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Long non-coding RNA (H19) in patients with spondyloarthritis: association with disease parameters and ultrasonographic findings

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Abstract

Background Spondyloarthritis is a well-known chronic inflammatory disorder; despite recent advances, its genetic basis remains poorly understood. Recently, long non-coding RNA (H19) was identified to be associated with various human cancers and studied in some autoimmune diseases. Musculoskeletal ultrasound has been shown to have good sensitivity and specificity in detecting enthesitis. The Belgrade Ultrasound Enthesitis Score (BUSES) has recently been proposed as a comprehensive ultrasound enthesitis scoring system for differentiating between individuals with enthesitis associated with spondyloarthritis and those without spondyloarthritis.

Aim The current work aimed to study the role of long non-coding RNA (H19) as a potential biomarker in axial spondyloarthritis and its relationship with the different disease parameters (clinical and laboratory), disease activity, and functional status as well as the relation between long non-coding RNA (H19) and articular manifestations using ultrasonographic assessment of enthesitis.

Results Long non-coding RNA (H19) expression was statistically higher in axial spondyloarthritis patients than controls; there are no statistically significant correlations between long non-coding RNA (H19) relative expression and any of the listed parameters (ESR, CRP, ASDAS-CRP, BASDAI, BASFI, BASMI, BUSES, SPARCC index, mSASSS). As regards the Spearman correlation of Belgrade Ultrasound Enthesitis Score, it showed a statistically significant positive correlation with ASDAS-CRP, BASDAI, and BASMI (*p* value: 0.002, 0.02, and 0.046, respectively).

Conclusion Both long non-coding RNA (H19) and Belgrade Ultrasound Enthesitis Score have good discriminative ability between patients with axial spondyloarthritis and normal population; this suggests a possible role in early diagnosis for patients with axial spondyloarthritis who do not fulfill ASAS classification criteria. Their role to monitor the disease activity still needs further studies to be established. Musculoskeletal ultrasound is much more accurate in the assessment of enthesis than clinical examination. Further studies are needed to study the correlation between ultrasonographic entheseal scores and activity markers either clinically or laboratory.

Keywords Axial spondyloarthritis, lncRNA H19, Enthesitis, Belgrade score, Musculoskeletal ultrasound

Background

Spondyloarthritis (SpA) is defined as a collective group of chronic inflammatory disorders that share common clinical, genetic, and etio-pathogenic features. Spondyloarthritis includes axial SpA (axSpA) and peripheral SpA (pSpA), such as psoriatic arthritis, reactive arthritis, and enteropathic arthritis [1]. Axial spondyloarthritis predominantly affects the axial skeleton. It includes

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both ankylosing spondylitis (AS) and non-radiographic axSpA (nr-axSpA) [2].

The diagnostic feature of AS is the presence of radiographic sacroiliitis, whereas in nr-axSpA there is no definitive radiographic sacroiliitis by X-ray, but sacroiliitis could be detected by magnetic resonance imaging (MRI). It is now believed that nr-axSpA and AS are a continuum of the same disease process [3].

Spondyloarthritis arises from a combination of genetic susceptibility and environmental influences. The presence of a crucial genetic element, mostly influenced by the major histocompatibility complex allele HLA-B27, constitutes a significant factor in this context. However, the already identified risk loci can only account for around 25% of the overall heritability. Various mechanisms, including epigenetic alterations, have been suggested as potential explanations for the unexplained heritability [4].

Epigenetic modifications have a crucial role in governing the activation states of genes. Consequently, the disruption of any of these interdependent systems can potentially give rise to atypical gene expression, thereby contributing to the onset and progression of many diseases. Various epigenetic profiles have been documented, particularly in the context of malignancies [5], and more recently in many chronic inflammatory conditions [6].

The study of epigenetic changes is believed to import a majority of different prospectives into SpA pathophysiology, as well as the development of new diagnostic and prognostic techniques and the identification of new therapeutic agents.

The long non-coding RNA H19 has gained significant recognition in the scientific community over the last two decades. It has been extensively utilized in a wide range of research endeavors to investigate the role of H19 in the process of genomic imprinting. Recent studies have brought attention to the pivotal role of H19 in regulating embryonic placental development and skeletal muscle differentiation [7].

Enthesitis has been demonstrated to be among the initial manifestations and occasionally may be the sole characteristic of spondyloarthritis (SpA), particularly in the case of psoriatic arthritis (PsA). Nevertheless, physicians and patients alike often fail to fully appreciate or adequately acknowledge its significance, and in some cases, may even misinterpret its implications [8].

