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HOTAIR and THRIL Long Non Coding RNAs and Their Target Genes in Rheumatoid Arthritis patients

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Abstract

Background: Rheumatoid arthtritis (RA) is a chronic systemic inflammatory autoimmune disease characterized by irreversible joint damage and deformity. The aim of this study is to investigate THRIL and HOTAIR serum expression and their target genes in Egyptian RA patients and to evaluate their relationship to the clinico-pathological data.

Methods: The present study included fifty-two RA patients and fifty-six healthy controls. RA patients were classified according to DAS28 score. All subjects were subjected to full history taking and clinical examination. Quantitative real time PCR was done to estimate the expression levels of serum THRIL and HOTAIR as well as their target genes tumor necrosis factor alpha (TNF-α) and metalloproteinase 2 (MMP-2) were estimated by ELISA techniques.

Results: Results revealed that both THRIL and HOTAIR were statistically over expressed in RA patients compared to healthy group with p-value< 0.05. Results showed as well that the target genes for those long-non coding RNAs, TNF-α and MMP-2, were also significantly higher in RA patients compared to healthy controls.

Conclusions: Both THRIL and HOTAIR associated with their target genes, can be considered as diagnostic markers for RA.

Keywords: HOTAIR, Matrix metalloproteinase 2 and tumor necrosis factor alpha, Rheumatoid arthritis, THRIL.

Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune syndrome associated with several environmental, genetic and epigenetic factors which affect the articular joints contributing to cartilage and bone damage (1). RA causes a decrease in quality of life, marked physical disability and a substantial economic cost. Onset is most frequent during middle age and women are affected 2.5 times as frequent as men. The clinical expression of disease varies, ranging from mild self limiting forms to a very aggressive and rapid evolution that culminates with the destruction of the affected joint and the resulting disability (2). The etiology is still not clear (1). The pathogenesis of RA is complex and involves different cell populations related to the innate and adaptive immune response. RA primarily starts as a state of continuous activation of cells leading to autoimmunity and immune complexes in joints and other organs where it manifests (3).

Long non coding RNAs (lncRNAs) are functionally varied family of non-coding RNAs, which their functions have been detected in the last few years. LncRNAs have been involved in modifying gene expression

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through DNA epigenetic modifications, transcription, translation, immune response and disease states. The molecular mechanisms of lncRNAs are very numerous (4,5). Several lncRNAs are deregulated in autoimmune diseases such as RA and systemic lupus erythematosis (SLE) leading to aberrant gene expression that may contribute to the disease progression (6). It has also been recently found to take an important part in the pathogenesis of osteoporosis (5).

Homebox antisense intergenic RNA (HOTAIR) has been demonstrated to be expressed differentially in RA. HOTAIR, the first lncRNA to be identified, was existed on chromosome 12 and has been shown to have a role in regulation of chromatin state and epigenetic mechanisms within its different target transcripts (7). Elevated levels of inflammatory mediators are associated with autoimmune diseases accompanied by chronic or repeated inflammation. Many cytokines such as interleukine 6 (IL-6) and tumour necrosis factor alpha (TNFα) play an important role in RA and SLE diseases (2).

TNF and heterogeneous nuclear RNPL (hnRNPL)-related immunoregulatory lincRNA(THRIL) was identified in macrophages as a lncRNA that could form a complex with hnRNPL within the nucleoplasm. The long noncoding RNA THRIL regulates the expression of TNF-α through its interaction with hnRNPL (8).

The aim of our study is to investigate the expression of THRIL, HOTAIR and their target genes in RA patients and to evaluate their relationship to the clinical manifestations and disease activity.

Materials and Methods

Fifty-two RA patients diagnosed according to the 2010 American College of Rheumatology (ACR)/ European League against Rheumatism (EULAR) criteria for the classification of RA (9) and were fulfilling the rules of the Declaration of Helsinki 1975. Ethical committee approval was taken from the Faculty of Medicine, Cairo University as well as written consent from each subject before the start of the study as well.

Fifty-six healthy control studies were included as well. Full history taking, clinical examinations, as well as laboratory investigations were performed on all subjects. The disease activity score (DAS28) (10) was assessed. The activity was further subgraded into mild, moderate and asevere activity as well as remission. Patients with diabetes, neoplasia, inflammatory diseases, other autoimmune diseases or receiving anti-TNFα were excluded from our study.

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; Lnc RNA extraction and detection of the fold change for HOTAIR and THRIL expression were done using real time polymerase chain reaction (PCR) and measuring of MMP2 and TNF-α using ELISA techniques.

