Role of LncRNA NEAT-1 in pathogenesis of psoriasis in Egyptian patients

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Abstract:

Introduction: Psoriasis is a chronic, debilitating disease affecting 1–2% of the whole population.

Aim of the study: We aimed to investigate the expression of LncRNA NEAT-1 in psoriasis patients and the possibility of using their serum level as a potential marker for the diagnosis of the disease.

Subjects and Methods: 60 subjects were recruited from a dermatology outpatient clinic for this study and were divided into 2 groups: 40 psoriatic patients and 20 age- and sex-matched healthy control subjects. LncRNA NEAT-1 expression was detected by quantitative real-time polymerase chain reaction (qRT-PCR).

Results: Our results showed a statistically significant increase in the expression of NEAT-1 in the serum of psoriatic patients compared to controls. ROC curve analysis revealed the diagnostic value of NEAT-1 as a predictor in differentiating between cases of psoriasis and controls (p-value<0.001, AUC=1).

Conclusions: This study highlights the contribution of LncRNA NEAT-1 in the pathogenesis of psoriasis. The high expression of serum NEAT1 in psoriasis clarified its role as a potential marker for diagnosis and paved the way for the development of a potential therapy.

Keywords: psoriasis, NEAT-1, PCR.

1. Introduction

Psoriasis is a chronic, painful, and disabling disease with no known cure that has a substantial adverse effect on people's quality of life [1]. Psoriasis has a wide variation in reported frequency among nations, from 0.09 to 11.4 percent [2], making psoriasis a major issue
all around the world. Erythematous, pruritic, well-defined plaques coated in silvery scales are the classic clinical appearance. Psoriasis's etiology remains unknown. Genetic predisposition, environmental factors, skin barrier breakdown, and immune system malfunction are all proposed as major contributors to this complex disease [3].

RNA transcripts longer than 200 nucleotides that are not translated into proteins are called long non-coding RNAs. RNA polymerase II (RNA Pol II) is the primary transcriber of LncRNAs [4]. LncRNAs regulate gene expression at several stages, involving histone modification, transcription, post-transcription, translation, & post-translation. Dysregulation of LncRNAs participates in the pathogenesis of multiple autoimmune diseases [5].

NEAT1 is a new nuclear long non-coding RNA that clocks in at ~3.2 kb. It regulates gene expression through diverse mechanisms, including mRNA retention, mRNA breakage, A-to-I editing, and protein capture. Although NEAT-1's role in psoriasis remains unclear, it is possible that it contributes to the disease's development in a variety of ways. Overexpression of NEAT1 in SLE individuals' monocytes was found to elevate IL-6, CCL2, & CXCL10 production [6]. They participate in the development of psoriasis. Moreover, NEAT-1 acts through the MAPK-related axis, which plays a role in psoriasis pathogenesis [7].

The current study aimed to determine the expression levels of LncRNA NEAT-1 in the serum of psoriasis patients. Also, to investigate its function in the pathogenesis of the disease and whether it can be used as a potential diagnostic marker for psoriasis.

2. Subjects and methods

2.1. Subjects

This study was carried out on a total of sixty participants; group I consisted of forty people diagnosed with psoriasis who had not been taking any therapy for at least one month prior to the start of the trial. They received care at the dermatology outpatient clinic located inside the Faculty of Medicine at Fayoum University Hospital. Psoriasis, chronic dermatological conditions, or systemic disorders involving renal or liver diseases, as well as malnutrition, did not develop in any of the twenty healthy participants who served as
controls in Group II. These individuals were age- and gender-matched.

**Exclusion criteria**

- Age below 20 and above 60 years.
- Patients with other autoimmune diseases.
- Patients receiving treatment.
- Pregnant or lactating females.
- cases with hematological or solid malignancies.
- cases with concurrent or present history of systemic or cutaneous infections.

All study participants gave their written informed permission. The Ethics Committee of Fayoum University's School of Medicine has given the green light to all research including human subjects. All procedures involving human subjects complied with the Helsinki Declaration, an ethical norm established by the World Medical Association.

**2.2. Methods**

**Sample collection and storage**

- A complete medical history and physical examination were performed on all subjects.
- Each participant's antecubital vein was venipuncture for 10 ml of blood after they fasted overnight and under sterile circumstances. They were divided into 2 tubes: 2 ml in a tube containing Ethylene Diamine Tetra Acetic Acid (EDTA) for CBC and 8 ml in a serum separator tube, and allowed to clot for 15 minutes, then centrifuged at 3000 rpm for 10 minutes to separate serum. Serum was collected in 2 aliquots and stored at -80°C until used for measurement of total cholesterol, triacylglycerols, HDL, ALT, AST, urea, creatinine, and lncRNA NEAT1.