Enthesitis can be clinically identified through the elicitation of tenderness following the application of pressure on the enthesis using the thumb, till blanching of the nail bed is observed. Furthermore, it is possible that this condition is correlated with swelling resulting from the proliferation of soft tissues and/or the development of new bone formations, commonly referred to as enthesophytes.

In recent times, there has been evidence to support the efficacy of musculoskeletal ultrasound (MSUS) as a non-invasive imaging modality with a notable sensitivity in the evaluation of soft tissue lesions among individuals suffering from chronic inflammatory conditions. Furthermore, it exhibited a higher level of superiority compared to MRI in the identification of enthesitis among patients diagnosed with SpA [9–11].

Additionally, when MSUS is used early in a patient's illness, it is more sensitive than a clinical examination at distinguishing SpA from other inflammatory arthropathies such as crystalline arthropathies, polymyalgia rheumatica, and rheumatoid arthritis. This lowers the number of differential diagnoses that a clinician has to consider. The findings observed in patients with SpA include synovitis, tenosynovitis, and enthesitis [12].

The Outcome Measures in Rheumatology (OMERACT) consensus define ultrasonographic enthesitis as an "abnormally hypoechoic (loss of normal fibrillar architecture) and/or thickened tendon or ligament at its bony attachment (may occasionally contain hyperechoic foci consistent with calcification), seen in two perpendicular planes that may exhibit Doppler signal and/or bony changes including enthesophytes, erosions or irregularity" [13].

The most widely used MSUS enthesitis scores are Glasgow Ultrasound Enthesitis Scoring System (GUESS), Sonographic Enthesitis Index (SEI), the French scores, and Madrid Sonographic Enthesitis Index (MASEI) as well as the Belgrade Ultrasound Enthesitis Score (BUSES) [14].

The Belgrade Ultrasound Enthesitis Score was recently developed as a universal ultrasound enthesitis score for differentiating patients with enthesitis associated with spondyloarthritis (SpA) from those with enthesitis not associated with SpA. The discriminatory ability of this score was assessed using a cut-off value of ≥ 7, which demonstrated a high level of specificity (90.2%) in diagnosing SpA. The reliability of BUSES was assessed based on a high level of agreement among operators, as indicated by an intra-class correlation coefficient (ICC) of 0.990, with a 95% confidence interval (CI) of (0.985, 0.993). Additionally, BUSES has demonstrated a good feasibility based on the clear definition and interpretation of its components, acceptable time for patients and operators, and not requiring additional financial resources [15].

Methods

Study design and subjects

This study is an observational case control study; it was conducted on forty axial SpA patients aged above 16 years old presented to the rheumatology clinic at the Main University Hospital. All patients fulfilled the Assessment of SpondyloArthritis international Society (ASAS) Classification Criteria 2010 [16]. Forty healthy controls (age and sex matched) were also recruited. An informed consent was signed from all candidates and approval of the ethics committee was obtained before the beginning of the study.

Exclusion criteria included

Patients with axial spondyloarthritis aged below 16 years old, patients with coexisting autoimmune diseases, patients with history of cancer and also patients with active HCV, HBV, and patients with acute infections.

Clinical evaluation

Through baseline physical examination emphasizing on the musculoskeletal system was conducted in the clinic, socio-demographic data was obtained. In addition, each patient was interrogated thoroughly about symptoms, onset, course, and duration of the disease. Furthermore, recent and previous medications used, co-morbid illness, extra-articular manifestations, and family history were also obtained.

Laboratory investigations

Complete blood picture (CBC) [17], erythrocyte sedimentation rate (ESR) [18], C-reactive protein (CRP) [19], kidney and liver function [20], and HLA-B27 [17].

All were done for each patient.

Disease assessment measures

The disease was assessed using a core set of parameters for assessment of AS disease activity that includes Ankylosing Spondyloarthritis Disease Activity Score (ASDAS-CRP). Patients were categorized into 4 disease activity statuses, including inactive disease (< 1.3), moderate disease activity (< 2.1), high disease activity (< 3.5), and very high disease activity (> 3.5) [21].

Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) [22]. The functional limitation of patients with axSpA was assessed by using Bath Ankylosing Spondylitis Functional Index (BASFI) [22]. Assessment of 16 points of enthesitis was done using Spondyloarthritis Research Consortium of Canada (SPARCC) enthesitis index [23].