Molecular Biology Techniques

For HOTAIR and THRIL expression levels estimation in serum, RNA extraction was performed using miRNeasy mini kit and protocol for purification of serum total RNA, including non coding RNAs (Qiagen, Valencia, CA, USA). Quantitation and assessment of RNA purity was performed using the NanoDrop® (ND)-1000 spectrophotometer (NanoDrop Technologies, Inc. Wilmington, USA). Reverse transcription of LNC RNAs into complementary DNAs (cDNAs) was carried out on total RNA in a final volume of 20 uL RT reactions using the miScript II RT kit (Qiagen, Valencia, CA, USA). Quantitative Real-time PCR for Detection of Lnc RNAs HOTAIR and THRIL was performed using miScript SYBR® Green PCR kit and protocol for Lnc RNAs quantitative detection (Qiagen, Valencia, CA, USA). cDNA was used as a template in a total volume of 20 uL reaction

with the following conditions: Initial activation step at 95 ºC for 10 min. followed by 45 cycles of denaturation at 95 ºC for 15 sec. then 60 ºC for 60 sec. After completion of the PCR cycles, melting curves analyses were performed to validate the specific generation of the expected PCR products. Glyceraldehyde

Phosphate dehydrogenase (GAPDH) was used as an internal control. The expression levels for LNC RNAs; HOTAIR and THRIL were evaluated using the Δ*Ct* method. The cycle threshold (*Ct*) value is the number of qPCR cycles required for the fluorescent signal to cross a specified threshold. Δ*Ct* was calculated by subtracting the *Ct* values of GAPDH from those of target LNC RNAs. ΔΔ*Ct* was calculated by subtracting the Δ*Ct* of the control samples from the Δ*Ct* of the disease samples. The fold change in HOTAIR and THRIL expression was calculated by the equation $2^{-\Delta\Delta Ct}$.

For determination of TNF alpha and MMP-2 in serum, Enzyme Linked Immuno-Sorbant Assay (ELISA) kits and protocol for quantitative detection of TNF-α and *MMP-2* in serum. The kits were provided from SUNLONGBIOTECH (Shanghai, China).

Statistical Analysis

Data Analysis was performed using the statistical package of social science (SPSS 17.0) on windows 8.1. For quantitative parametric data, an independent student t-test was used to compare measures of 2-independent groups as well a One-way ANOVA test was used for comparing more than 2-independent groups with Benferroni Post-Hoc to test significance at p-value< 0.05. While for quantitative nonparametric data, the Kruskal-Wallis test and Mann-Whitney test were used to compare more than 2-independent groups. For measuring the correlation between qualitative data, Bivariate Pearson correlation test to find out the association between different groups with a two-tailed to test the significance. Sensitivity and specificity tests were generated for testing a new test with ROC Curve (Receiver Operating Character). P-value< 0.05 was considered as a cutoff value for significance.

Results

There were 52 RA subjects with a mean age of (39.52±10.8). The study included 56 aged healthy control (41.07±11.18 years). Results showed that there was not statistically significant between the two groups with respect to age and gender with p-values of 0.47 and 0.733, respectively (Table 1).

RA patients were classified according to DAS28 score into inactive (Remission) (23 patients) and active (29 patients). The active patients were subdivided into mild/low (5 patients), moderate (18 patients), and severe/High (6 patients).

As regards to the activity of RA, results show that there is a highly statistically significance between DAS28 and the activity of the disease with p-value 0.001. There is not statistically significance between disease activity (39.93±11.08) and remission (39.5±10.8) with p-value 0.73. There is also not statistically significance between the disease activity and the duration of RA where p-value> 0.05 (Table 2).

Table 1. Demographic data of rheumatoid arthritis patients and control groups.

Clinical Data		RA n= 52	Control n= 56	p-value
Age (years)		39.52±10.80	41.07±11.18	0.47
Sex	Female	44(84.5%)	46(82%)	0.733
	Male	8(15.5%)	10(18%)	

Table 2. Comparison of the age, disease duration, and DAS28 according to the disease activity and inactivity in RA patients.

Variables	DAS28 Score in RA Patients (n= 52) Mean±SD		p-value
	Remission **(n= 23)**	***Active*** **(n= 29)**	
DAS28	1.5±0.5	4.27±1.15	0.001*
Age (Years)	39.5±10.8	39.93±11.08	0.73
Disease Duration (Years)	7.00±5.06	6.56±3.89	0.06

** Significant at p-value <0.05, DAS28: Disease Activity Score in 28 joints.*

As regards to the laboratory investigations, results shows that there was a significant increase in the mean levels of ESR and TLC in RA patients compared to the control group (p< 0.001). There was a significant decrease in Hb value in RA patients compared to the control group (p= 0.023). No significant difference was detected in platelet count (p= 0.149), ALT (p= 0.06) and Creatinine (p= 0.25) levels (Table 3).

Table 3. Laboratory investigations of RA and control groups.

Variables	RA *(n= 52)* Mean±SD	Control (n= 56) Mean±SD	p-value
ESR ***(1mm/1sthr)***	41.79±23.79	7.5±2.37	< 0.001*
Hb ***(gm/dL)***	11.72±1.546	12.4±1.6	0.023*
TLC ***(x 103/mm^3)***	7.67±2.37	6.13 ± 1.46	< 0.001*
PLT ***(x 103/mm^3)***	322.12±291.76	264.2±58.0	0.149
ALT***(U/ml)***	29.77±22.98	23.9±6.0	0.06
Cr ***(mg/dL)***	0.93±0.816	0.8 ±0.2	0.25

ESR: erythrocyte sedimentation rate, Hb: hemoglobin, TLC: total leucocytic count, Pl: platelets, ALT: alanine tranaminase, Cr.: creatinine.

