using the **Table (3)** as a guide, mixed thoroughly and briefly centrifuged.

Table (3): PCR reagent per sample

Component	Amount
HERA SYBR® Green Master Mix (2x)	10µl
Forward primers 20x (200nM)	1µl (up to 1µM each)
Reverse primers 20x (200nM)	1µl (up to 1µM each)
Nuclease free water	To 20µl
DNA template (up to 250ng)	Volume varies

3. The master mix was aliquoted into separate 0.2mL PCR tubes before adding the DNA template.
4. Gently mixed together and centrifuged briefly.
5. **Table (4)** was used as a guide to set up the thermal profile, considering the melting temperature of the primers. ROX was as the reference dye.

Table (4): Temperature and Times for the PCR protocol

Stage	Temperature	Time
Stage 1	95°C	2 min
Stage 2.1	95°C	*10sec
Stage 2.2	60°C	20-30sec
	Repeat stage 2 for 40 cycles	

6. Reactions were added and incubated into the qPCR thermocycler. During the last stage, HERA SYBR® Green intercalated with the amplicon to generate a fluorescent signal, which was detected by the qPCR instrument.
7. Once the reaction was completed, Ct value data was analysed for gene expression.

d) Calculation of results:

After completion of the PCR cycles, melting curve analyses were performed to validate the specific generation of the expected PCR product. Due to the lack of an endogenous reference housekeeping gene of CircRNA in the serum, (U6) was used to normalize the expression pattern and for relative quantification of the target miRNAs. The expression level of Circ RNA 0001073 was evaluated using the ΔCt method.

The cycle threshold (Ct) value is the number of qPCR cycles required for the fluorescent signal to cross a specified threshold.

ΔCt was calculated by subtracting the Ct values of (U6) from those of target micro-RNAs.

ΔΔCt was calculated by subtracting the ΔCt of the control samples from the ΔCt of the disease samples.

The fold change in CircRNA0001073 a expression was calculated by the equation 2–ΔΔCt.

The mathematical relationship between Ct, ΔCt, ΔΔCt and FC (Rq) is: ΔCt(RA patients)=

Ct (miRNA) – Ct (Endogenous control) ΔCt(control) = Ct (miRNA) – Ct (Endogenous control) ΔCt(RA patients)= Ct (ΔΔCt(RA patients)) – Ct (control) FC (Rq) = 2- ΔΔCt

If the FC is positive it means that the miRNA is upregulated; if the FC is negative it means it is downregulated. Control value was assumed equaled 1, because –ΔΔCt for control subjects equals zero and 2 0 equals.

- **Procedure:**

Primer selection:

For the selection of the ideal primer pair, the considered factors are included melting temperature (Tm: 60–65 °C), GC content (40%–60%) and amplicon length of about 90 - 200 bp.

Primer sequence for the studied target genes (CircRNA0001073) and reference housekeeping gene (18sR) were shown in **table (5)**.

Table (5): Primer sequence of circRNA 0001073 and β-actin

Gene symbol	Primer sequence from 5′- 3′
Circ RNA0001073	forward: 5′-AGTCAGTTCCTTGTGGAGCC-3′, reverse: 5′-GCATGGGTTCTGACGGACAT-3′).
β-actin	forward: 5′-CTCTGCCCGCATGAACCT-3′, reverse: 5′-CCACCATCCACATCCCAC-3′)

Figure (1): StepOne Real Time PCR, Applied Biosystems.

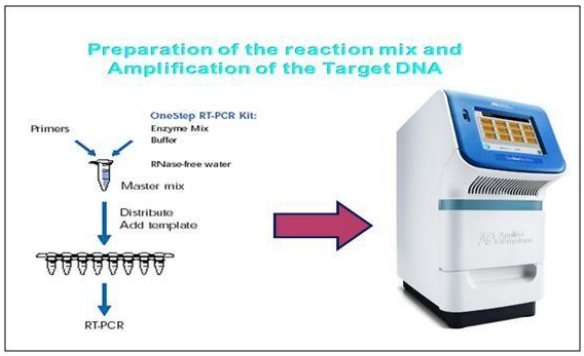

Figure (2): Preparation of master mix and amplification of the target DNA.

Statistical Methods:

Data were coded and entered using the statistical package SPSS version 22. Data were statistically described in terms of mean, standard deviation, analysis of variance (ANOVA) with multiple comparison post hoc test were used to compare quantitative variables between the studied groups. Correlations between quantitative variables

were done using Pearson correlation coefficient.

