Original Article

Increased LncRNA TUG1 Expression Level Impacted Ankylosing Spondylitis Risk, Association with Disability and Patients' Quality of Life

Heba A. Esaily[1], Gehan Abd-Elfatah Tawfeek[2] and Dina S. Fotoh[1]

Department of [1]Rheumatology and Rehabilitation, [2]Clinical Pathology, Faculty of Medicine, Menoufia University, Egypt.

ABSTRACT

Background: The role of expression level of long noncoding RNA (lncRNA) taurine upregulated gene 1 (TUG1) as a risk of ankylosing spondylitis (AS) development is unclear.

Aim: The aim of the current study is to investigate LncRNA TUG1 expression level in relation to AS and to the degree of disability and quality of life which has not been adequately studied.

Patients and Methods: In the present study, 50 Patients of AS and 50 healthy controls of matched age and gender were included. Patients were categorized into two groups based on Bath AS disease activity index (BASDAI): active AS patients and inactive AS cases. For AS patients, disease duration, clinical assessment, Quality of life was assessed using AS quality-of life-questionnaire (ASQoL). Mobility and functional limitations were assessed by Bath AS metrology index (BASMI) and Bath AS functional index scores (BASFI).

Results: Structural damage was assessed using modified stroke ankylosing spondylitis spinal score (MSASSS). Laboratory investigations were done including: HLA-B27, ESR and CRP, Vitamin D levels by enzyme immunoassay method and measurement of LncRNA TUG1 by quantitative real time PCR (qRT-PCR). There was upregulation of LncRNA TUG1 in AS patients than control (p <0.001), at cutoff >6.2. TUG1 has a sensitivity of 88% and specificity of 84%. Active AS patients have significant higher level of LncRNA TUG1 than inactive AS (p <0.001) with a sensitivity of 84% and a specificity of 88%. Moreover, TUG1 could discriminate AS with structural damage from those without structural damage (p= 0.008). LncRNA TUG1 was positively correlated with CRP, BASDAI, VAS, BASDAI, BASMI, BASFI and MSASSS (p <0.001) and was not correlated with disease duration, ESR, Vit D or HLA-B27 (p >0.05).

Conclusion: These results indicated that for the first time, upregulation of LncRNA TUG1 increased the risk of AS and was associated with increased disease activity, structural damage, disability and poor quality of life.

Key Words: Ankylosing spondylitis, Disability, Disease activity, LncRNA TUG1 gene expression, Quality of life.

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Corresponding Author: Gehan Abd-Elfatah, MD, Department of Clinical Pathology, Faculty of Medicine, Menoufia University, Egypt. **Tel.:** 00201018972270, **E-mail**: dr.gehansh7977@gmail.com

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INTRODUCTION

Ankylosing spondylitis (AS) is an axial skeleton chronic, inflammatory disease affecting young adults and presenting with several clinical manifestations, it is characterized by the chronic low back pain and stiffness (**Murphy *et al*., 2022**). The sacroiliac joint is the most commonly affected in the axial skeleton. The inflammatory process affects peripheral joints, tendons, and cartilage producing irreversible damage (**Fotoh *et al*., 2020**).

This disease also affects several extra-skeletal organs causing inflammatory bowel disease, acute anterior uveitis, and cardiac problems (**Chetrit *et al*., 2020; Wallman *et al*., 2020**). The pathological process of AS is thought to begin with inflammation progressed to new bone formation causing cartilage erosion, bone destruction, followed by ankyloses. However, the exact pathogenesis is still unclear. Early detection of Sacroiliitis and syndesmophytes (radiographic AS) is achieved by magnetic resonance imaging (MRI) but they can also be detected by plain radiography (**Kanwal and Fazal, 2018**). As a result, AS has many complications causing disability and affecting the patient quality of life including vertebral fragility fractures, atlantoaxial subluxation, spinal cord injury, and, rarely, cauda equina syndrome (**Zhu *et al*., 2019**).

Human leukocyte antigen (HLAB-27) is the best known diagnostic marker of AS, and C-reactive protein (CRP) is a suitable marker for assessing disease activity, determining treatment efficacy, and structural progression. However, HLAB-27 is responsible for about only 30% of the genetic factors for AS, indicating that other genetic disorders contribute to AS pathogenesis (**Lan *et al*., 2018; Ma *et al*., 2020**).

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Treatment of AS is a major challenge due to the unclear pathogenesis and the need to identify of novel molecular treatment targets. Changes in expression levels of long non-coding RNAs (LncRNAs), are thought to have critical roles in human diseases (**Huang *et al*., 2021; Sun *et al*., 2022**).

