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## LncRNA HOTAIR as a novel biomarker in Psoriasis

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### Abstract:

**Introduction:** Psoriasis is an inflammatory skin disease. Immunological, genetic, and environmental factors have all been implicated in the development of psoriasis. (HOTAIR) is a lncRNA that regulates gene expression through epigenetic modulation and post-translational gene regulation.

**Aim of the study:** To compare the expression of HOTAIR in psoriasis patients with that of normal, healthy subjects.

**Subjects and Methods:** A case-control study was conducted on 40 psoriatic patients that were recruited from a dermatology outpatient clinic and 20 age- and sex-matched healthy control subjects. Serum samples were collected, and lncRNA HOTAIR expression was detected by quantitative real-time polymerase chain reaction (qRT-PCR).

**Results:** Our results demonstrated a statistically significant up-regulation of the HOTAIR gene expression in the serum of psoriasis patients in comparison with the control subjects ( $P < 0.001$ ).

**Conclusion:** This elevated level of Lnc-HOTAIR in the serum of psoriasis patients suggests its possible contribution to the pathogenesis of the disease.

**Key words:** Psoriasis; LncRNA; HOTAIR; qRT-PCR.

## 1. Introduction

1–3% of the population worldwide is affected by psoriasis, which is a multi-system non-infectious inflammatory disease that mainly affects the skin [1]. Immunological, genetic, and environmental factors participate in the development of psoriasis by causing

dysregulation of cytokines and cellular function [2].

LncRNAs are non-protein-coding RNAs with 200 or more nucleotides that regulate gene expression [3], and their

dysregulation may play a key role in autoimmune diseases [4].

HOTAIR is an antisense lncRNA produced from the HoxC gene of the 12q13.13 chromosome [5]. It has been linked to the development of different autoimmune diseases due to its regulatory role on the inflammatory responses through mediating the secretion of TNF $\alpha$ , IL-17, IL-1 $\beta$  and IFN $\gamma$  inflammatory cytokines [6] as well as activation of the

NF- $\kappa$ B transduction pathway [7], which mediate the inflammatory reactions during the pathogenesis of psoriasis and dysregulate the differentiation and proliferation of keratinocytes [8].

This work aimed to investigate the levels of HOTAIR gene expression in psoriasis patients' serum and its possible contribution to the development of the disease, in addition to the possibility of being a diagnostic marker for psoriasis.

## 2. Subjects and Methods

### 2.1. Subjects

60 participants were included in the study; group 1 (n = 40) included psoriasis patients aged from 20 to 60 years old, not receiving any treatment for at least one month before the study. They were selected from Fayoum University Hospital's outpatient dermatology clinic. Group 2 (n = 20) included 20 participants who were matched for age and sex as controls and had no manifestations of psoriasis or other dermatological or systemic disorders.

### Exclusion criteria

- age below 20 and above 60 years.
- patients with other autoimmune diseases.
- patients receiving treatment.
- pregnant or lactating females.
- patients with malignancies.
- Patients having other dermatological or systemic disorders

### 2.2. Methods

This study was designed as a case-control study. The ethical board at the Faculty of Medicine has reviewed and accepted the study, which was done

according to the human experimentation ethical code (Helsinki's Declaration).

An informed consent and a full medical history were provided to all the participants. A thorough clinical assessment was performed for each subject to determine the PASI score, which evaluates the severity of the disease.

### Sample collection

After an overnight fast and under strictly aseptic conditions, 10 ml of venous blood were collected by venipuncture from the antecubital vein of every participant. They were then divided into 2 tubes: 2 ml in an EDTA-containing tube for CBC and 8 ml in a serum separator tube left for 15 minutes for clotting. The serum was separated by a 10-minute centrifugation at 3000 rpm. Serum was preserved in 2 aliquots at -80°C until used for measurement of cholesterol, TG, HDL, creatinine, urea, ALT, AST, and lnc-HOTAIR.

