

## Expression of Long Non-coding RNA in Psoriasis

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### Abstract

**Background:** Psoriasis is a chronic hyper proliferative inflammatory disease, in which genetic and environmental factors have an important role, but the exact cause is yet unknown. Long non coding RNA (lncRNAs) act as a key regulator for the inflammatory gene expression by collaborating with the transcription factors.

**Aim:** Detection of the expression of long non coding RNA in psoriatic skin of diseased patients as compared to normal control persons to investigate the possible role of long non coding RNA in the psoriasis pathogenesis

**Subjects and methods:** The present study included 30 psoriatic patients and 30 age, gender-matched healthy controls. 4 mm punch skin biopsy was taken from patients (psoriatic lesion) & control and it was kept in lysis solution for the stability of the studied parameters and was kept frozen at -80°C till analysis of long non coding RNA by qRT PCR.

**Results:** The tissue level of Long Non Coding RNA values was significantly higher among patients with psoriasis as compared with normal control.

**Conclusion:** Long Non Coding RNAs (lncRNAs) could contribute to psoriasis pathogenesis through its immunomodulatory role.

**Keywords:** Psoriasis; lncRNAs; Real-time polymerase chain reaction

### Introduction

Psoriasis is a chronic inflammatory skin disease that affects around 0.5%-1% of children and 2%-3% of the world's population [1]. Psoriasis is believed to be multifactorial with multiple key components such as genetic susceptibility, environmental triggers together with skin barrier disruption and immune dysfunction [2].

Long noncoding RNAs (lncRNAs) are defined as RNA transcripts equal to or longer than 200 nucleotides that do not encode for proteins [3]. lncRNAs was shown to participate in multiple biological processes such as the control of gene transcription, DNA replication, RNA splicing and stability, protein synthesis, and protein modification [3].

The aim of our study was to evaluate lncRNA role in pathogenesis of psoriasis through its evaluation in the tissue biopsies from the normal and psoriatic skin.

### Subjects and Methods

This case-control study included 30 patients with chronic plaque psoriasis and 30 age and sex matched healthy controls. The patients and controls were recruited from individuals attending the outpatient clinic of Beni-Suef University Hospitals in the period from November 10, 2017 to April 2, 2018.

Exclusion criteria included the use of any topical or systemic treatment for psoriasis in the last 3 months, Patients with other types of psoriasis or with associated systemic or dermatological diseases.

Patient information was collected by one dermatologist including age, sex, type of psoriasis, affected body surface area (BSA) according to the rule of nines [4] and disease activity determined by psoriasis area and severity index (PASI) score.

The aim of our study was explained to each patient, the protocol of the study conforms to ethical guidelines of the 1975 Declaration of Helsinki as reflected in the prior approval by Institution Human Research Committee.

### Detection of long non-coding RNA expression in tissue

RNA extraction: miRNA was isolated using miRNA extraction kit supplied by mirVana™ PARISTM Kit, ambion, USA.

Sample preparation by adding 300 µL of Binding Buffer solution (L3) to skin sample, then mixed well by vortexing and 300 µL 70% ethanol was added. The previously prepared sample was added to a spin cartridge in a collection tube. It was centrifuged at 12,000 × g for 1 minute. One hundred percentage ethanol was added to the flow and mixed well by vortex. Seven-hundred µL were transferred to a second spin cartridge in collection tube and were centrifuged at 12,000 × g for 1 minute, so the small RNA molecules bind to the spin cartridge, and

the flow through was discarded. The spin cartridge was then washed with a 500 µL wash buffer (W5) with ethanol, centrifuged at 12.000 × g for 1 minute and then the flow through was discarded. The wash step with 500 µL wash buffer was repeated once. The collection tube was discarded then spin cartridge was placed in a wash tube supplied with the kit.

The spin cartridge was then centrifuged at a maximum speed for one minute for removal of the residual wash buffer (W5). The spin cartridge was then placed in a clean 1.7 ml suitable recovery tube supplied with the kit. The miRNA was eluted with 50-100 µl sterile RNase free water supplied with the kit.

Cartridge was incubated at room temperature for 1 minute. The spin cartridge was then centrifuged at a maximum speed for one minute to elute micRNA. The recovery tube contains purified small RNA molecules, and the spin cartridge was discarded. Purified micRNA was stored at -70°C. Nanodrop® spectrophotometer was used to measure the actual absorbance of the isolated RNA at 260 nm, 280 nm and 230 nm.