Serum biomarkers level in RA patients and control group

TNF-α and MMP-2 expressions were assessed in serum regarding RA group compared to healthy controls. Both expressions were highly statistically significant with a p-value of 0.001 each (Fig. 1).

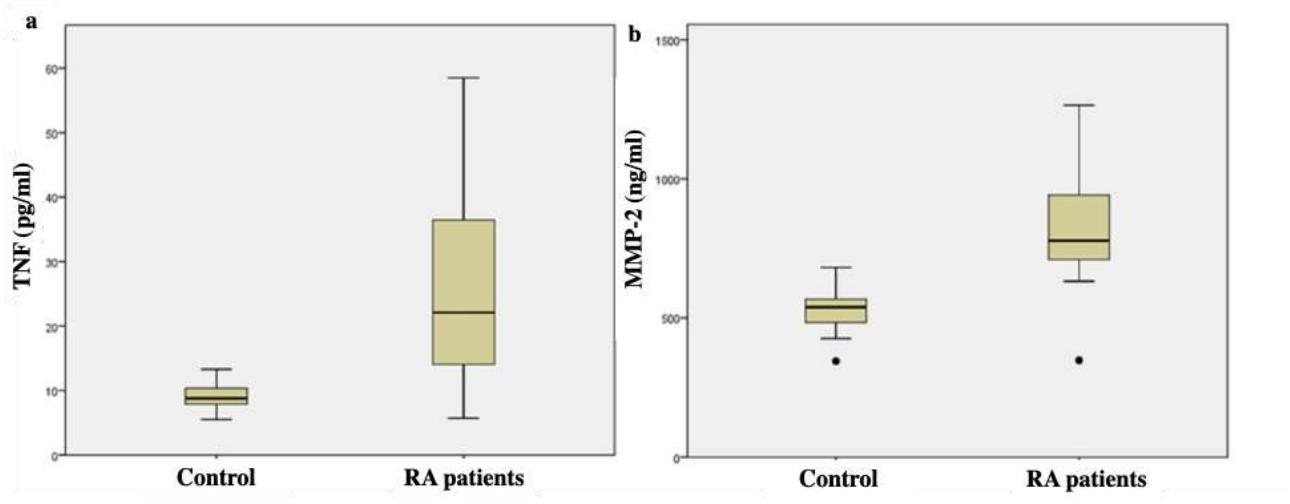

Fig. 1. Relative serum expression of (a) TNF-α (b) and MMP-2 among RA patients and control group.

Furthermore, Long Non-Coding THRIL and HOTAIR fold changes in RA patients and control groups were assessed. Results revealed that the relative expressions of Long Non-Coding THRIL and HOTAIR were significantly higher in RA patients compared to the control group (p= 0.001). The relative expression of THRIL and HOTAIR tended to increase in RA patients with a 3.66- and 15.72-fold change, respectively (Fig. 2).