### Biochemical investigations

CBC was measured by an Automated Hematology Analyzer, Sysmex

XT-4000i, Lincolnshire, IL, USA. Cholesterol, TG, HDL, urea, creatinine, ALT, and AST were measured by colorimetric and kinetic methods using the Spectrophotometer 5010 V5+. Serum Lnc-HOTAIR expression was measured using qPCR.

### ***Methods for detecting Lnc-HOTAIR in serum***

#### **a. RNA Extraction**

According to the manufacturer's instructions, RNA extraction was performed using the MiRNeasy mini Kit (Qiagen, Valenica, CA, USA), and the concentration of RNAs was calculated by the NanoDrop spectrophotometer IMPLN. GmbH, Munich, Germany.

#### **b. cDNA formation by Reverse transcription (RT) of RNA**

Reverse transcription of the total RNA was done using RT2 first strand KIT Cat. No. 330404 (Qiagen, Maryland, USA), with a total volume of 20µl of RT reactions following the manufacturer's instructions.

#### **c. Detection of lncRNA HOTAIR Using qPCR**

The RT2 SYBR Green ROX quantitative PCR (q PCR) Master mix, RT2 lncRNA q PCR Assay Cat. No. 330520 (Qiagen, Maryland, USA), was used to prepare the reaction mix in a nuclease-free tube with a reaction volume of 25µl per well. The (DNA-technology thermocycler, DT-Lite 4S1, Russian) qPCR was set to start with one cycle of 10-minute incubation at 95°C, followed by 40 cycles of 15-second incubation at 95°C, and then incubation at 60°C for 60s. Using

pre-made primers for HOTAIR and for GAPDH (the internal control), the level of Lnc-HOTAIR in the serum was measured. The HOTAIR catalogue number was 330701 LPH07360A, and the GAPDH catalogue number was 330701 LPH31725A.

The gene expression was compared to the internal control, and the relative fold change (FC) was calculated using the equation  $2^{-Ct}$ . A FC value higher than 1 indicates up-regulation of the gene, while a FC value below 1 indicates down-regulation of the gene. The control value was assumed to equal 1 [9].

### ***2.3. Statistical Methods***

Data analysis was carried out using IBM® SPSS® (SPSS Inc., IBM Corporation, NY, USA) Statistics V. 25-2017. for verification of the normal data distribution, the Shapiro-Wilk test was used [10, 11]. Parametric statistical tests were applied as the data showed a normal distribution. The demographic parameters of age and gender were expressed as counts and percent. For presenting continuous variables, mean  $\pm$  standard error (SE) were used. An independent student t-test was applied to compare the measurements of two independent groups for quantitative parametric data. The Bivariate Pearson correlation test was utilized to identify the relationship between different groups. Also, the chi-square test was applied for qualitative data. Finally, tests for sensitivity and specificity were created to evaluate a new test using the Receiver Operating Character (ROC) Curve. statistical tests used were all 2-tailed tests; A p-value of 0.05 or less was regarded as statistically significant.

### 3. Results

This study included 60 participants; group 1 included 40 patients with psoriasis, and group 2 included 20 age- and sex-matched healthy participants as controls. Demographic data for both groups were presented in Table 1. It

showed a statistically non-significant difference comparing both groups regarding the distribution of age and sex variables (p-value  $>0.26$  and  $> 1$ , respectively).

**Table 1:** Demographic data of patients and control groups

		Patients Group (n=40)	Control group (n=20)	P-value
Age (years)	Range	20- 60	28 - 58	
	Mean $\pm$ SE.	37.8 $\pm$ 1.99	41.4 $\pm$ 2.08	$>0.26$
Gender	Males	20 (50%)	10 (50%)	
	Females	20 (50%)	10 (50%)	$>1$

The description of PASI score in the patients' group is presented in Table 2, No significant difference in PASI score was found between both sexes, with  $P >$

$0.447$ . However, there was a statistically significant earlier onset of the disease in females with a  $P < 0.034$  (Table 3).