Long non-coding RNAs (LncRNAs) are non-coding, regulatory RNAs longer than 200 nucleotides in length of medical importance because of their roles in biological functions like apoptosis, cell proliferation, and the release of pro-inflammatory cytokines. LncRNAs can modulate gene expression at the epigenetic, transcriptional, and post-transcriptional level. Deregulated LncRNAs level has been reported in multiple diseases, such as degenerative disorders, cancers, cardiovascular disease, and autoimmune/inflammatory disorders (**Safa *et al*., 2020; Sun *et al*., 2022**).

Recently, multiple studies suggested that lncRNAs have a critical role in developing of bone diseases, such as arthritis, scoliosis and AS. Dysregulation of non-coding RNAs, including miRNAs and LncRNAs, may contribute to AS pathogenesis via modulation of immune system, as cytokine release and T-cell survival. This give rise to the hypothesis that LncRNAs could be used as potential prognostic markers for AS (**Li *et al*., 2020; Zhang *et al*., 2021a**). LncRNA taurine up-regulated gene 1 (TUG1) is a 7.1-kb lncRNA up-regulated by taurine, firstly discovered as epigenetic risk factor in carcinogenesis (**liu *et al*., 2017; Wei *et al*., 2019; Alkhathami *et al*., 2022**) and recently there are reports about its role in some autoimmune/ inflammatory diseases such as osteoarthritis (**Duan *et al*., 2021**), and rheumatoid arthritis (**Zhang *et al*., 2021b**). However, its role in AS is still unclear. Accordingly, the present study aimed to investigate the relation of LncRNA TUG1 expression level to AS development and progression in addition to its relation to the patients' quality of life which has not been adequately studied before.

PATIENTS AND METHODS:

1. Study design and patient groups

This case-control research included 50 patients with AS, recruited from the outpatient clinic of the Rheumatology, Physical, and Rehabilitation Department, diagnosed according to the modified New York criteria 1984 AS (**Van der Linden and Cats, 1984**), 50 healthy controls of matched age and gender were investigated. Patients were categorized into two groups based on CRP, ESR, and BASDAI: active AS patients with a CRP level >8mg/l and/ or with a BASDAI score ≥4 and ESR >20 mm/h, while other patients were defined as inactive AS cases.

2. Exclusion criteria

Patients with various autoimmune illnesses, chronic infection, lymphoproliferative disorders, cancer, pregnancy, and patients on biologics were excluded.

3. Clinical assessment

Demographic data were collected from all subjects. For AS patients, disease duration, special habits, particularly, smoking, clinical assessment, and history of current treatment were taken. Quality of life was assessed using the AS quality-of life-questionnaire (ASQoL) (**He *et al*., 2022**) A 10 cm visual analog scale (VAS) was used to record pain (**Akad *et al*., 2013**).

CRP (mg/l) and ESR (mm/h) in combination with Bath AS disease activity index (BASDAI) were used to assess disease activity (**Garrett *et al*., 1994**). Mobility and functional limitations were assessed by Bath AS metrology index (BASMI) (**Martindale *et al*., 2012**) and Bath AS functional index (BASFI) (**Calin *et al*., 1994**).

4. Radiological assessment

Lateral radiographs of the lumbar and cervical spines were done to evaluate the structural damage in accordance to the presence or absence of syndesmophytes using the modified stroke ankylosing spondylitis spinal score (MSASSS) from the anterior margins of the lower border of C2 to the upper border of Th1 and from the lower border of Th12 to the upper border of S1. This score is graded from 0 to 3 points each (0: normal; 1: erosion, sclerosis, or squaring; 2: syndesmophytes; 3: bone bridge) with a total score of 0-72 (**Creemers *et al*., 2005**).

5. Laboratory evaluation

1. laboratory investigations were done including (HLA-B27) antigen by flowcytometry; inflammatory markers ESR by the Westergren method and Highly sensitive C-reactive protein (Hs CRP) by immunoturbidimetric assay (Orion Diagnostica Turbox). 25-hydroxyvitamin D [25(OH) D] levels by ELISA (Abcam, UK), Vitamin D considered as sufficient (>30ng/ml), insufficient (15- 30ng/ml) and deficient.