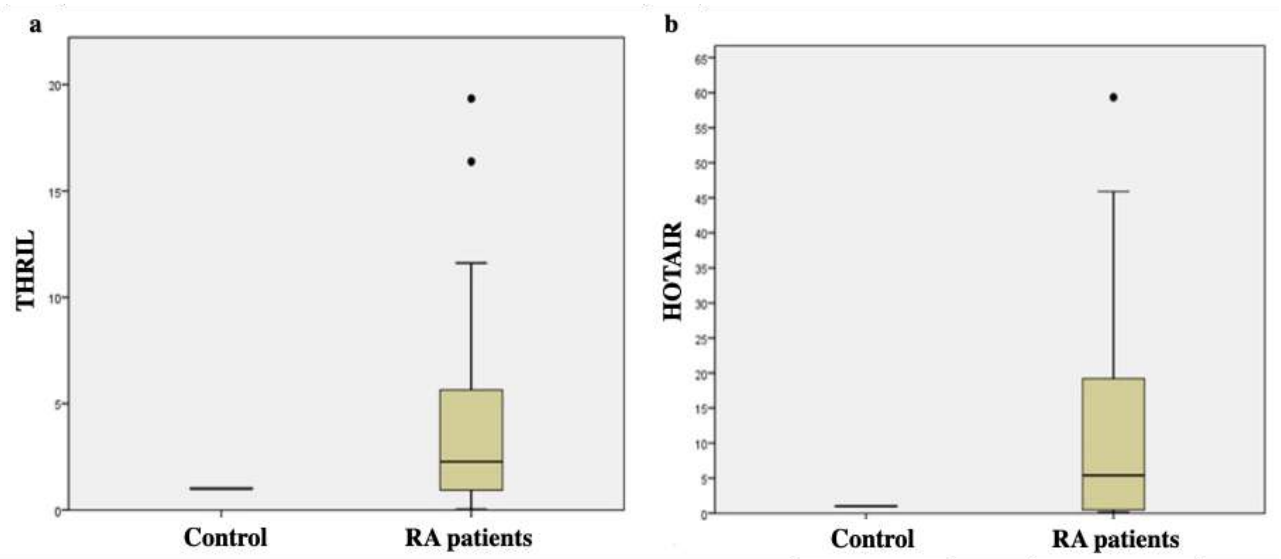

Fig. 2. Relative long-non coding serum expression of (a) THRIL and (b) HOTAIR among RA patients and control group.

Relation of DAS Score to TNF-α, MMP-2, THRIL and HOTAIR in RA patients

After classifying the data according to DAS28 score and activity, results showed that there is statistically significant difference between TNF-α and DAS28 score as regard to activity with p-value 0.013. There is also statistically significant as regard to activity and MMP-2 with p-value 0.005. There is not statistically significant difference as regards to activity and fold change of THRIL and HOTAIR with p-value> 0.05 (Table 4).

Table 4. Relation of DAS Score to TNF-α, MMP-2, THRIL and HOTAIR in RA patients.

Variables	DAS28 Score in RA Patients (n= 52) Mean±SE		p-value
	***Remission* (n= 23)**	***Active* (n= 29)**	
TNF-α	17.17±1.902	32.84±2.66	0.013*
MMP-2	709.61±8.20	931.97±32.68	0.005*
THRIL	3.93±0.76	3.44±0.802	0.718
HOTAIR	19.75±5.62	12.52±3.83	0.190

According to TNF-α and DAS28 score and fold change of THRIL with DAS28 score, there is no correlation between them where p value> 0.05, while there is correlation between DAS28 score activity of RA disease and MMP-2 as well as LNC HOTAIR with p-value 0.001 and 0.024 respectively (Table 5).

Table 5. Relation of disease severity to TNF-α, MMP-2, THRIL and HOTAIR in RA patients.

Parameters	DAS28 Score in RA Patients (n= 52) Mean±SEM				p-value
	Remission (n= 23)	*Mild/low* (n= 5)	*Moderate* (n= 18)	*Severe/High* (n= 6)	
TNF-α	17.17±1.902	28.38±3.01	29.13±3.31	47.67±4.35	0.395
MMP-2	709.61±8.20	769.40±6.90	900.33±33.73	1162±40.65	0.001*
THRIL	3.93±0.76	2.05±1.13	4.01±1.15	2.91±1.58	0.895
HOTAIR	19.75±5.62	11.47±6.56	15.81±5.79	3.54±1.89	0.024*

Relationship between serum TNF-α and MMP-2 levels and clinical manifestations of RA patients

Serum TNF-α and MPP-2 levels were significantly higher (p= 0.016 and p= 0.035, respectively) in RA patients with morning stiffness ≥30 mins than RA patients with morning stiffness <30 mins. In addition, RA patients suffering from arthritis had significantly (p= 0.029) higher serum levels of MMP-2. Disease duration, presence of deformities, fever, rheumatic nodules, and RF positivity, showed no statistically significant difference as regards serum TNF-α and MMP-2 levels. There is highly statistically significant between VAS and MMP-2 with p-value 0.004 (Table 6).

Table 6. Serum TNF-α and MMP-2 levels in relation to the clinical manifestations of RA patients.

Variables		TNF-α Mean±SD	p-value	MMP-2 Mean±SD	p-value
Disease Duration (Years)	≤5	24.88±15.56	0.586	862.38±159.92	0.832
	>5	26.60±13.98		814.13±182.11	
Morning Stiffness (Min)	<30	24.33±12.20	0.016*	775.13±131.41	0.035*
	≥30	28.05±17.25		913.36±194.24	
Arthritis	Yes	27.04±15.07	0.280	860.49±185.52	0.029*
	No	21.67±11.86		733.45±43.60	
Deformities	Yes	27.54±14.90	0.669	857.59±190.78	0.103
	No	22.81±13.63		788.33±127.89	
Fever	Yes	20.77±13.03	0.330	718.67±179.52	0.544
	No	26.98±14.72		857.67±164.31	
Rheumatoid Nodule	Yes	19.83±10.52	0.073	738.90±176.67	0.546
	No	27.35±15.05		856.17±167.01	
Rheumatoid Factor	Yes	25.98±13.77	0.814	838.89±179.06	0.074
	No	23.42±15.18		790.85±117.43	
VAS	≤5	27.61±15.34	0.251	873.97±193.91	0.004*
	>5	22.68±12.57		757.39±89.04	
TJC	≤12	30.94±4.64	0.452	900.50±57.59	0.297
	>12	24.39±2.19		813.55±25.67	
SJC	≤12	36.82±4.90	0.174	961.18±55.92	0.508
	>12	22.98±1.98		799.39±24.25	