**Table 2:** Description of PASI score in the patients' group

	Minimum	Maximum	Mean	Std. Error
PASI score	2.00	35.00	10.41	1.15

**Table 3:** Age of onset and PASI score in different genders

	Males	Females	P-value
Age of onset	31.1000	23.4000	$< 0.034$
PASI	11.2950	9.5250	$>0.447$

Regarding the laboratory data presented in Table 4, statistically significant differences existed in measurements of neutrophil/lymph ratio

(NLR) ( $P \leq 0.003$ ), cholesterol ( $P \leq 0.001$ ), triglycerides ( $P \leq 0.017$ ), HDL ( $P \leq 0.01$ ), and ALT ( $P \leq 0.007$ ).

**Table 4: Laboratory data of patients and control groups**

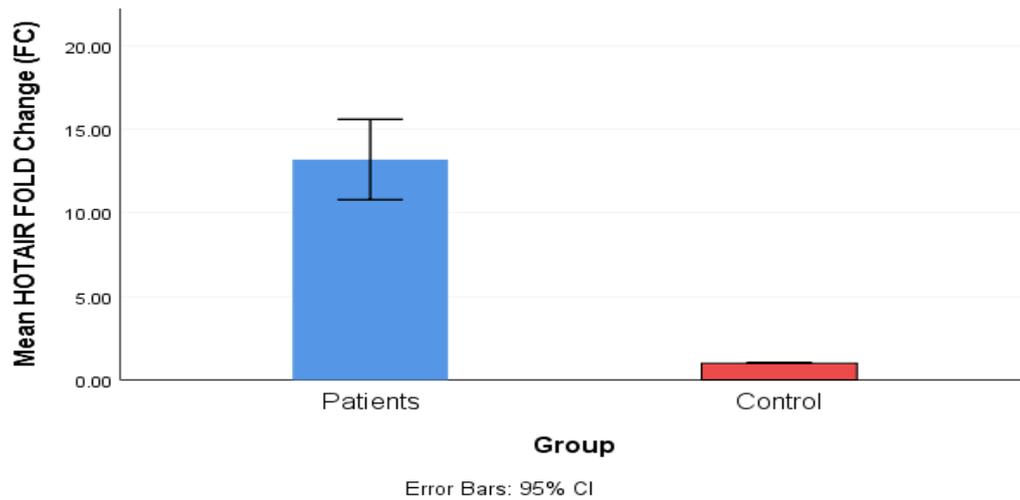
Mean $\pm$ SE	Patients group	Control group	<i>P</i> -value
<b>Hemoglobin</b>	13.4 $\pm$ 0.269	13.48 $\pm$ 0.378	> 0.864
<b>WBCs</b>	7.06 $\pm$ 0.344	6.39 $\pm$ 0.292	> 0.206
<b>Platelets</b>	272.78 $\pm$ 9.640	275.6 $\pm$ 7.595	> 0.848
<b>(NLR)</b>	1.84 $\pm$ 0.131	1.214 $\pm$ 0.109	$\leq$ 0.003
<b>(PLR)</b>	125.7 $\pm$ 6.242	117.54 $\pm$ 3.595	> 0.380
<b>Cholesterol</b>	218.13 $\pm$ 7.3	180.10 $\pm$ 6.26	$\leq$ 0.001
<b>Triglycerides</b>	146.50 $\pm$ 13.20	98.30 $\pm$ 8.60	$\leq$ 0.017
<b>HDL</b>	37.50 $\pm$ 1.18	43.15 $\pm$ 1.86	$\leq$ 0.010
<b>Urea</b>	21.87 $\pm$ 0.665	23.02 $\pm$ 1.242	> 0.372
<b>Creatinine</b>	0.75 $\pm$ 0.031	0.78 $\pm$ 0.048	> 0.542
<b>ALT</b>	14.8 $\pm$ 0.95	10.70 $\pm$ 0.81	$\leq$ 0.007
<b>AST</b>	20.75 $\pm$ 1.00	18.9 $\pm$ 1.497	> 0.3

A highly significant statistical difference with  $P \leq 0.001$  existed between the mean HOTAIR fold change of the

patient group (13.18) and the mean of the control group (1.00), as shown in Table 5 and Figure 1.