2. Measurement of LncRNA TUG1 by quantitative real -time PCR (qRT-PCR)

Total RNA was extracted from fresh plasma using RNeasy Plus Mini Kit (Qiagen) according to the manufacturer's instructions. Two-Step PCR was performed, 8µL of RNA was reverse transcribed into cDNA using MultiScribe Reverse transcriptase according to manufacturer's protocol using GeneAmp Gold RNA PCR Reagent Kit (Applied Biosystem), first strand cDNA was synthesized from RNA elute using RT Reaction Mix of 20µL containing: Buffer: 4.0µL, 25 mM Magnesium Chloride 2.0µL, 10mM dNTP Blend: 2.0µL, RNase Inhibitor 0.5µL,100 mM DTT 2.0µL, Random Hexamerb 0.5µL, MultiScribe ReverseTranscriptase (50 Units/µL) 0.3µL, RNA: 8µL, RNase free water to 20µL. Then cycling parameters of RT- step at 10min at 25°C and 12min at 42°C. The developed cDNA was stored at -20°C till further using.

The second step, quantitative real-time PCR of LncRNA was performed using SYBR® Green Real-Time PCR Master Mixes. Sequences of primers used in PCR reactions were: 5'-TAGCAG TTC CCC AAT CCT TG -3' (sense) and 5'-CAC AAATTC CCA TCA TCC C -3' (antisense) for TUG1; 5'-GACCTCTATGCCAACACAGT-3' (forward) and 5'-AAC GCT TCA CGAATT TGC GT -3' (reverse) for U6. A volume of 5µL of cDNA was added to a final PCR reaction mixture of 25 µL containing 12.5µL Master Mix SYBR Green Dye (Applied Biosystem), 1.5µL of each Primer, 4.5µLRNase free water. Reaction conditions of PCR (Biometra T professional thermocycler 070-851, Germany): 40 cycles of 95°C for 30s, 95°C for 5s, and 60°C for 34s. Ct values were processed using 2-ΔΔCT method, and TUG1 expression was normalized to U6 endogenous control (amplification plot are illustrated in (Figure 1).

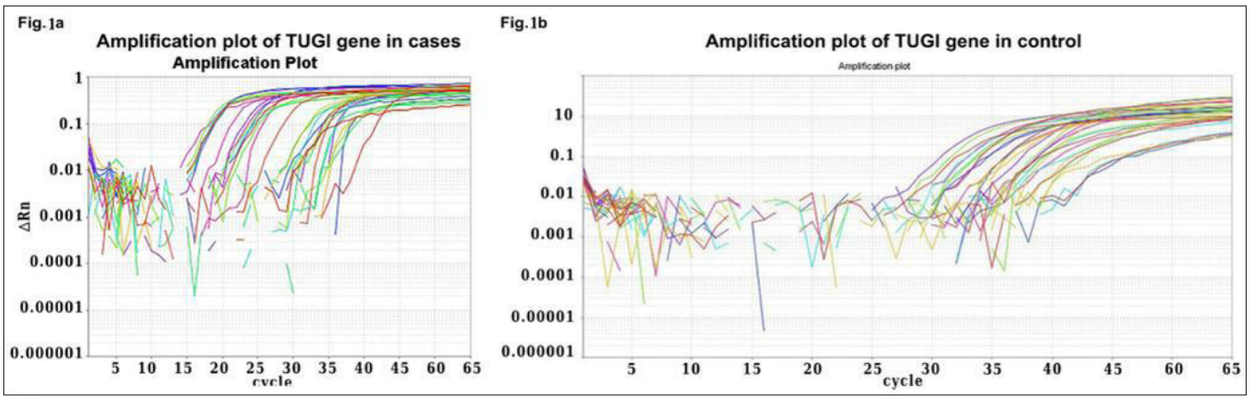

Figure 1: Post-amplification analysis in cases (Fig. 1a) and in control (Fig. 1b).

Statistical analysis

IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp) was used to analyze the data. The data were represented as numbers and percentages. The differences between the variables were assessed by the chisquare test, Mann Whitney or Kruskal–Wallis one-way analysis. Quantitative data were presented as mean, standard deviation, range and median. Student t-test was used to compare two groups while one way ANOVA test was used for comparing the three studied groups and followed by Post Hoc test. Correlation between quantitative variables was done by the Spearman coefficient. The receiver operating characteristic curve (ROC) was used to determine the diagnostic performance of the markers, area more than 50% gives an acceptable performance and area about 100% is the best performance for the test. Logistic regression analysis was used to detect the most independent factor for affecting Active AS. 5% level was used to determine the significance of the obtained results.