Relationship between serum THIRL and HOTAIR levels and clinical manifestations of RA patients

Table 7 shows that in RA patients with a disease duration> 5 years, there was a significant (p= 0.049) increase in the mean levels of THRIL fold change. While, the mean levels of HOTAIR fold change where significantly higher (p= 0.001) in RA patients with a disease duration≤ 5 years. In patients with morning stiffness< 30mins, the mean levels of HOTAIR fold changes where significantly increased (p=0.006). Also, the mean levels of THRIL fold change where significantly higher in patients with deformities and rheumatoid nodules (p= 0.05 and p= 0.001), respectively. In addition, the mean levels of HOTAIR fold change where significantly increased (p= 0.032) in patients who were rheumatoid factor positive.

Table 7. THRIL and HOTAIR fold change in relation to the clinical manifestations in RA patients.

Variables		THRIL (Fold change) Mean±SD	p-value	HOTAIR (Fold change) Mean±SD	p-value
Disease Duration (Years)	≤5	2.86±2.43	0.049*	23.76±30.55	0.001*
	>5	4.20±4.75		10.27±15.93	
Morning Stiffness (Min)	<30	3.59±3.42	0.236	20.63±28.06	0.006*
	≥30	3.75±4.77		9.03±13.83	
Arthritis	Yes	4.08±4.26	0.207	14.27±22.02	0.163
	No	2.10±2.43		21.42±29.57	
Deformities	Yes	4.26±4.57	0.05*	14.42±23.79	0.786
	No	2.52±2.33		16.30±24.09	
Fever	Yes	2.66±2.41	0.228	13.23±28.91	0.992
	No	3.87±4.25		16.24±22.78	
Rheumatoid Nodule	Yes	6.79±6.72	0.001*	14.25±15.14	0.270
	No	2.91±2.66		16.07±25.411	
Rheumatoid Factor	Yes	3.80±3.73	0.961	18.51±26.74	0.032*
	No	3.63±5.01		9.33±12.23	
VAS	≤5	3.37±0.69	0.777	13.85±3.46	0.148
	>5	4.19±0.94		19.25±6.94	
TJC	≤12	3.53±0.89	0.511	21.19±7.49	0.434
	>12	3.70±0.67		14.08±3.64	
SJC	≤12	3.52±1.05	0.955	22.38±8.78	0.107
	>12	3.70±0.65		13.93±3.44	

Correlations TNF-α, MMP-2 and clinical manifestations of RA patients

As regard to the morning stiffness the number of patients have morning stiffness> 30 minutes are n= 15 where there is positive correlation with TNF-α with patients having MS> 30 min. While there is negative correlation between morning stiffness and TNF-α with r= -0.330 and p-value= 0.016 for patients having MS< 30 min n= 37. There is also a positive correlation between morning stiffness and MMP-2 with r= -0.177 and p-value 0.035. there is positive correlation between SJC and TNF-α and MMP-2 with (r= 0.57 and p-value= 0.0001) and (r= 0.61 and p-value= 0.0001) respectively. There was also a positive correlation between both TNF and TJC and between MMP-2 and TJC with (r= 0.63 and p-value= 0.0001) and (r= 0.64 and p-value= 0.0001) respectively.

Correlations THRIL, HOTAIR and clinical manifestations of RA patients

Results showed that there was a statistically negative significant correlation between morning stiffness and LNC THRIL fold change (r= -0.02 and p-value= 0.049). As well as a negative significant correlation between morning stiffness and LNC THRIL fold change (r= -0.20and p-value= 0.001). There was no correlation between ESR and THIRL nor between HOTAIR and ERS.

Correlations all serum biomarkers

There was a positive correlation between TNF-α and MMP-2 in RA patient groups with r= 0.788 and p-value 0.0001. There was also a positive correlation between THRIL and HOTAIR fold change in RA patient groups with r= 0.29 and p-value 0.0369 (Fig. 3).