**Table 5: Serum HOTAIR fold change in patients and control study groups**

HOTAIR	Patients group (n=40)	Control group (n=20)	<i>P</i> -value
<b>Minimum</b>	0.48	1.00	
<b>Maximum</b>	64.89	1.00	
<b>Range</b>	64.42	0.00	$\leq$ 0.001
<b>Mean <math>\pm</math>SE</b>	13.18 $\pm$ 2.41	1.00 $\pm$ 0.00	



**Figure 1:** Simple bar chart with error bar showing mean HOTAIR Fold change in patients and control groups.

Results showed no statistically significant difference in serum HOTAIR fold change between males and females

with a *P-value* > 0.454, as presented in Table 6.

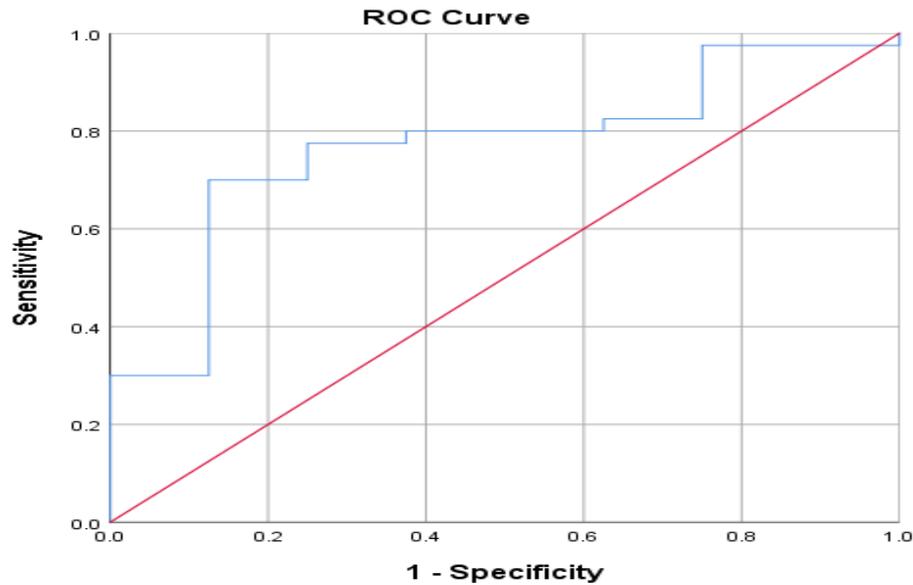
**Table 6:** Comparison of serum HOTAIR fold change in both genders

	Males	Females	P-Value
<b>HOTAIR fold change</b>	15.0141	11.3512	>0.454

Results showed that there were no significant statistical correlations between HOTAIR fold change and other variables among the patient group (*P* > 0.05).

Figure 2 and Table 7 illustrate the ROC curve of the serum HOTAIR to discriminate patients from healthy

controls. Serum HOTAIR levels gave the lowest diagnostic efficacy among the different parameters, with an area under the curve (AUC) of 0.769 [95% confidence interval (CI): [0.604-0.934], sensitivity equals 70%, and specificity equals 87.5% with *P* > 0.017.