Plain X-ray cervical and lumbosacral lateral view was done for each patient to assess spinal damage and radiographic progression by using the modified Stoke Ankylosing Spondylitis Spine Score (mSASSS) [24].

Ultrasound examination

The ultrasound examiner (E.S.) was unaware of the clinical data and enthesitis symptoms. The machine used for the ultrasonography examination of enthesitis was the Esaote MyLab X5. The study employed a high frequency linear probe operating at a frequency range of 6–19 MHz, accompanied by a low wall filter. The color gain setting was modified to a level that is situated beneath the noise floor. The power Doppler settings were modified to optimize the detection of low flow.

The research used the Belgrade Ultrasound Enthesitis Score as a tool for evaluating different sites of enthesitis [25].

All the laboratory and clinical measures were obtained within 1 week maximally of the ultrasonographic examination.

Assessment of LNCRNA H19 relative expression in peripheral blood mononuclear cells (PBMCs)

Relative quantification of lncRNA H19 expression in PBMCs of axSpA patients and controls was done by using real-time reverse transcription polymerase chain reaction (RT-PCR).

RNA extraction

The extraction of total RNA from peripheral mononuclear cells (PBMCs) was performed using the miRNeasy Mini Kit (Qiagen, Germany) in accordance with the instructions provided by the manufacturer. The assessment of RNA concentration and purity was conducted using the NanoDrop 2000/2000c spectrophotometer (Thermo Scientific, Rockford, IL, USA).

Quantitative reverse transcription polymerase chain reaction

The synthesis of cDNA was performed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Waltham, MA, USA) following the instructions provided by the manufacturer. One thousand nanograms of RNA was combined with a reverse transcription master mix of 10 µL to yield a final volume of 20 µL. Subsequently, the incubation process was carried out at a temperature of 25 °C for a duration of 10 min, followed by a temperature of 37 °C for a duration of 120 min, and finally at a temperature of 85 °C for a duration of 5 min.

The qPCR test was conducted using Maxima SYBR Green/ROX qPCR Master Mix (Thermo Scientific, Rockford, IL, USA) and primers (10 pmol/reaction) for the long non-coding RNA H19, as well as the GAPDH gene which served as the normalization gene. The cDNA was

included in a total volume of 20 µL, following the instructions provided by the manufacturer. The Stratagene MX3000P PCR System was utilized to conduct thermal cycling, which involved the initial step of activating Taq polymerase at a temperature of 95 °C for a duration of 10 min. This was followed by a series of 40 cycles, each consisting of denaturation at 95 °C for 15 s, and subsequent annealing and extension at 60 °C for 60 s. A negative control, specifically a no template control, was incorporated into every experimental run. The lncRNAH19 forward primer: TTTCATCCTTCTGTCTCTTTGT, reverse primer: CAACCAGTGCAAATGACTTAG were used. GAPDH: forward primer: TGTTGCCATCAATGACCC CTT, reverse primer: CTCCACGACGTACTCAGCG were also used.

Data analysis

Comparative CT method (2-ΔΔCt) was used to calculate lncRNA H19 expression in PBMCs of patients with axSpA normalized to GAPDH expression and relative to healthy controls.

Statistical analysis

The data was entered into the computer system and subsequently analyzed using the IBM SPSS software program, namely version 20.0. (Armonk, NY: IBM Corp). The representation of categorical data involved the utilization of numerical values and percentages. The chi-square test was utilized to conduct a comparison between two groups. On the other hand, the normality of continuous data was assessed using the Shapiro–Wilk test. The quantitative data were displayed using a range of statistical measures, such as the minimum and maximum values, mean, standard deviation, and median. The Student's *t*-test was used to compare two groups with normally distributed quantitative variables, while the Mann–Whitney test was used to compare two groups with non-normally distributed quantitative variables. The collected results were evaluated for statistical significance at a significant level of 5%.

Results

This study represented a comparison between two groups, axSpA patient group ($n=40$) and healthy control group ($n=40$). As regards the demographic data, there were no statistically significant differences between patients and control groups regarding the gender, age, smoking, and BMI at p value: 0.99, 0.853, 0.99, and 0.671, respectively.

Table 1 displays the clinical and laboratory data for patients with axSpA; it shows that 9 patients (22.5%) had extra-articular manifestations with 5 (12.5%) having uveitis, 2 having psoriasis (10%), and 2 having IBD (10%). Half of the patients were diagnosed as non-radiographic SpA.