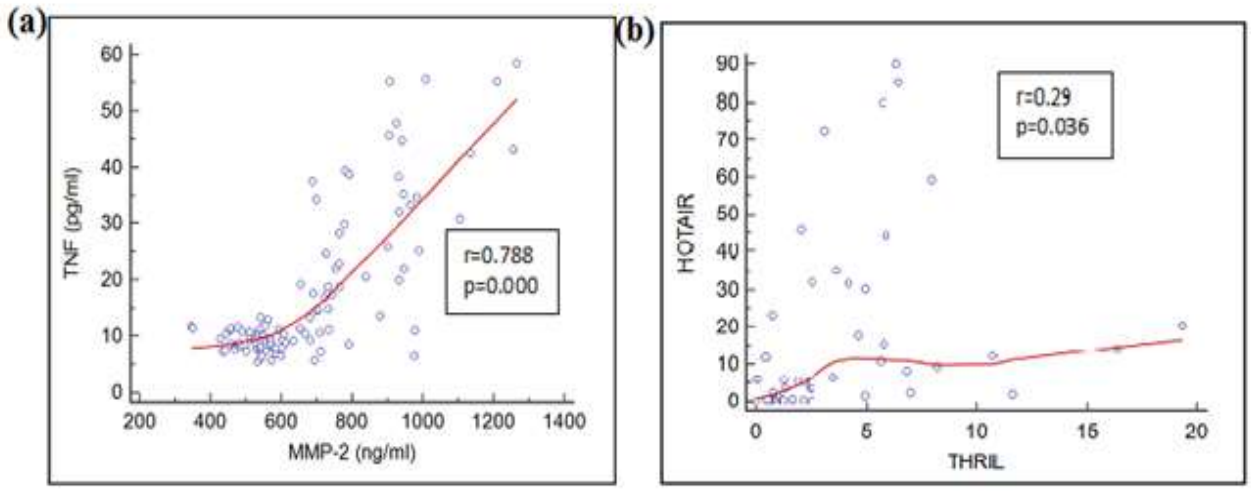

Fig. 3. Correlation between (a) TNF-α and MMP-2 (b) THRIL and HOTAIR fold change in RA patients.

ROC curve analysis for serum biomarkers

The ROC curve analysis of serum TNF-α, MMP-2, THRIL and HOTAIR for RA patients is determined in Fig.4. Diagnostic performance of different genes expression as markers of RA group at different cutoff points using (ROC) curve. For discrimination of RA group versus control group, regarding TNF-α, the AUC= 0.90 with a sensitivity of 78.8% and specificity of 98.2%, while for MMP-2, the AUC was of 0.98 with a sensitivity of 98.0% and specificity of 98.21% (Fig. 4).

Regarding lnc THRIL and HOTAIR, (AUC= 0.73 with a sensitivity of 73.08% and specificity of 100%) and (AUC= 0.69 with a sensitivity of 61.58% and specificity of 100%) respectively (Fig. 5).

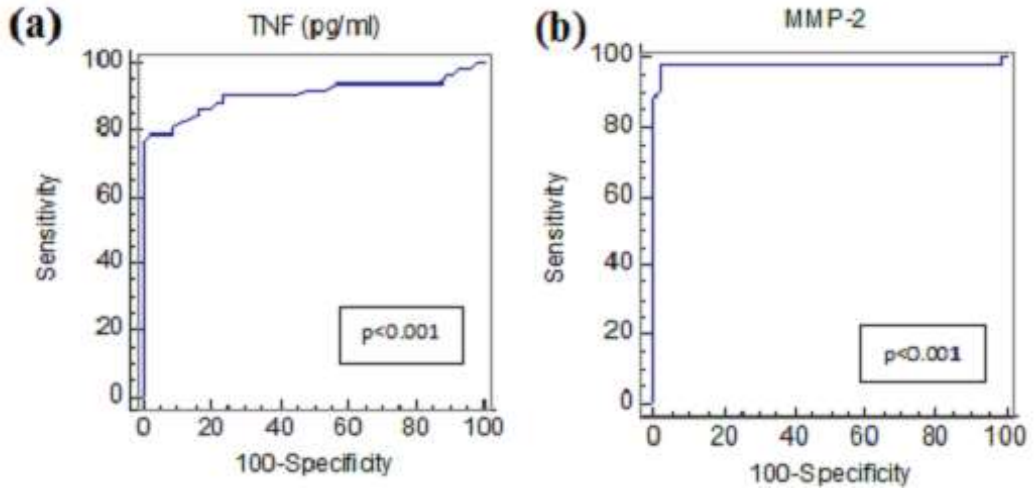

Fig. 4. ROC curve for (a) TNF-α (b) MMP-2 in RA patients group.

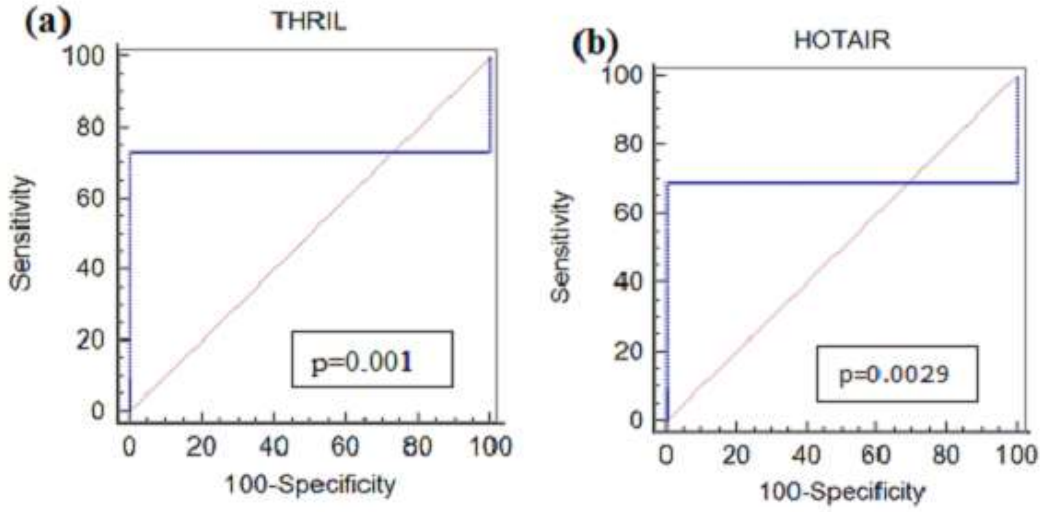

Fig. 5. ROC curve for (a) THRIL (b) HOTAIR in RA patients group.