RESULTS:

General characteristics of the study subjects

Fifty AS patients with equal number of age and sex matched healthy control are included in this study. The patients with AS were divided into active and inactive groups based on BASDAI (26 of the patients have an active disease and 24 have an inactive disease). There was no significant difference between patients and control regarding age, sex, occupation and smoking condition (p >0.05). However, there was a significant difference between patients and control regarding ESR, CRP and Vit D (<0.001). It was found that active AS was significantly different from inactive AS regarding CRP, HLA-B27 (p <0.001) but no significant difference regarding disease duration, ESR and VIT D (p= 0.120, 0.963, 0.850) (Table 1).

Active vs inactive AS regarding certain clinical and radiological indicators

It was found that active AS patients have higher score of pain recording (VAS), quality of life (As Qol), disability and functional limitation (BASFI and BASMI) (p <0.001). Moreover, active AS patients have higher MSASSS score indicating more liability for structural damage than inactive AS (p <0.001) (Table 1).

Results of LncRNA TUG1 expression in the studied groups

There was a significantly higher value of LncRNA TUG1 in AS patients than the control (p <0.001), as illustrated in (Figure 2 and Table 1). To assess the accuracy of LncRNA TUG1 as diagnostic marker of AS, ROC curve analysis was performed. The AUC was 0.874 (p <0.001), and at cutoff >6.2 the sensitivity and specificity were 88% and 84% respectively (Table 2). To evaluate the role of LncRNA TUG1 in disease activity, it was found that active AS patients have a significantly higher level of LncRNA TUG1 than inactive AS (p <0.001) and LncRNA TUG1 was positively correlated with BASDAI. Additionally, the ROC curve was used to detect the diagnostic performance of LncRNA TUG1 in disease activity at a cutoff >16.7, the sensitivity and specificity were 84% &88% respectively (p <0.001), (Tables 1, 2).

Relation of LncRNA TUG1 to structural damage

The patients were divided into two subgroups according to the Presence (n= 26) or absence (n= 24) of syndsmophytes by radiological investigation and MSASSS scoring. LncRNA TUG1 could significantly detect the structural damage in AS at a cutoff 16 with a sensitivity of 69.23% and specificity of 66.67% (p= 0.008). Furthermore, active AS patients have higher MSASSS score than inactive patients, (Table 1, 2).

Correlation of LncRNA TUG1 to different parameters in AS

In AS patients, LncRNA TUG1 was positively correlated with CRP, VAS, BASDAI, BASMI, BASFI and MSASSS (p <0.001) and not correlated with disease duration, ESR, Vit D or HLA-B27 (p >0.05), (Table 3, 4).

Univariate and multivariate analysis in AS

As shown in table 5, it was found that only HLA-B27, MSASSS and LncRNA TUG1 were the independent predictor of AS disease activity using univariant logistic regression analysis (p= 0.008, 0.035, 0.005) respectively.

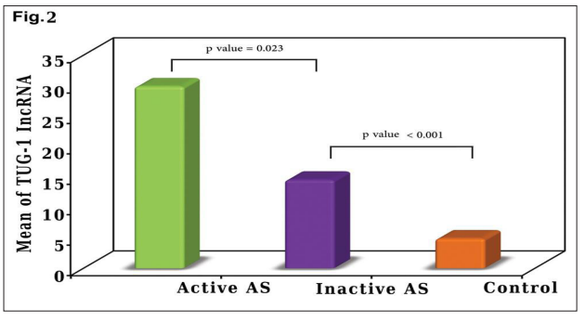

Figure 2: Post-amplification analysis in cases (Fig. 1a) and in control (Fig. 1b).

Table 1: Comparison between the three studied groups according to different parameters:

	Active AS (n= 25)	Inactive AS (n= 25)	Control (n= 50)	Test of Sig.	p	Post Hoc Test		
						p_1	p_2	p_3
(Age/years)								
Mean±SD.	35.36±8.16	37.60±9.98	41.08±9.90	F= 3.265*	0.042*	0.684	0.042*	0.299
Median (Min.–Max.)	35.0(22.0–52.0)	35.0(22.0–60.0)	39.0(20.0–64.0)					
Sex								
Male	18(72.0%)	20(80.0%)	28(56.0%)	χ^2= 4.813	0.090	–	–	–
Female	7(28.0%)	5(20.0%)	22(44.0%)					
Occupation								
Worker	18(72.0%)	19(76.0%)	33(66.0%)	χ^2= 0.795	0.672	–	–	–
Non worker	7(28.0%)	6(24.0%)	17(34.0%)					
Smoking								
Non smoker	11(44.0%)	9(36.0%)	10(20.0%)	χ^2= 5.143	0.076	–	–	–
Smoker	14(56.0%)	16(64.0%)	40(80.0%)					
E.S.R								
Mean±SD.	23.04±3.37	23.24±4.12	3.75±0.65	F= 651.133*	<0.001*	0.963	<0.001*	<0.001*
Median (Min.–Max.)	23.0(16.0–29.0)	25.0(16.0–29.0)	3.75(2.70–4.70)					
Hb (g/dl)								
Mean±SD.	12.21±1.35	12.45±1.37	12.47±1.36	F= 0.315	0.731	–	–	–
Median (Min.–Max.)	12.0(10.2-15)	12.6 (10.7-14.6)	12.25 (10.6-15.3)					
S. Creatinine (mg/dl)								
Mean±SD.	0.69±0.20	0.65±0.21	0.64±0.16	F= 0.619	0.541	–	–	–
Median (Min.–Max.)	0.70(0.40-1.0)	0.70(0.38-1.0)	0.66(0.36-0.95)					
Hs C.R.P (mg/L)								
Mean±SD.	53.04±7.14	7.27±0.96	3.42± 0.89	F= 1664.76*	<0.001*	<0.001*	<0.001*	<0.001*
Median (Min.–Max.)	54.0(42.0–70.0)	7.30(5.80–10.2)	3.50(2.0–5.0)					
Vitamin-D (ng/mL)								
Mean±SD.	17.02±1.08	17.72±1.79	36.39±6.28	F= 215.846*	<0.001*	0.850	<0.001*	<0.001*
Median (Min.–Max.)	16.9(15.4–19.3)	18.0(14.6–20.7)	34.7(30.0–52.0)					
Disease duration (years)								
Mean±SD.	11.12±5.42	8.86±4.94	–	U= 232.50	0.120	–	–	–
Median (Min.–Max.)	11.0(2.0–25.0)	9.0(2.50–20.0)	–					
BASADI								
Mean±SD.	5.44±0.66	2.63±0.67	-	t= 14.925	<0.001*	<0.001*	-	-
Median (Min.–Max.)	5.6(4.10-6.40)	2.60(1.50-3.6)						

Con. Table 1: Comparison between the three studied groups according to different parameters:

BASFI								
Mean±SD.	5.03±0.92	2.40±0.54	–	t= 12.320*	<0.001*	–	–	–
Median (Min.–Max.)	4.90(3.40–6.80)	2.50(1.40–3.20)	–					
BASMI								
Mean±SD.	4.69 ± 0.42	2.41±0.57	–	t= 16.086*	<0.001*	–	–	–
Median (Min.–Max.)	4.80 (4.0–5.30)	2.50(1.20–3.10)	–					
MSASSS								
Non structural damage (18.6)	6(24.0%)	18(72.0%)	–	$C2$= 11.538*	0.001*	–	–	–
Structural damage (≥18.6)	19(76.0%)	7(28.0%)	–					
AS QoL								
Mean±SD.	10.90±1.88	6.92±8.18	–	U= 25.00*	<0.001*	–	–	–
Median (Min.–Max.)	10.50(8.60–16.50)	6.40(1.40–45.0)	–					
VAS								
Mean±SD.	7.24±1.09	3.80±0.82	–	U= 0.000*	<0.001*	–	–	–
Median (Min.–Max.)	7.0(6.0–9.0)	4.0(3.0–5.0)	–					
HLAB27 Negative Positive	 4(16.0%) 21(84.0%)	 17(68.0%) 8(32.0%)		χ^2= 13.875*	<0.001*			
TUG-1 expression								
Mean±SD. Median (Min.–Max.)	29.84±11.22 34.0(2.0–40.6)	14.48±5.16 15.0(1.30–31.0)	14.48±5.16 15.0(1.30–31.0)	<0.001*	<0.001*	0.023*	<0.001*	<0.001*

SD: Standard deviation; χ^2: Chi square test; MC: Monte Carlo; t: Student t-test; U: Mann Whitney test; F: F for One way ANOVA test, Pairwise comparison bet. each 2 groups were done using Post Hoc Test (Tukey); H: H for Kruskal Wallis test, Pairwise comparison bet. each 2 groups were done using Post Hoc Test (Dunn's for multiple comparisons test) p: p value for comparing between the three studied groups; p_1: p value for comparing between Active AS and Inactive AS; p_2: p value for comparing between Active AS and Control.