**Figure 2:** ROC curve presents the diagnostic accuracy of serum HOTAIR between patients and control.

**Table 7:** Performance characteristics of serum HOTAIR

	Sensitivity	Specificity	95% CI	AUC	P-value
<b>Serum HOTAIR</b>	<b>70 %</b>	<b>87.5 %</b>	<b>0.604-0.93</b>	<b>0.769</b>	<b>&lt;0.017</b>

### 3. Discussion

Psoriasis is classified as a multifactorial autoimmune disorder that mainly affects the skin [12]. The interplay of genetic and environmental factors determines disease susceptibility and phenotypic presentation. The epigenetic mechanisms, which in turn might be activated by environmental factors, can modify the genetic component [13].

LncRNAs are non-protein-coding RNAs with 200 or more nucleotides that exert epigenetic modulation and post-translational modifications. Studies have demonstrated the role of lncRNA dysregulation in a number of autoimmune diseases, including psoriasis [14].

This work aimed to evaluate the suggested role of HOTAIR in psoriasis pathogenesis and severity. Using real-time PCR, expression of the HOTAIR gene in 40 psoriatic patients' serum was assessed in comparison with its level in the serum of 20 matched control subjects. It showed a highly significant statistical result of up-regulation in the expression of the HOTAIR gene in the serum of psoriatic cases compared to the control participants ( $p\text{-value} \leq 0.001$ ), which suggested the role of HOTAIR in developing the disease. However, there was no statistically significant correlation with the disease severity represented by the PASI score.

The laboratory blood tests showed that psoriatic patients had a significantly higher lipid profile and higher neut./lymph. ratio (NLR) in comparison to control subjects, which correlates with previous research in the same aspects [15].

In agreement with our results, two studies have proven the connection between a higher risk of having psoriasis and some genomic variants in HOTAIR. By analyzing different SNPs associated with psoriasis, results showed a positive correlation with some of these genotypes, which supports the involvement of HOTAIR in the pathogenesis of psoriasis [16, 17].

Our results were also consistent with different studies that investigated the role of the HOTAIR gene in immune cells like macrophages during the inflammatory response after lipopolysaccharide stimulation. These studies concluded that HOTAIR was up-regulated and was responsible for activating the NF- $\alpha$  signalling pathway and for increasing inflammatory cytokine secretion, which includes IL-6, IL-1 $\beta$ , IFN, TNF $\alpha$ , and IL-17 [6, 18]. NF- $\alpha$  was approved to be more activated in psoriasis skin and to affect the proliferation and differentiation of keratinocytes in psoriasis, besides increasing the transcription of the inflammatory cytokines involved in psoriasis [19]. In addition, IL-6, IL-1 $\beta$ ,

#### **Ethical approval and consent:**

The work was done with the review and acceptance of the ethical board in the Faculty of Medicine, Fayoum University, all subjects were submitted to an informed consent

IFN, TNF $\alpha$ , and IL-17 constitute the main intermediates involved in keratinocytes proliferation and in the inflammatory cascades found in psoriasis [8]. Moreover, in agreement with our results, HOTAIR was proven to induce the secretion of IL-1 $\beta$  and TNF $\alpha$  through activating the Nlrp3 component of inflammasomes [20], which are also important players in the early phase of psoriasis [21].

Contrary to our results, a protective role of HOTAIR in psoriasis was suggested by a study that was conducted in vitro on a psoriasis cell model through interacting with miR-126, inhibiting the proliferation of cells activated by IL-22, and inducing apoptosis [22].

In addition, another study was conducted on 15 psoriasis patients to assess the lnc-HOTAIR gene expression in the skin of those patients. They compared the levels of HOTAIR gene expression in skin biopsy lesions to their levels in the surrounding non-lesional healthy skin in the same patients, but a statistically non-significant decrease in psoriasis lesional skin was found compared to healthy skin with a p-value < 0.218 [23].

**Conclusion:** Our study indicated that an increased level of Lnc-RNA HOTAIR in the serum of psoriatic patients suggests its possible contribution to the pathogenesis of the disease.

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**Conflicts of Interest:** No author has disclosed any conflicts of interest.

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