**Clinical assessment**

- Extent of disease: (%): using rule 9 [8].
- Assessment of disease severity: using the PASI score. The Psoriasis Area and Severity Index (PASI) assesses erythema, scaling, and lesion thickness, with area of involvement acting as a weighting factor assessing the cranium, trunk, and upper and lower extremities. The PASI scale varies from 0 to 72 and is calculated as the extent of involvement × (score for erythema + score for scaling + score for thickness) × area multiplier, where the extent of involvement is categorized as: 0 (0%), 1 (1–9 percent), 2 (10–29 percent), 3 (30–49 percent), 4 (50–69 percent), 5 (70–89 percent), or 6 (90–100 percent), in addition to scores for erythema, scaling, and thickness from 0 to 4 with area multiplier is 0.1 for head and neck, 0.2 for upper
Biochemical investigations

The following were assessed:

- CBC by cell counter (Sysmex XT-4000i Automated Hematology Analyzer, Lincolnshire, IL, USA).
- Total cholesterol, triacylglycerols, HDL-cholesterol, urea, Alt, AST, creatinine using spectrophotometer 5010 v5+.
- lncRNA NEAT-1.

Methods for detecting NEAT-1 in serum

A. RNA extraction

Serum was analyzed for RNA content and purity utilizing a NanoDrop spectrophotometer (IMPLEN. GmbH, Munich, Germany) after being extracted employing a miRNeasy mini-Kit (Qiagen, Valenica, CA, USA) extraction kit.

B. Reverse transcription reactions

A total of eleven microliters of RNA, two microliters of genomic DNA elimination (GE), and seven microliters of reverse transcription mix were combined into a final volume of twenty microliters to execute reverse transcription on total RNA. The RNAs were reverse transcribed into cDNAs with an RT-PCR kit utilizing RT2 first strand KIT Cat. No. 330404 (Qiagen, Valencia, CA, USA), in accordance with the instructions provided by the manufacturer.

C. Quantitative Real-time PCR (qPCR) for Detection of Lnc RNA NEAT-1

RT-qPCR was performed using the Rotor-gene Q real-time PCR system (Qiagen, USA). We used RT2 SYBR Green ROX qPCR Mastermix, (Cat. NO.330520, Qiagen, Maryland, USA), and a predesigned specific primer for lncRNA NEAT-1(Catalog no: 330701 LPH15809A, Accession no: NR_028272.1) and the housekeeping gene (GAPDH; Catalog no: 330701 LPH31725A, Accession no: ENST00000496049.0) in a total volume of 25 μL. The PCR cycling procedure for quantifying lncRNA NEAT-1 begins with a 10-minute incubation at 95°C, followed by 40 cycles at 95°C for 15 seconds and 60°C for 60 seconds. $2^{-\Delta\Delta Ct}$ was utilized to calculate the serum fold changes of NEAT-1. The values for FC controls were set to one.

2.3. Statistical Analysis

Statistical Package for the Social Sciences (SPSS) version 22 was employed for data collection, codification & analysis. Categorical data were compared using the Chi-square ($\chi^2$) test. The mean and standard deviation were employed to summarize numerical data. Both the independent-t test and
the Mann-Whitney U test were used to assess the degree of similarity between the two sets of information. When comparing courses, the Kruskal-Wallis test was utilized. ROC analysis was done to detect the diagnostic value of serum NEAT-1 for individuals with psoriasis. Pearson's correlation coefficient was utilized to establish associations amongst quantitative variables [10].

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.

The age and sex distribution of the individuals and control groups demonstrated a statistically non-significant variation comparing groups (\( P = 0.8, 0.26 \), respectively).

Table 1: Demographic data of control subjects and psoriasis patients.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n=20)</th>
<th>Psoriasis (n=40)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>37.2±8.3</td>
<td>36.7±12.6</td>
<td>0.8</td>
</tr>
<tr>
<td>Male</td>
<td>14 (70%)</td>
<td>21 (52.5%)</td>
<td>0.3</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>6 (30%)</td>
<td>19 (47.5%)</td>
<td></td>
</tr>
<tr>
<td>Age of onset (years)</td>
<td>-----------</td>
<td>27.7±11.4</td>
<td></td>
</tr>
<tr>
<td>PASI score</td>
<td>-----------</td>
<td>10.4±7.2</td>
<td></td>
</tr>
</tbody>
</table>

*Data were expressed as Mean ± SD, p value <0.05 was significant.*

**Figure 1** shows that there was a significant increase in lncRNA NEAT-1 levels in psoriasis cases compared with controls (\( P<0.001 \)). **Table 2** shows no significant distinction in NEAT-1 levels among male and female psoriasis patients (\( P = 0.76 \)), and between different courses of the disease (\( P =0.4 \)).
**Figure 1:** IncRNA NEAT-1 fold change in Psoriasis patients compared to controls.