Regarding the lncRNAH19 relative expression, it was significantly higher in patients with axSpA, with a higher median (IQR) expression level (2.5 (1.4–6.6)) compared to the control group (1.1 (0.7–1.6)) at $p<0.0001$ as shown in Fig. 1.

When investigating the associations between lncRNAH19 level and the clinical and laboratory features of patients with axSpA using Spearman correlation, there was no statistically significant correlations between lncRNA H19 relative expression and the laboratory parameters either ESR or CRP (p value: 0.62 and 0.18, respectively), also there was no statistically significant correlation between lncRNA H19 relative expression and different disease indices ASDAS, BASDAI, BASFI, and BASMI (p value: 0.75, 0.69, 0.69, and 0.49, respectively).

There was no statistically significant correlation between lncRNA H19 relative expression and entheses assessment scores either clinically using SPARCC index or ultrasonographically using BUSES (p value: 0.14 and 0.23, respectively) as shown in Table 2.

Finally, when investigating the correlation between lncRNA H19 relative expression and radiographic progression using mSASSS, there was no statistically significant correlation (p value: 0.16).

By ultrasonographic entheseal examination using BUSES, we found that 23 of our patients had enthesitis at the Achilles tendon on the right side (57.5%) and 26 on the left side (65%) making it the most common affected site. The plantar fascia enthesis was involved in 6 patients (15%) on the right side and 7 on the left side (17.5%), the distal patellar tendon enthesis was involved in 3 patients on the right side (7.5%) and 5 on the left side (12.5%) while the proximal, patellar tendon enthesis was involved in 4 patients on the right side (10%) and 6 on the left side (15%), the quadriceps tendon was involved in 5 patients on the right side (12.5%) and 5 patients on the left side (12.5%), while the common extensor tendon was involved in 9 patients on the right side (22.5%) and 6 on the left side (15%). This is well demonstrated in Table 3 and Fig. 2.

Regarding the Spearman correlation of Belgrade score, it showed a statistically significant positive correlation with ASDAS-CRP (r: 0.47, p: 0.002), BASDAI (r: 0.36, p: 0.02), and BASMI (r: 0.32, p: 0.046). However, there was no statistically significant correlation between Belgrade score and ESR, CRP, BASFI, or SPARCC index as shown in Table 4.

Regarding the diagnostic performance of lncRNA H19 relative expression, the lncRNA-H19 level can act

Table 1 Comparison between the two studied groups according to different parameters

	axSpA patients ($n=40$)	Control ($n=40$)	Test of sig	*p*
Gender				
Male	24 (60%)	25 (62.5%)	$\chi^2=0.053$	0.818
Female	16 (40%)	15 (37.5%)		
Age (years)	36.4 ± 10.7	36.8 ± 9.7	$t=0.186$	0.853
Smoking	9 (22.5%)	8 (20.0%)	$\chi^2=0.075$	0.785
BMI (kg/m^2)	27.3 ± 4.6	26.9 ± 4.3	$t=0.427$	0.671
Disease duration (years)	8.5 (1.0–25.0)			
Extra-articular manifestations	9 (22.5%)			
Uveitis	5 (12.5%)			
Psoriasis	2 (5%)			
IBD	2 (5%)			
Recent treatment				
Biological naïve	20 (50%)			
Anti-interleukin 17A	13 (32.5%)			
Anti-tumor necrosis factor	7 (17.5%)			
Laboratory findings				
ESR, mm/h	27.1 (5.1–70)			
CRP, mg/L	7.3 (0.2–55)			
HLA-B27				
Negative	19 (47.5%)			
Positive	21 (52.5%)			
ASDAS-CRP				
Inactive	1 (2.5%)			
Moderate	3 (7.5%)			
High	16 (40%)			
Very high	20 (50%)			
BASDAI	6 ± 1.8			
Inactive	8 (20%)			
Active	32 (80%)			
BASFI	6.3 (0.8–9.5)			
BASMI	0 (0–10)			
SPARCC index	1.5 (0–9)			
Radiological findings				
Non-radiographic	20 (50%)			
Radiographic	20 (50%)			
mSASSS	0 (0–60)			
Belgrade score	2.5 (0–31)			