3. Results:

The current study included 40 acne vulgaris patients from both sexes **(Table 6).** They all presented to dermatology department at Beni-Suef University hospital. The acne vulgaris patients were (9) males and (31) females patients, their age ranged from (18) to (32) years, the average age was; 23.20±4.9. And 40 healthy controls were taken, they were age and sex matched to the acne vulgaris cases **(Table 7).**

Table (6): Data of Studied acne vulgaris Patients

No.	Gender	Age (years)	Severity	Disease Duration (years)	CIRCULAR RNA 0001073
1	Female	25	Mild	6	0.11
2	Female	18	Severe	0.1	1
3	Male	22	Moderate	0.1	1.2
4	Female	25	Severe	1.5	0.11
5	Female	20	Severe	2	0.5
6	Male	24	Mild	4	0.59
7	Male	20	Severe	0.2	0.23
8	Female	32	Mild	8	0.12
9	Male	30	Mild	2	0.73
10	Female	18	Moderate	0.2	0.58
11	Female	22	Severe	1	0.24
12	Female	18	Moderate	3	0.73
13	Female	31	Mild	7	0.2
14	Male	23	Mild	2	0.55
15	Female	19	Moderate	1.5	0.62
16	Male	20	Moderate	0.3	0.59
17	Female	27	Moderate	0.3	0.67
18	Female	19	Moderate	4	0.15
19	Female	32	Severe	0.1	0.7
20	Female	30	Mild	0.6	0.9
21	Female	18	Mild	2	0.21

22	Female	28	Moderate	5	0.28
23	Female	18	Mild	1	0.57
24	Female	19	Severe	3	0.3
25	Female	29	Severe	4	0.23
26	Female	32	Moderate	0.8	0.55
27	Male	20	Moderate	1	0.73
28	Female	26	Mild	0.1	0.74
29	Male	18	Moderate	2	0.56
31	Male	21	Severe	2	0.51
31	Female	30	Severe	0.5	0.9
32	Female	30	Moderate	0.7	0.13
33	Female	23	Severe	5	0.2
34	Female	23	Severe	3	0.24
35	Female	18	Severe	0.7	0.16
36	Female	18	Severe	0.2	0.73
37	Female	21	Mild	0.5	0.6
38	Female	23	Moderate	1	0.45
39	Female	18	Moderate	5	0.18
40	Female	20	Severe	1	0.19

Table (7): Data of Studied Healthy Controls

No.	Gender	Age (years)	CIRCULAR RNA 0001073
1	Female	30	1
2	Female	25	1.02
3	Female	35	1
4	Female	22	1.01
5	Male	25	1
6	Female	28	1.08
7	Female	21	1
8	Female	25	1.04
9	Female	20	1.02
10	Female	30	1
11	Female	22	1.05
12	Female	23	1
13	Female	25	1
14	Female	18	0.98
15	Female	20	0.95
16	Female	25	1.1
17	Female	21	1.06
18	Female	20	1
19	Female	18	1.03
20	Female	18	1
21	Female	19	1
22	Female	21	1.07
23	Female	22	1
24	Female	30	1.02
25	Female	25	0.99
26	Female	20	1.08
27	Male	22	1.2
28	Female	18	1.06
29	Female	25	1
30	Male	23	1.01

31	Male	26	1.05
32	Male	30	1
33	Female	21	1.09
34	Female	22	0.97
35	Female	18	1
36	Female	25	1
37	Male	30	1.06
38	Female	30	1.02
39	Female	25	1.05
40	Female	18	1

1) Descriptive Statistics

Table (8): Sex Distribution of the acne vulgaris Cases and Healthy Controls; (N= 80)

		N (%)		Total N= 80	p-value*
		acne vulgaris Patients N= 40	Healthy Controls N= 40		
Sex	**Male**	9 (22.5)	6 (15.0)	15 (18.8)	0.568
	Female	31 (77.5)	34 (85.0)	65 (81.3)	

**p-value >0.05 is considered non-significant by Chi-Square test.*

Table (8) demonstrates gender distribution of the cases and the controls; the acne vulgaris cases; 22.5% of them were males and 77.5% were females, while the controls; 15% were males and 85% were females.

There was no statistically significant difference between the cases and the control groups regarding sex (p-value> 0.05).