Discussion

Rheumatoid arthritis is a common systemic and autoimmune disease in which the pathophysiology underlying its development and progression is a result of dysregulations of the immune system (11). Its primary pathological characters are synovitis and vasculitis. A critical role of non-coding RNAs (ncRNAs) in inflammation and autoimmune regulation, primarily including microRNA (miRNA), long non-coding RNA (lncRNA), and circular RNA (circRNA) has been implicated (12). RA is characterized by destruction of cartilage and bones as well as consistent synovitis leading to multiple joints deformities (13). It is also characterized by hyperplasia, autoantibody production and systemic features. Chronic inflammation and bone erosion are the central characteristics of RA (14,15). Chronic inflammation in RA is caused by the imbalance between cytokines of pro- and anti-inflammation and the induction of autoimmunity (16).

Potential triggers of RA include autoantibodies such as ACPA and rheumatoid triggers, and pro-inflammatory cytokines like TNF-α and IL-6 (16). TNF-α, particularly, plays a pivotal role in the pathogenesis of RA. In rheumatoid synovial tissue TNF-α is the principal cytokine which regulates the formation of other pro-inflammatory cytokines (17). Production of connective tissue growth factor is mediated by the activation of synovial

fibroblasts by TNF-α. This in turn promotes the hyperactivation of osteoclasts and thus the destruction of joints (18). In the present study, serum TNF-α levels were significantly higher in patients than the control. Moreover, it was significantly higher in active patients than in remission.The present work agrees with others (19-21) reporting an elevation of serum TNF-α in RA. On the contrary, Ebrahimi et al found no significant differences in serum level of TNF-α between RA patients and the control (22).

Rheumatoid arhritis is characterized by progressive joint destruction with loss of bone and cartilage as well as the aggressive activation of synovial fibroblasts (SFs) bearing a tumor-like appearance (23, 24). MMPs play a critical role in the pathogenesis of RA. RA SFs secrete various proteases, including MMPs that degrade ECM components, mainly proteoglycans and collagens, of articular cartilage in the affected joints (25). MMP-2 (gelatinase A) is important in collagen degradation, through digestion of denatured collagen (gelatin), generated by collagenases. Additionally, it digests other substrates, including fibrillar collagen I and II and aggrecan, which predominantly exist in cartilage (26). In the present study, serum MMP-2 levels were significantly high in patients especially those with activity. In harmony, Giannelli et al. and Chang et al. reported a significant increase in MMP-2 in RA patients (27, 28).

Initiation and development of RA is influenced by both environmental and genetic factors. It has been postulated that a high-risk genetic background, together with epigenetic elements and environmental exposures, leads to a cascade of events initiating RA (29). The ncRNAs involved in epigenetic mechanisms are divided into two principal subgroups: the lncRNAs with more than 200 nucleotides and small ncRNAs with less nucleotides (30). Thousands of lncRNAs have been identified and they play an important role in regulation of the gene expression (31). Functions assigned to lncRNAs include transcriptional interference, initiation of chromatin remodeling, promoter inactivation by binding to basal transcription factors, oligomerization of activator protein, activation and transport of transcription factors, activation of accessory protein and epigenetic repression of genes or gene clusters (32). Accumulating evidence has shown that lncRNAs participate in the process of inflammation, aberrant proliferation, migration, invasion and apoptosis (33) and their roles in autoimmune diseases, such as SLE, MS, RA and Sjogren's syndrome have also been demonstrated (34).

In this work, the expression of two lncRNAs was investigated; THRIL and HOTAIR. THRIL induce TNF-α expression and is essential in the innate immune response and inflammatory diseases (8). The expression of both were found to be significantly upregulated in serum of the RA patients. In concordance, Moharamoghli et al. indicated that T cells of RA patients significantly upregulate gene expression of THRIL (35). In accordance, Fouad et al. reported that THRIL was significantly upregulated in the serum of SLE patients (36). Moreover, Song et al. reported that expression of HOTAIR was upregulated in patients with RA (14). Shaker et al. who assessed the serum expression levels of HOTAIR in RA and investigated their role as novel noninvasive biomarkers in diagnosis of RA reported that HOTAIR was significantly upregulated (37).