SD Standard deviation, χ^2 Chi-square test, *t* Student *t*-test, *U* Mann–Whitney test

p: *p* value for comparing between the studied groups

BMI Body mass index, *ASDAS* Ankylosing spondylitis Disease Activity Score, *BASFI* Bath Ankylosing Spondylitis Functional Index, *BASMI* Bath Ankylosing Spondylitis Metrology Index, *BASDAI* Bath Ankylosing Spondylitis Disease Activity Index, *IBD* Inflammatory bowel disease, *ESR* Erythrocyte sedimentation rate, *CRP* C-reactive protein, *SPARCC* The Spondyloarthritis Research Consortium of Canada, *mSASSS* modified Stoke Ankylosing Spondylitis Spine Score, *HLA* Human leukocyte antigen

as a good predictive marker for axSpA at a cut-off value of ≥ 2.44 with an area under the curve of 0.83 with reasonably high specificity (97%) and PPV (97%) but lower sensitivity (52.5%) and NPV (67.8%) as shown in the ROC curve in Fig. 3.

Discussion

Spondyloarthritis results from a combination of both genetic and environmental factors. SpA has a substantial genetic component that is mainly dominated by the major histocompatibility complex (MHC) allele HLA-B27. Many genome-wide association studies (GWAS)

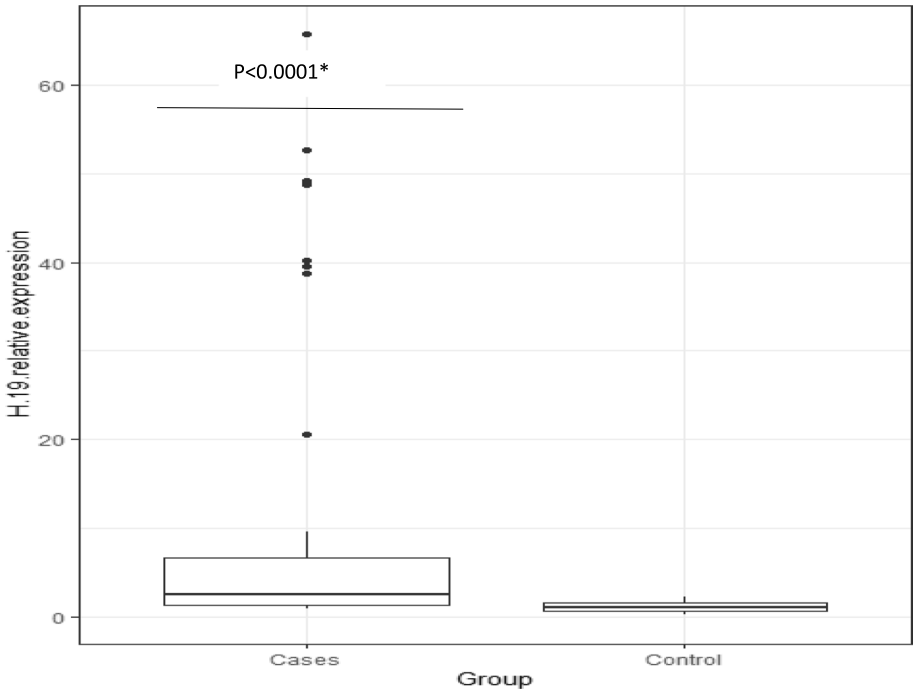

Fig. 1 Boxplot showing the comparison between the two studied groups as regards the lncRNA-H19 relative expression

Table 2 The Spearman correlation between lncRNA H19 relative expression and different parameters

	lncRNA H19 relative expression		
	Median (IQR)	***r***	***p* value**
ESR in mm/h	27.1 (15.3–45.6)	0.08	0.62
CRP in mg/dl	7.2 (3.6–13.0)	0.22	0.18
ASDAS	3.5 (3.1–4.13)	0.05	0.75
BASDAI	6.15 (4.25–7.21)	−0.06	0.699
BASFI	6.25 (4.48–7.5)	−0.06	0.69
BASMI	0 (0–3)	0.11	0.49
Belgrade score	2.5 (1.0–6.25)	0.19	0.234
SPARCC index	1.5 (0–4.0)	0.24	0.14
mSASSS	0.0 (0–60.0)	0.33	0.16

r: Pearson coefficient

ASDAS Ankylosing Spondylitis Disease Activity Score, *BASFI* Bath Ankylosing Spondylitis Functional Index, *BASDAI* Bath Ankylosing Spondylitis Disease Activity Index, *BASMI* Bath Ankylosing Spondylitis Metrology Index, *ESR* Erythrocyte sedimentation rate, *CRP* C-reactive protein, *SPARCC* The Spondyloarthritis Research Consortium of Canada, *mSASSS* modified Stoke Ankylosing Spondylitis Spine Score

have discovered more than 40 other susceptibility loci other than the MHC. However, only a small proportion (25%) of the heritability is explained by the recently known risk loci [4]. Different mechanisms, especially epigenetic changes, were proposed to be responsible for the unexplained heritability [26].