Table (9): Age Distribution of the Acne Vulgaris Cases and Healthy Controls; (N= 80)

Age (years)	Mean	SD	Minimum	Maximum	p-value
Acne Vulgaris Patients	23.20	4.9	18.00	32.00	0.752
Health y Controls	23.53	4.3	18.00	35.00	

**p-value >0.05 is considered non-significant by independent sample t-test.*

As illustrated in table (9); the average cases age was; 23.20±4.9 (SD) years, while average controls age was 23.53±4.3 (SD) years.

There was no statistically significant difference between cases and control groups regarding to age (p-value> 0.05).

Table (10): Severity of Acne among studied Acne Vulgaris Cases; (N= 40)

		Frequency	Valid Percent
Acne Severity	Mild	11	27.5
	Moderate	14	35.0
	Severe	15	37.5

Table (10) demonstrates that among studied Acne Vulgaris cases; 27.5% had mild acne, 35% had moderate acne and 37.5% of cases had severe acne disease.

Table (11): History of Disease Duration among studied Acne Vulgaris Cases; (N= 40)

	Mean	SD	Minimum	Maximum
Disease Duration (years)	2.06	2.1	0.10	8.0

Table (11) demonstrates the disease duration was ranged from (0.10) to (8) with a mean of 2.06 ±2.1 (SD) years of disease duration.

2) Analytical Statistics:

Table (12): Comparison between controls and Acne Vulgaries patients regarding CIRCULAR RNA 0001073 in expression blood samples

CIRCULAR RNA 0001073	**Mean**	**SD**	**Minimum**	**Maximum**	**p-value**
Acne Vulgaris Patients	0.47	0.3	0.11	1.20	**<0.001***
Healthy Controls	1.03	0.1	0.95	1.20	

** p-value < 0.05 is considered significant by independent sample t-test.*

As demonstrated in table (12); CIRCULAR RNA 0001073 in blood samples was significantly down-regulated in Acne Vagaris patients as compared with healthy controls; the mean Circular RNA levels (0.47 vs. 1.03) in blood samples from Acne Vulgaris patients and healthy controls respectively with a statistically significant p-value< 0.001

Table (13): Relation between severity of acne and Circular RNA 0001073

CRP	Mild acne (n=11)	Modrate acne (n = 14)	Severe acne (n = 15)	P
Min. – Max.	0.43– 0.69	0.44– 0.63	0.18– 0.39	<0.001*
Mean ± SD.	0.56± 0.19	0.53± 0.16	0.29± 0.19	
Sig. bet. Grps.	p1=0.725, p2<0.001*, p3<0.001			

p: p value for comparing between the studied groups

p1: p value for comparing between mild acne and moderate acne

p2: p value for comparing between mild acne and severe acne

p3: p value for comparing between moderate and severe acne

**: Statistically significant at p ≤ 0.05*

This table (13) shows that there is high significant relation between severity of acne and Circular RNA 0001073.

Table (14): Relation between CIRCULAR RNA 0001073 expression in blood samples and patients' gender in studied Acne Vulgaris patients; (N= 40)

	Gender	N	Mean	SD	Minimum	Maximum	p-value
CIRCULAR RNA 0001073	**Male**	9	0.63	0.3	0.23	1.20	0.055
	Female	31	0.43	0.3	0.11	1.00	

**p-value >0.05 is considered non-significant by independent sample t-test.*

As demonstrated in table (14); CIRCULAR RNA 0001073 expression in blood samples was slightly lower in female with Acne Vulgaris as compared with males; however no statistically significant difference was detected in relation between gender and CIRCULAR RNA 0001073 expression in blood samples (p-value= 0.055).

Table (15): Correlation between CIRCULAR RNA 0001073 expression in blood samples and Patients' Age in studied Acne Vulgaris patients; (N= 40)

	Age (years)	
CIRCULAR RNA 0001073	r = -0.045	p-value = **0.781**

r Spearman's correlation coefficient analysis

Table (15) demonstrates no detected significant linear correlation between CIRCULAR RNA 0001073 expression in blood samples and patients' age in studied Acne Vulgaris patients; (p-value >0.05).

Table (16): Correlation between CIRCULAR RNA 0001073 expression in blood samples and Disease Duration in studied Acne Vulgaris patients; (N= 40)

	Disease duration (years)	
CIRCULAR RNA 0001073	r = -0.592	p-value = **0.001***

r Spearman's correlation coefficient analysis

** p-value < 0.05 is considered significant*

Table (16) demonstrates a significant moderate negative linear correlation between CIRCULAR RNA 0001073 expression in blood samples and disease duration in studied Acne Vulgaris patients; (r= -0.592, p-value <0.001).