Table 2: Diagnostic performance for LncRNA TUG-1:

To discriminate	AUC	p	95% C.I	Cut off	Sensitivity	Specificity	PPV	NPV
AS patients vs. Control (50 vs. 50)	0.892	<0.001*	0.811–0.972	>6	90.0	80.0	81.8	88.9
Active vs. Inactive (25 vs. 25)	0.878	<0.001*	0.757–1.000	>16	88.0	80.0	81.5	87.0
Structural damage vs. Non-Structural damage (n= 26 vs. 24)	0.760	0.002*	0.623–0.897	>18.55	76.0	72.0	73.1	75.0

AUC: Area Under a Curve; p value: Probability value; CI: Confidence Intervals; NPV: Negative predictive value; PPV: Positive predictive value *: Statistically significant at $p \leq 0.05$.

Table 3: Correlation between LncRNA TUG-1 with different parameters:

LncRNA TUG-1	AS patients (n = 50)	
	r_s	p
Disease duration (years)	0.166	0.249
BASADI	0.652*	<0.001*
BASFI	0.714*	<0.001*
BASMI	0.756*	<0.001*
MSASSS	0.470*	0.001*
ASQoL	0.648*	<0.001*
V.A.S	0.697*	<0.001*
E.S.R	-0.108	0.456
C.R.P	0.664*	<0.001*
Hb	-0.110	0.445
S. Creatinine	0.100	0.491
Vitamin-D	-0.148	0.304

DISCUSSION

AS is a chronic inflammatory immune- mediated heterogeneous disorder, its pathogenesis and risk factors are still inconclusive and under investigation (**Chen *et al*., 2021**). Genetic factors have been reported as major risk factors for AS especially HLA-B27 which represent the most contributing genetic element. However, there is a proportion of patients do not have HLA-B27, thus studies are growing to detect more genetic factors contributing to AS (**Chen *et al*., 2017**). AS has a destructive property of joints and affects the ability to move as well as quality of life and the diagnostic and prognostic biomarkers are still challenging so it needs more research for new biomarkers.

Table 4: Relation between TUG-1 IncRNA and HLAB27 in total AS patients (*n*= 50):

HLAB27	No.	IncRNA TUG-1		*U*	*p*
		Mean±SD.	Median (Min.–Max.)		
Total AS patients (*n*= 50)					
Negative	21	19.81±8.90	15.50(11.80–40.60)	247.50	0.262
Positive	29	23.86±13.14	30.0(1.30–38.0)		

SD: Standard deviation; U: Mann Whitney test; *p*: *p* value for Relation between TUG-1 IncRNA and HLAB27.

Table 5: Univariate and multivariate logistic regression analysis for the parameters affecting AS (*n*= 25 vs. 25):

	Univariate		#Multivariate	
	P	OR (LL–UL 95% C.I)	*p*	OR (LL–UL 95% C.I)
Age (/years)	0.382	0.972(0.913–1.035)		
Male gender	0.509	1.556(0.419–5.779)		
Worker patients	0.530	1.490(0.429–5.172)		
Smoking	0.564	0.716(0.230–2.230)		
E.S.R	0.848	0.985(0.848–1.145)		
C.R.P	0.997	3.128(8.7×10-223–1.1×10223)		
Vitamin-D	0.103	0.719(0.483–1.069)		
TUG-1 IncRNA	<0.001*	1.182(1.088–1.285)	0.003*	1.180(1.057–1.317)
Disease duration (years)	0.134	1.092(0.973–1.224)		
BASFI	0.985	–		
BASMI	0.995	–		
MSASSS	<0.001*	1.168(1.071–1.273)	0.110	1.113(0.976–1.270)
AS QoL	0.014*	1.299(1.055–1.599)	0.096	1.108(0.982–1.250)
VAS	0.993	–		
Positivity of HLAB27	0.001*	11.156(2.864–43.464)	0.009*	45.920(2.572–819.99)

OR: Odd`s ratio; C.I: Confidence interval; LL: Lower limit; UL: Upper Limit; #: All variables with $p < 0.05$ was included in the multivariate; *: Statistically significant at $p \leq 0.05$.