The lncRNA H19, one of the new well-known imprinted genes, is located on human chromosome 11 and it is transcribed only from the maternally inherited allele [27]. Recent studies found that the H19 regulates cell differentiation and musculoskeletal system regeneration and plays a vital role in inflammatory disorders [28].

H19 expression was significantly higher in synovial tissue obtained from patients with rheumatoid arthritis and osteoarthritis compared to healthy controls [29]; a microarray analysis revealed 121 differentially expressed lncRNAs in osteoarthritis patients and H19 was upregulated [30].

These findings suggest that H19 plays a vital role in the pathogenesis of inflammatory diseases. Its role in the pathogenesis of axSpA is a novel area of interest. There were only a few studies in the literature to clarify the role of lncRNA in the pathogenesis of radiographic axSpA (AS) and non-radiographic axSpA. So, it was the trigger to initiate the current study on lncRNA H19 and SpA.

The current work aimed to study the role of lncRNA H19 as a potential biomarker in axSpA and its relationship with the different parameters of disease activity (clinical and laboratory), functional status, and studying the relation between lncRNA H19 in SpA and articular manifestations using ultrasonographic assessment of enthesitis.

In the current study, lncRNA H19 expression in peripheral blood mononuclear cells was significantly

Table 3 The distribution of ultrasound entheseal involvement using BUSES in axSpA patient group ($n=40$)

BUSES	Right		Left		Total	
	No	%	No	%	No	%
Achilles tendon enthesis	23	57.5	26	65.0	49	61.25
Plantar fascia enthesis	6	15.0	7	17.5	13	16.25
Distal patellar tendon enthesis	3	7.5	5	12.5	8	10
Proximal patellar tendon enthesis	4	10.0	6	15.0	10	12.5
Quadriceps tendon	5	12.5	5	12.5	10	12.5
Common extensor tendon	9	22.5	6	15.0	15	18.75

BUSES Belgrade Ultrasound Enthesitis Score

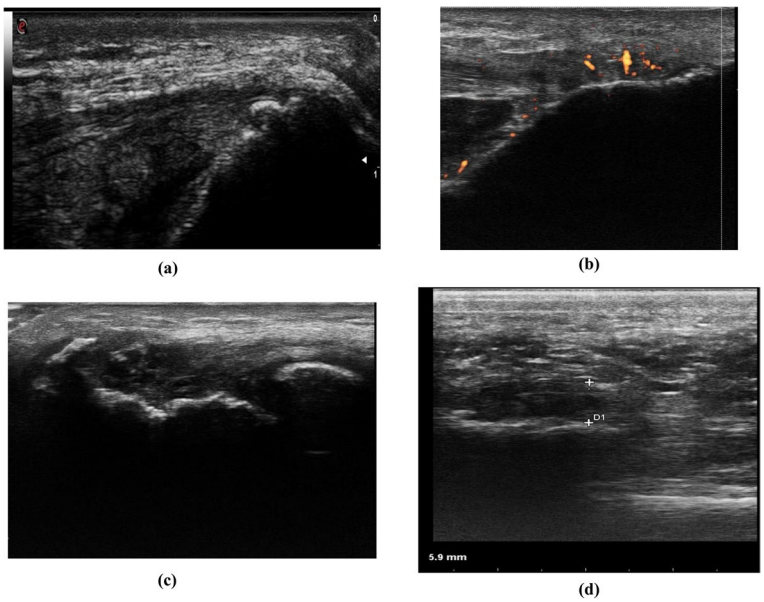

Fig. 2 **a** Longitudinal suprapatellar knee scan (gray scale): quadriceps tendon enthesis shows loss of fibrillar pattern, hypoechogenicity, and an enthesophyte at the superior pole of the patella. **b** Longitudinal infrapatellar knee scan showing the inferior patellar tendon enthesitis: there is thickening of the enthesis, hypoechogenicity, loss of the normal fibrillar pattern, and irregularity of the bony cortex with power Doppler signals at the enthesis. **c** Longitudinal scan right lateral elbow scan showing common extensor tendon enthesis (gray scale): there is thickening of the enthesis, hypoechogenicity, loss of the normal fibrillar pattern, irregularity of the bony cortex, and enthesophyte. **d** Gray scale longitudinal scan of left patellar fascia showing: increased thickness 5.9 mm, hypoechogenicity, loss of normal fibrillar pattern, and enthesophyte

higher in SpA patient group than healthy control (p value < 0.0001).