4. Discussion:

Acne is a common chronic skin disease arising from the pilosebaceous unit, characterized by inflammatory, non-inflammatory lesions, seborrhoea and/or various degrees of scarring (10).

It is one of the most prevalent skin conditions, affecting more than 85% of teenagers. The pathologic formation of an acne lesion requires complex interplay of follicular hyper keratinization, seborrhea, sebaceous gland hyperplasia, P. acnes and inflammation. Inflammation is a key constituent of acne, and the major reason for post inflammatory hyperpigmentation and scarring (11).

The pathophysiology of acne formation involves several key changes in the pilosebaceous unit—follicular hyper keratinization, sebum production, Propionibacterium acnes colonization, and inflammation. Androgen also contributes to this process by stimulating the growth and secretory activity of sebaceous glands. Acne lesions can be subdivided into two main categories: (i) noninflammatory or comedonal acne, which includes whiteheads and blackheads; and (ii) inflammatory acne lesions, which include papules, pustules, nodules, and cysts (12).

Recent research has revealed that circ RNAs can regulate alternative splicing and transcription, and modulate the parental gene expression by acting as a miRNA sponge in various diseases (13).

Skin diseases unconsciously present as psychological and pathological symptoms with patients' conditions waxing and waning, so the refractory. When exploring more diagnostic and therapeutic approaches, a novel biomarker Circular RNA (circRNA) has drawn considerable attention due to its stability, abundance and versatility. CircRNAs with a unique covalently closed loop can function as regulators to participate in tumour proliferation, apoptosis and invasion, and are more promising in serving the prognostic markers and

therapeutic targets in various diseases due to their stability, sensitivity and specificity (14).

Many circRNAs are abnormally expressed in skin disorders, and they are involved in the occurrence and development of skin diseases through a variety of molecular mechanisms, which provide more evidence for circRNAs as biomarkers for diagnosis, prognosis, and therapy of skin diseases (15).

This is why this study was selected to be conducted to evaluate Circular RNA Expression In Patient With Acne and to correlate its level with acne severity.

A case control study was conducted at conducted at Dermatology outpatient cinic at Beni-Suef University hospital including 40 with acne vulgaris and 40 normal as control. The duration of the study ranged from 6-12 months.

The main results of this study were:

As regard sociodemographic data, as regard gender distribution of the cases and the controls; the acne vulgaris cases; 22.5% of them were males and 77.5% were females, while the controls; 15% were males and 85% were females. There was no statistically significant difference between the cases and the control groups regarding sex (p-value> 0.05). As regard the average cases age was; 23.20±4.9 (SD) years, while average controls age was 23.53±4.3 (SD) years. There was no statistically significant difference between cases and control groups regarding to age (p-value> 0.05).

Our results were in agreement with study of **Kim et al. (16)** as they reported that there were no differences between male and female subjects for any of the baseline characteristics; therefore, the data for male and female subjects were combined. There was no difference in age and sex between groups.

However, in the study of **Mueller et al. (17)**, 43 subjects were enrolled in this 8-week study; 39 completed the trial per protocol (18 females, 21 males; mean age: 17.5 3.8 years, range: 14–29 years).

The present study showed that according to severity among studied Acne Vulgaris cases; 27.5% had mild acne, 35% had moderate acne and 37.5% of cases had severe acne disease. The disease duration was ranged from (0.10) to (8) with a mean of 2.06 ±2.1 (SD) years of disease duration.

However, **Chan et al. (18)** found that 84% of their studied group had mild disease severity and 16% of them had moderate disease severity.

Sharara et al. (19) reported that there were 20 cases with mild disease severity (50%) and 20 with severe disease severity (50%).

To our knowledge, there are so little studies which evaluate circ RNA among acne patients.

The current study showed that Cir RNA 0001073 in blood samples was significantly down-regulated in Acne Vulgaris patients as compared with healthy controls; the mean Cir

RNA levels (0.47 vs. 1.03) in blood samples from Acne Vulgaris patients and healthy controls respectively with a statistically significant p-value< 0.001.