LncRNA TUG1 gene expression may play a role in AS development and its prognosis. In the present study, TUG1 gene expression was evaluated as diagnostic and prognostic marker reflecting disease activity, disability and quality of life. Active AS patients were younger than inactive cases (35.36±7:9 years) with longer disease duration (11.12±5.42) and were mostly males (72%), which is consistent with the common pattern of the disease having male predominance (**Tsur *et al*., 2022**). Our findings are in consistent with several studies reflecting that the disease being more prevalent in the second and third decades of life with an aggressive nature among the young adults (**Chen *et al*., 2021**). Also, our results support the hypothesis that young age and longer disease duration are poor prognostic indicators for AS (**Nossent *et al*., 2019; Gordon *et al*., 2018**).

CRP and ESR which are common markers of inflammation were higher in patients than control; however CRP was characterized as higher in active disease than inactive compared to unchanging ESR. According to the literature, CRP is more sensitive and accurate as an acute phase reactant and more useful in mentoring disease activity (**Tennant, 2013**). Vit D role in inflammation was proved in many studies (**El-Sharkawy and Malki, 2020; Ismailova and White, 2022; Vernia *et al*., 2022**) in the present study it was significantly lower in patients than control but could not differentiate between active and inactive disease which might indicate the role of Vit D deficiency in AS pathogenesis but not in disease activity, these findings are in consistent with other several studies (**Mitulescu *et al*., 2016; Kocyigit and Akyol, 2018; Žagar *et al*., 2019; Pillar *et al*., 2022**) and contrast study by **Zhao *et al.*, 2014**. who found that there was correlation between Vit D deficiency and AS activity. HLA-B27 is used as a main genetic biomarker in AS, it has reported that 83.3% of AS patient have HLA-B27 positivity in Spanish population (**Arévalo *et al*., 2018**) and 62.5% of Blacks, 85.3% of Whites, and 86.7% of Latinos (**Jamalyaria *et al*., 2017**). However **Cortes *et al*., 2013**, stated that it was 20% in east Asian population. In our study, it was positive in 58% of the AS patients and was independent risk factor for AS according to multivariate regression analysis. This difference may be due to different ethnic population and its hereditary. Additionally, in the current study it was present in 84% of active AS versus 32% in inactive disease, some studies found an association between HLA-B27 positivity and disease activity (**Chung *et al*., 2011; Popescu *et al*., 2014**). Others reported association of HLA-B27 positivity and younger age of onset, longer disease duration, more inflammation on MRI, peripheral and hip arthritis and more uveitis (**Zhang *et al*., 2020; Diaconu *et al*., 2022**).

Recently, LncRNA has a growing interest in understanding the pathogenesis of certain diseases as its critical role in controlling target genes at both transcriptional and post-transcriptional levels has been discovered (**Chen *et al*., 2018**). So, LncRNA might be implicated in AS diagnosis and prognosis. In the present study TUG1 expression was increased in AS patients than control and also, it has ability to discriminate AS cases from healthy control with high sensitivity of 90% and specificity of 80%, also it was independent risk factor by multivariate regression analysis and this is the first report that indicate an increased TUG1 in AS, the only single previous study by **Lan *et al*., 2018** was against our result in which TUG1 was down regulated, the current study is different from that done by **Lan *et al*., 2018** in the patient selection as the previous study include all patients with AS (newly diagnose, under treatment, patients completed treatment, patients completed follow up) also they include patients without other severe diseases and patient,s families so the TUG1 marker was investigated from collection of different patient categories. However, our study included the newly diagnosed patients who under treatment and exclude the patients completed treatment, patients completed follow-up, patient,s families AS patient with any other autoimmune diseases either mild or severe and we did not include patient families and also this critical difference in patient selection may explain the contradictory results between the two studies. The mean age and male gender are higher in our study than the previous one. Also, we collect fresh plasma however, the previous study investigated the TUG1 marker on serum and sacroiliac biopsy and this is another difference between the two study.

Supporting to our results, studies that done on other autoimmune diseases which have close phenotype to AS reported upregulation of TUG1 in multiple sclerosis (**Yue *et al*., 2019**) and Rheumatoid arthritis (**Zhang *et al*., 2021b**).

In cancerous cells, TUG1 is upregulated in cancer bladder, gastric cancer, esophageal squamous cell carcinoma, colon cancer, hepatocellular carcinoma, and therefore promotes tumor progression (**Huang *et al*., 2015; Dong *et al*., 2016; Iliev *et al*., 2016; Wang *et al*., 2016; Zhang *et al*., 2016**) while downregulated in in glioma and lung cancer with uncertain causes. however, the pathogenesis of colorectal cancer and osteosarcoma has some relation to the AS. Interestingly, TUG1 is upregulated in both diseases (**Li *et al*., 2016; Lin *et al*., 2016**).