In accordance with our study, Zhang et al. [28] revealed that relative expression of lncRNA H19 in peripheral blood mononuclear cells of ankylosing spondylitis patients was significantly higher in comparison to normal individuals. Moreover, H19 increased the release of IL-17A and IL-23 cytokines by competitively binding to vitamin D receptor (VDR) via acting as a competing endogenous RNA (ceRNA) for miR22-5p and interacting with miR675-5p.

Similarly, Esawy et al. [31] found that H19 expression was significantly higher in patients with AS than healthy controls.

In contrast to the previous studies [28, 31] which were conducted on patients with AS, the current study was the first one to study lncRNA H19 in patients with non-radiographic SpA as well as patients with AS.

Zhang et al. [28] performed ROC curve analysis to determine the most representative lncRNA among the dysregulated lncRNAs between patients with AS and healthy controls and found that H19 had a higher area

Table 4 The Spearman correlation between BUSES and different parameters

	BUSES		
	Median (IQR)	***r***	***p* value**
ESR in mm/h	27.1 (15.3–45.6)	0.22	0.16
CRP in mg/dl	7.2 (3.6–13.0)	0.06	0.73
ASDAS	3.5 (3.1–4.13)	0.47	0.002*
BASDAI	6.15 (4.25–7.21)	0.36	0.02*
BASFI	6.25 (4.48–7.5)	0.29	0.07
BASMI	0 (0–3)	0.32	0.046*
SPARCC index	1.5 (0–4.0)	0.25	0.12

r: Pearson coefficient

*: statistically significant at $p \leq 0.05$

ASDAS Ankylosing Spondylitis Disease Activity Score, *BASFI* Bath AS Functional Index, *BASDAI* Bath Ankylosing Spondylitis Disease Activity Index, *ESR* Erythrocyte sedimentation rate, *CRP* C-reactive protein, *SPARCC* The Spondyloarthritis Research Consortium of Canada, *BASMI* Bath Ankylosing Spondylitis Metrology Index

under curve and a greater power than LOC101929023 suggesting that it has a higher diagnostic value.

It was found that lncRNAH19 has specificity of 97% and sensitivity of 52.5% with area under the curve (AUC) of 0.83.

These findings may point that lncRNA H19 can be used for discrimination of patients with axial spondyloarthritis from normal population.

This suggests a probable role of lncRNA H19 for diagnosis of patients with early axSpA who do not fulfill ASAS criteria and to differentiate between patients with axSpA and patients with fibromyalgia.

In current study, there was no significant correlation between relative expression of lncRNAH19 and laboratory findings including ESR and CRP and different indices including BASDAI, ASDAS-CRP, BASFI, and BASMI. Measuring acute-phase reactants can be a potent assessment method for monitoring various inflammatory disorders [32]. In ankylosing spondylitis, only 50–70% of those with active illness have elevated levels of C-reactive protein and erythrocyte sedimentation rate. It has been proposed that measuring these acute-phase reactants has limited efficacy in assessing disease activity [33, 34]. As regards BASDAI, ASDAS-CRP, and BASFI, the lack of significant correlation is not fully understood but it may be due to the subjective nature of these questionnaires. The correlation between BASMI and lncRNA H19 was not studied before. While investigating the correlation between lncRNA H19 relative expression and entheses assessment scores in patients with axSpA either clinically