Our results were supported by study of **Liang et al. (20)** as they reported that a total of 35 782 circRNAs from lesioned skin and 27 700 circRNAs from non-lesioned skin were detected. The distribution of different types of circRNAs (total circRNAs, exonic circRNAs, intronic circRNAs, intergenic circRNAs) was almost similar between lesioned skin and non-lesioned skin. The majority of the circRNAs (87% in lesioned skin; 84% in non-lesioned skin) derive from exons of protein-coding genes, whereas others derive from intronic and/or intergenic genomic regions. The variations of circRNA expression between the two groups were assessed using scatter plot, and differentially expressed circRNAs with statistical significance were displayed through volcano plot.

Circ RNA had been detected in other skin diseases. Keloid or HS originates from aberrant wound healing, which is characterized by an over-proliferation of fibroblasts. Highthroughput sequencing and bioinformatics analysis revealed a series of significant differentially expressed lncRNAs, mRNAs and circRNAs between HS and normal skin tissues, containing 2479 upregulated and 990 downregulated lncRNAs, 345 upregulated and 191 downregulated mRNAs, 10 upregulated and 1 downregulated circRNAs **(21)**.

Recently, **Wang et al. (22)** have illustrated that the expression profiles of lncRNAs, mRNAs and circRNAs in keloid are different from those in healthy controls. The potential functions of differentially expressed circRNAs and lncRNAs by GO and KEGG path analyses, then circRNA-miRNA interaction gene co-expression networks were constructed. There are 71 upregulated, and 83 downregulated circRNAs. The upregulated circRNAs regulate actin cytoskeleton and focal adhesion, whereas the downregulated circRNAs are closely related to the PI3K-Akt signalling pathway following the KEGG pathway analysis results. Those differentially expressed circRNAs were identified as interacting with 8271 miRNAs.

In the study in our hands, Circ RNA 0001073 expression in blood samples was slightly lower in female with Acne Vulgaris as compared with males; however no statistically significant difference was detected in relation between gender and Circ RNA 0001073 expression in blood samples (p-value= 0.055). There was no detected significant linear correlation between Circ RNA 0001073 expression in blood samples and patients' age in studied Acne Vulgaris patients; (p-value >0.05). There was high significant negative correlation between Circ RNA 0001073 and disease severity (p-value <0.001). There was significant negative linear correlation between Circ RNA 0001073 expression in blood

samples and disease duration in studied Acne Vulgaris patients; (r= -0.592, p-value <0.001).

To our knowledge, the only study which evaluate Circ RNA among acne patients, conducted by **Liang et al. (20)**, and revealed that Using high throughput RNA sequencing and bioinformatics analysis, 538 differentially expressed circRNAs were detected, in which 271 circRNAs are significantly upregulated and 267 circRNAs are downregulated in lesioned skins compared with their paired adjacent non-lesioned skins (n = 3). GO and KEGG pathway analysis revealed that the aberrantly expressed circRNAs were associated strongly with inflammatory, metabolism and immune responses, which is involved in the pathophysiology of acne. Among 11 selected circRNAs for validation, five circRNAs (circRNA_0084927, circRNA_0001073, circRNA_0005941, circRNA_0086376 and circRNA_0018168) were further confirmed to be significantly downregulated between additional paired lesioned skin and non-lesioned skin (n = 4) by PCR, Sanger sequencing and qRT-PCR, which are consistent with RNA sequencing data, and likely contribute to the initiation and progression of severe acne.

In the study of **Liu et al., 2017,** flow cytometry analysis showed that miR-338-3p attenuated lipogenesis to a level similar to that in control cells (no TNF-α treatment). Further analysis with TLC showed that there was an equal reduction in sebum components, including cholesterol esters, triglycerides, and free fatty acids, in the miR-338-3p-treated cells. Quantitative assay showed that, on average, a greater than 50% (n = 3, P < 0.05) reduction in each lipogenesis component. Thus, miR-338-3p attenuated TNF-α-induced lipogenesis in human sebocytes. These findings indicate that miR-338-3p suppresses the TNF-α-induced lipogenesis in sebocytes by targeting PREX2a and down-regulating the PI3K/AKT pathway.

5. Conclusion:

From findings of this study we concluded that there was high negative correlation between RNA 0001073 and acne severity. A further study with a larger sample size and the detail mechanisms based on the circRNA– miRNA–mRNA network is warranted to confirm our findings in severe acne. These results provided a novel insight into the mechanisms underlying in severe acne.

Collectively, circRNA is a novel and favourable potential biomarker for skin diseases because of its structural and functional properties (stability, specificity and sensitivity). With the development of biotechnology and bioinformatics, there is no doubt that more and more circRNAs will be identified as promising biomarkers for clinical applications of skin diseases.

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