### Reverse Transcription-Polymerase chain reaction (RT-PCR)

In the reverse transcription (RT) step, cDNA was reverse transcribed from total RNA samples using the specific micRNA primers from the TaqMan® MicroRNA Assays and the reagents from the TaqMan® MicroRNA Reverse Transcription Kit. In the PCR step, PCR products had been amplified from the cDNA samples using TaqMan® MicroRNA assay together with the TaqMan® Universal PCR Master Mix.

### Statistical analysis

Data had been coded and entered using statistical package SPSS (Statistical Package for Social Sciences) version 22. Data had been summarized using mean, standard deviation in the quantitative data and using frequency (count) and relative frequency (percentage) for the categorical data. Suitable statistical tests were used (Chi-square ( $\chi^2$ ), one way ANOVA, one sample t-test, Person's and Spearman's correlation) whenever needed, P-values equal to or less than 0.05 were considered statistically significant.

### Results

The gender ratio, age were not substantially different for each variable among patients with psoriasis (10 women, 20 men, mean ± SD age 48.20 ± 11.50 Years), and healthy controls (4 women, 26 men; mean ± SD age 50.40 ± 6.6 Years). Clinical data of participants are presented in (Table 1).

### The tissue long non coding RNA expression

The level of Long Non Coding RNA values were significantly higher among patients with psoriasis as compared with normal control where the level was (4.71 ± 1.8 vs. 1.03 ± 0.1) in psoriatic patients and controls respectively with a significant p-value <0.001 (Table 2).

We found no relation between patient age, sex, disease duration, family history, PASI score of psoriatic patients with the Long non coding RNA expression and p-value >0.05 in all.

	Patients (n=30)	Control (n=30)
<b>Gender n (%)</b>		
Males	20 (66.7)	26 (86.7)
Females	10 (33.3)	4 (13.3)
<b>Age (Years)</b>		
Mean ± SD	48.20 ± 11.50	50.40 ± 6.6
<b>Duration of psoriasis (Months)</b>		
Mean ± SD	114.47 ± 83.49	
<b>Family history of psoriasis n (%)</b>		
Negative	3 (10%)	
Positive	27 (90%)	
<b>PASI</b>		
Mean ± SD	6.99 ± 6.20	

**Table 1:** Demographic data, clinical characteristics of the psoriasis patients and controls, PASI: Psoriasis Areas and Severity Index, SD: Standard Deviation.

	Patients (n=30)	Control (n=30)
<b>lncRNA level</b>		
Mean ± SD	4.71 ± 1.8	1.03 ± 0.1

**Table 2:** Tissue level of long non coding RNA, lncRNA: long non coding RNA.

### Discussion and Conclusion

The pathogenesis of psoriasis is linked to pathways affecting the innate and adaptive immunity, also epidermal differentiation. There are two well established hypotheses about the process that occurs in the psoriasis development. The first one considers psoriasis as a disease with an excessive growth and reproduction of the skin cells; the problem here is shown as a fault of the epidermis and its keratinocytes. In the second hypothesis, psoriasis is considered as an immune-mediated disorder, the excessive reproduction of the skin cells occurs secondary to factors produced by the immune system [5]. Over the past few years, advances in the genome analyses have identified an emerging class of non-coding RNAs which play important roles in regulation of the gene expression and epigenetic reprogramming [6]. Studies have shown the noncoding RNAs role in the epidermal development and also in the keratinocyte differentiation [7]. LncRNAs act as the key regulator for inflammatory gene expression through collaborating with the transcription factors [8].

Researches on lncRNAs in the immune cells (dendritic cells, T-cells, neutrophils, monocytes, macrophages, and B cells) revealed the fact that the level of expression of lncRNA was associated with the differentiation, development and activation of immune cells [9].

All the above mentioned prompted us to investigate lncRNA role in pathogenesis of psoriasis through its evaluation in the tissue biopsies from the normal and psoriatic skin.

Our results showed that there were significantly higher values among patients with psoriasis as compared with normal control with a statistically significant p-value <0.001.

Consistent with our study, Gupta et al. reported a significant higher lncRNAs expression in psoriatic skin lesion as compared to normal skin. Also in the same study the authors further observed differential higher expression of lncRNAs in psoriatic skin lesion before (PP) and after treatment (PT) with TNF-alpha inhibitor adalimumab [10].

### Limitations of this Study

Additional studies on large number of cases are needed to determine its exact role in pathogenesis of Psoriasis.

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