**Table 2:** Risk factors among the studied groups.

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IncRNA NEAT-1</td>
<td>17.3±12.2</td>
<td>18.3±13.6</td>
<td>0.8</td>
</tr>
</tbody>
</table>

**Table 3** shows that there was a statistically significant positive association between PASI score and age ($P = 0.007$) and platelet/lymphocyte ratio ($P = 0.027$) in psoriasis patients. Also, there was a statistically significant positive correlation between age of onset and each of age ($P = 0.001$) and triglycerides ($P = 0.04$), while there was a significant negative correlation between age of onset and HDL ($P = 0.025$). Also, there was a significant positive correlation among the ratios of platelets to lymphocytes and neutrophils to lymphocytes ($P = 0.003$).

**Table 3:** Correlations between various studied parameters in psoriasis patients.

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Onset age</th>
<th>NEAT</th>
<th>PASI</th>
<th>TC</th>
<th>TG</th>
<th>HDL</th>
<th>Neut ratio</th>
<th>Plt. ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r$</td>
<td>1</td>
<td>0.713**</td>
<td>-0.222</td>
<td>0.422**</td>
<td>0.003</td>
<td>0.09</td>
<td>-0.135</td>
<td>0.1</td>
<td>0.108</td>
</tr>
<tr>
<td>$P$</td>
<td>0</td>
<td>0.168</td>
<td>0.007</td>
<td>0.984</td>
<td>0.581</td>
<td>0.407</td>
<td>0.541</td>
<td>0.507</td>
<td></td>
</tr>
</tbody>
</table>
The ROC analysis showed that the diagnostic value of lncRNA NEAT-1 as a predictor in differentiating between cases of psoriasis and controls \( (p < 0.001, \text{AUC} = 1) \) at a cutoff value of 1.47 with sensitivity of 97%, specificity of 99%, & accuracy of 98 (Table 4, Figure 2).

Table 4: Sensitivity and specificity of lncRNA NEAT-1 in diagnosis of psoriasis.

<table>
<thead>
<tr>
<th>AUC</th>
<th>P value</th>
<th>95% CI</th>
<th>cut off value</th>
<th>sensitivity</th>
<th>specificity</th>
<th>accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&lt;0.001</td>
<td>1-1</td>
<td>1.47</td>
<td>97%</td>
<td>99%</td>
<td>98%</td>
</tr>
</tbody>
</table>

*Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed).

TC: Total cholesterol; TG: Triglycerides; OCT: optical coherence tomography; t: Independent t-Test.

**Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed).
4. Discussion

Psoriasis is a chronic, stressful, and disabling disease for which there is no cure and has a great negative influence on individuals’ quality of life. Genetic factors play a significant role in the pathogenesis of the disease. Immunity also plays a role in the pathogenesis of psoriasis through T cells, dendritic cells, natural killer cells, Langerhans cells, and neutrophils [11]. In agreement with the findings of investigations of LncRNAs in immune cells (dendritic cells, neutrophils, T cells, monocytes, macrophages, & B cells), it was discovered that the degree of expression of LncRNA was connected with the differentiation, development, and activation of immune cells [12]. NEAT1 is a conserved, long, noncoding RNA whose significance in immunity is still largely unknown. The purpose of this study was to examine the expression levels of LncRNA NEAT1 in the serum of psoriasis patients, its role in the pathogenesis of the disease, and whether it could be used as a potential marker for the diagnosis of psoriasis. To the best of our knowledge, this was the first study to detect the level of NEAT-1 in the serum of psoriatic patients.

In the current research, the expression of LncRNA NEAT-1 was detected by RT-PCR in psoriatic patients and contrasted with controls. The serum expression of LncRNA NEAT-1 was significantly higher in patients than controls. According to the ROC test, NEAT1 showed a highly significant value in psoriasis (p value < 0.001, AUC = 1) at a cutoff value of 1.47 with sensitivity of 97%, specificity of 99%, &...
accuracy of 98%, which could be useful to establish a new diagnostic marker. There were many studies that showed that many other autoimmune disorders involving SLE had previously implicated NEAT1. It had been established that NEAT1 was overexpressed in SLE patients' monocytes. In order to increase the expression of IL-6, CCL2, and CXCL10, this lncRNA phosphorylates JNK & ERK. Thus, it is obvious that NEAT-1 increases the level of IL-6. The latter plays a role in the development of psoriasis. This might be considered a mechanism for the action of NEAT1 in psoriasis development.