Interestingly, previous studies proved the role of TUG1 in inflammatory disorders. A study by *Wang et al.,* proved the role of microglia TUG1 in neuroinflammation and silencing of TUG1 shifted M1 to M2 and down regulated the inflammatory cytokines in addition to suppression of nuclear factor- κB (NF-κB) pathway (**Wang *et al*., 2019**). Furthermore, *Zhang et al.* found that TUG1 overexpression enhance the inflammatory response and cell proliferation through sponging of miRNA133a and its knockdown improves the atherosclerosis by decreasing the hyperlipidemia and inflammation in an in-vitro and in-vivo studies (**Zhang *et al*., 2018**). Another recent study discovered positive correlation between TUG1 and NBAT1 which is a lncRNA contributing to carcinogenesis by enhancing cell proliferation and this may explain the role of overexpressed TUG1 in development of AS via the new bone formation in addition to its role in increasing the inflammation process (**Yan *et al*., 2017; Mohammed *et al*., 2022**).

In the present study there was no relation between TUG1 and HLA-B27 which may indicate that TUG1 has different pathway different from HLA-B27.

Disease activity is a critical factor that reflects disease rate progression and outcome, TUG1 was highly expressed in active than inactive AS and can discriminate between active and inactive disease with strong sensitivity of 88% and specificity of 80%, in addition to its positive correlation with BASADI score. This may be explained by its role in increased inflammation which is indicated in our study by the positive correlation with CRP and its role in increasing structural damage.

Our results found a significant positive correlation between TUG1 and MSASSS, BASMI and BASFI scores which indicates structural damage, mobility and function limitation. Such findings raised the possibility of contributing the role of TUG1 to structural damage and new bone formation characteristics of AS with subsequent functional limitation and disability.

Structural damage is considered a significant disability in AS begining from new bone formation and syndesmophyte up to ankylosis of the sacroiliac joints and vertebral column. In the current study the Structural damage obtained by the MSASSS score was higher in active AS than inactive AS. TUG1 had the ability to differentiate between patients with structural damage from cases without it with a sensitivity of 76% and specificity of 72% in addition to positive correlation of TUG1 with MSASSS and this could indicate the adverse impact of TUG1 on bone remolding. It is reported that TUG1 promotes osteogenic differentiation by upregulating RUNX-2 through direct interaction with miR-204-5p (**Yu *et al*., 2018**).

Tang *et al*., 2020 documented that TUG1 could affect the cell survival and ECM degeneration in the intervertebral disc by regulating the miR-26a/HMGB1, which may be included in the activation of NF-κB pathway. Such activation of NF-KB pathway was confirmed in another study to be related to new bone formation in enthesis and amp; to radiographic progression in AS through TNF induced NF-κB activation upregulates the DKK1 transcript level (**Tang *et al*., 2022**).

Previous studies investigated the effect of AS on quality of life using EASi-QoL questionnaires and they reported that AS negatively affect the quality of life especially the physical aspect due to functional limitation, stiffness, fatigue and pain but other aspects of quality of life were also affected including psychological and social aspects (**Rosenbaum *et al*., 2019; Rehab and Amany, 2022**), the associated comorbidities in AS such as uveitis, cardiovascular and osteoporosis are another factors affecting the quality of life (**Shen *et al*., 2016**). Therefore, it is important to us to investigate the relation between TUG1 expression and the quality of life and this is the first report to handle this issue, we found that high expression of TUG1 associated with poor quality of life using EASi-QoL questionnaires, this could be explained by the possible role of TUG1 in the pathogenesis of structural damage, new bone formation, mobility and functional limitation in AS which is reflected by other indicators such as MSASSS, BASMI and BASFI.

The limitation of this study is the small sample size and more studies on a larger scale are needed to validate the results.

CONCLUSION

In conclusion, there is controversy about the exact role of TUG1 in AS pathogenesis. In the current study, the upregulation of LncRNA TUG1 was found to be a promising marker for AS, disease activity, disability and poor quality of life and could open a new era of studies on therapeutic implications of TUG1 in AS.

ABBREVIATIONS

Ankylosing spondylitis (AS); AS quality-of life-questionnaire (ASQoL); visual analog scale (VAS); Bath AS disease activity index (BASDAI); Bath AS metrology index (BASMI); Bath AS functional index (BASFI); modified stroke ankylosing spondylitis spinal score (MSASSS); LncRNA taurine up-regulated gene 1 (TUG1).

CONFLICT OF INTEREST

There are no conflicts of interest.

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