In this work a significant correlation between THRIL and HOTAIR expression was detected in patients which indicates their role in the pathogenesis of the disease. There was a significant correlation between THRIL expression and serum TNF-α. THRIL was recently identified in macrophages as a lncRNA that is important for the processing, function, and stabilization of mRNAs. Critically, it was demonstrated that the THRIL-hnRNPL complex binds to the promotor of TNF and induces its expression following TLR-2 activation (8). On the contrary, Moharamoghli et al. did not find a link between THRIL and TNF-α in RA as the sample size was very small (20 patients) (35). The characteristic inflammation of RA occurs due to the abundance of proinflammatory cytokines.

TNF-α is key and regulates the formation of other inflammatory mediators in the synovial tissue such as IL-1 and MMPs, involved in its degradation (18).

In the present study, serum levels of TNF-α and MMP-2 showed a significant relation to the disease activity in RA patients. While, HOTAIR expression was higher in RA patients in remission. A significant negative correlation was found between HOTAIR expression and serum MMP-2 levels in patients. This coincides with Song et al., who reported that expression of HOTAIR was upregulated in PBMCs and the serum exosome of patients with RA, but conversely HOTAIR was downregulated in differentiated osteoclasts and rheumatoid synoviocytes (14). Increased HOTAIR expression might enhance the recruitment of macrophages to target tissues in RA, whereas decreased levels in osteoclasts and synoviocytes can increase MMP-2 and MMP-13 expression, which disintergrate the bone and cartilage matrix and when overproduced, can lead to joint destruction. These results indicate that aberrant expression of HOTAIR is involved in the pathogenesis of RA.

A significant correlation between serum TNF-α and MMP-2 was found in patients. Chu et al. reported that TNF-α upregulates the expression of MMPs and increase MMP-9 production in all synovial and some meniscal cultures (38). Furthermore, Scian et al. reported that the main inducer of MMP-2 secretion in synoviocytes was TNF-α (39). A preponderant role of TNF-α as an MMP inducer has been determined (40).

Serum TNF-α and MMP-2 levels were significantly higher in patients with morning stiffness≥ 30 mins. MMP-2 was significantly higher in patients with VAS≤ 5. In addition, those with arthritis had significantly higher serum levels of MMP-2. TNF-α and MMP-2 significantly correlated with morning stiffness, SJC and TJC. The present work agrees partly with a study conducted by Klimiuk et al. who found that serum TNF-α correlated not only with ESR but also with CRP, the number of swollen joints, and the DAS (41). Others observed a significant correlation of serum TNF-α with the ESR, and CRP (42).

In the present study, patients with a disease duration> 5 years had a significant increase in the levels of THRIL while, HOTAIR was significantly higher in patients with a DD≤ 5 years. In patients with morning stiffness< 30 mins or positive RF, the HOTAIR was significantly increased. THRIL was significantly higher in patients with deformities and RN. In the present study, a significant correlation was detected between THRIL expression and ESR. On the contrary, Moharamoghli et al. observed no correlation yet their sample size was small and they are detecting mRNA expression levels of TNF-α not the protein level (35).

In the present study THRIL significantly predicted RA with a sensitivity 73.08% and specificity 100% at AUC 0.73; while HOTAIR at AUC 0.69 the sensitivity was 61.58% and specificity 100% suggesting that THRIL and HOTAIR may be potential diagnostic biomarkers for RA. In agreement, Shaker et al. reported that lncRNA HOTAIR could discriminate RA patients from controls (37). Also, Moharamoghli et al. reported that THRIL significantly predicted RA thus providing better diagnostic efficiency (35). Furthermore, at AUC 0.91, serum TNF-α predicted RA at a sensitivity of 78.8% and specificity 98.2%; while MMP-2 at 0.98 the sensitivity was 98.1% and specificity 98.2% suggesting that serum TNF-α and MMP-2 may be potential diagnostic biomarkers for RA.

Large multicenter studies on a large population should be performed to give more accurate results about the expression variability of the studied lncRNAs among Egyptian RA patients, as well as verifying their diagnostic and prognostic values. Screening of different lncRNAs should be carried out among Egyptian RA patients to detect which are exclusively or remarkably expressed in our ethnic group. To investigate whether the expression pattern is mirrored in different specimens, further studies should be performed to compare the expression in RA synovial fluids, plasma, PBMCs or tissues. Multiple endogenous housekeeping

genes should be tested to find the most appropriate one for data normalization. Manipulation of HOTAIR and THRILL expressions may represent a new therapy for several human diseases. TNF-α and MMP-2 can be used in therapy monitoring.

In conclusion, the lncRNAs, HOTAIR and THRIL contribute to the pathogenesis of RA through activation of their target genes MMP-2 and TNF-α, respectively. Serum levels ofTNF-α and MMP-2 were significantly higher in RA patients compared to control and they were positively correlated to some clinical manifestations of RA. LncRNAs THRIL and HOTAIR were highly over expressed in RA patients compared to control and could be used as diagnostic markers for RA.

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