In another study about psoriasis, they found that CXCL10 was the most highly ranked gene and indicated positive relationships with the other six hub genes, suggesting it may be the most important gene. The CXCL10 RT-qPCR data further confirmed this forecast. Psoriatic skin lesions and serum CXCL10 levels have been found to be elevated in numerous investigations [13].

In SLE, NEAT1 increases the level of CXCL-10. The latter plays a role in the pathogenesis of psoriasis [14], so this might be considered another mechanism for the role of NEAT-1 in the development of psoriasis.

Cell proliferation, differentiation, gene expression, and apoptosis are all regulated by the MAPK kinases, which together form an important collection of signaling pathways [15]. MAPKs are activated by phosphorylation, and once activated, they phosphorylate other intracellular kinases and transcription factors. One of the targets of the p38 MAPK signaling cascade is a protein kinase called MAPK-activated protein kinase 2 (MK2). Psoriatic lesions were shown to have larger quantities of activated MK2 than other tissues [16]. These findings could support our result, as P38MAPK participates in the development of psoriasis and NEAT-1 acts through the MAPK-related axis. NEAT-1 knockdown inhibited the Ras-MAPK pathway [17]. This might be considered an explanation for the role played by NEAT1 in psoriasis development. Multiple inflammasomes (NLRP3, NLRC4, and AIM2) & pro-inflammatory cytokines (CXCL10, IL-6, & IL-1β) are released when NEAT-1 is upregulated, leading to an immunological response in inflammatory and immune disorders [18]. For example, upregulation of NEAT1 was reported in bronchial asthma [19] and SLE [20]. Overexpression of NEAT1 has also been demonstrated to increase levels of reactive oxygen species, which are known to aggravate immunological responses, herpes simplex infection, and inflammatory responses [21]. We hypothesized that NEAT-1 has a role in the immunological and inflammatory responses.
observed in psoriasis based on the available information.

Consistent with previous research, we found a link between NEAT1 expression and disease activity in SLE [20]. In addition, Wang et al. (2017) discovered that blocking NEAT1 results in better skin lesion healing [21].

In contrast with our results, another study was performed to clarify the mechanism underlying the influence of paeoniflorin (PF) on the proliferation and emigration of psoriatic keratinocytes. The expressions of NEAT-1 and Galectin-7 in skin tissues from psoriatic patients and healthy subjects were evaluated, and there was a reduced expression of NEAT-1 and Galectin-7. PF suppressed the proliferation and emigration of psoriatic HaCat cells by increasing the expressions of NEAT-1 and Galectin-7 [22]. Also, in another study performed to decipher the role of IncRNAs in psoriasis utilizing RT-qPCR, they found that Meg9 and NEAT-1 illustrated a trend towards reduced expression, but the results were not statistically significant [23].

Serum triglyceride (TG) and cholesterol levels were found to be significantly higher in psoriasis patients compared with controls ($p = 0.02, 0.001$ correspondingly), whereas serum high-density lipoprotein (HDL) levels were found to be significantly lower ($p = 0.01$). This was consistent with another study performed to estimate the lipid profile in psoriasis patients in comparison with controls, where the results were as follows: Individuals with psoriasis had considerably higher TG levels and significantly lower high-density lipoprotein (HDL) levels in contrast to people without psoriasis ($p = 0.001$ and 0.013, respectively) [24].

Our study also showed that there was an increase in neutrophil/lymphocyte ratio and platelet/lymphocyte ratio in psoriasis patients compared with controls, and this was consistent with another study performed to estimate these parameters in psoriasis patients, and they found that they were all significantly higher in moderate to severe psoriasis patients [25]. Also, there was no significant variation among the two groups concerning AST and ALT; this was consistent with another study that found the activity and concentration of liver function markers (ALT and AST) did not differ significantly between healthy individuals and cases with psoriasis [26]. Our study showed that there was no significant distinction amongst the two groups concerning serum creatinine levels, and this is consistent with another study that found the median levels of serum creatinine were not significantly different among psoriatic cases and healthy controls [27].
Conclusion

This research revealed the role of NEAT1 in the onset of psoriasis. Serum NEAT1 levels were shown to be significantly higher than those for other papulosquamous illnesses, suggesting that this circulating marker may be useful in the diagnosis of psoriasis. These findings also suggest that manipulation of this marker might lead to the development of a viable therapeutic for psoriasis.

Ethical consideration and patient consent:
The study was approved by the Faculty of Medicine, Fayoum University Research Ethical Committee. Approval and consent to participate were gained by obtaining informed written consent from individuals who were invited to take part in the research.

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Conflicts of Interest: All authors declare they have no conflicts of interest.

References


23. Akkara Veetil BM, Matteson EL, Maradit-Kremers H, McEvoy MT, Crowson CS. Trends in lipid profiles in...