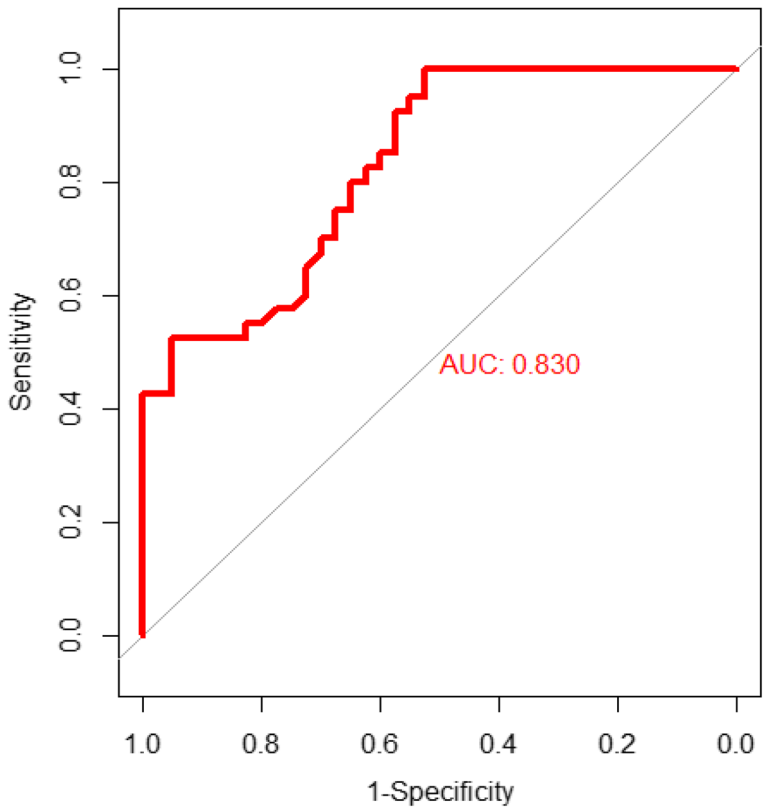

Fig. 3 ROC curve for LNCRNA-H19 to discriminate patients with axSpA from normal population

(SPARCC) or ultrasonographically (BUSES), it was not statistically significant. Meanwhile, Esawy et al. [31] studied the correlation between lncRNA H19 expression and different indices in patients with AS and it was found that H19 expression was significantly correlated with laboratory findings ESR and CRP and also correlated with BASDAI, ASDAS-CRP, and BASFI. Zhang et al. [28] did not study the correlation between H19 expression and disease activity scores. So further studies will be needed to determine the role of lncRNA H19 in monitoring disease activity. To the best of our knowledge, this is the first study to investigate the correlation between lncRNA and entheseal involvement clinically and by ultrasound.

Enthesitis plays an essential role in the pathogenesis of SpA. According to the literature, enthesitis was identified as a significant indication of disease activity, with individuals experiencing more active disease when enthesitis was present compared to those without this condition. Furthermore, there was a significant correlation between enthesitis and increased disease severity, as well as diminished quality of life. Therefore, early detection and management of enthesitis could significantly influence the overall prognosis of SpA [35].

The clinical identification of enthesitis lacks specificity, as it may result from various factors such as mechanical microdamage, fibromyalgia, or hyperalgesia [36]. Furthermore, certain entheses, such as those associated with the cruciate ligaments, provide difficulties in terms of clinical assessment. The complexity arises from the fact that the absence of localized discomfort does not necessarily rule out the existence of enthesitis. The estimated prevalence of clinical enthesitis in psoriatic arthritis (PsA) ranges from 30 to 50%. However, it is important to note that the reported burden of enthesitis may be underestimated, indicating that the true incidence could be higher than currently documented. Hence, there has been a growing interest in the role of imaging as it has the potential to provide insights beyond the limitations of the clinical examination [14].

In our study, we assessed enthesitis clinically by using SPARCC index; the results were correlated with the ultrasonographic findings by using Belgrade score.

The Belgrade Ultrasound Enthesitis Score has recently been employed as a comprehensive ultrasound enthesitis scoring system in order to distinguish between individuals with enthesitis associated with spondyloarthritis and those without spondyloarthritis [25].

In current study, there was not a significant correlation between BUSES and SPARCC index in patients with axSpA. This was contradictory to Florescu et al. [37], who studied the correlation between BUSES and SPARCC index in patients with AS and reported that there was a significant correlation between them. Furthermore, we found a significant correlation between Belgrade sore and disease indices ASDAS-CRP (p value 0.002), BASDAI (p value 0.02) and BASMI (p value 0.046), while there is no significant correlation with BASFI and laboratory parameters ESR and CRP.

A discrepancy exists between the objective measures of erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), and the subjective symptoms reported by patients. This discrepancy leads to a weak connection between these objective measures and both the clinical disease activity and radiographic advancement [38]. The subjective nature of the BASFI questionnaire, along with the absence of specific inquiries regarding the quantification of enthesitis/enthesopathy, results in inconsistencies between the index and the evaluation of entheseal conditions [37].

Florescu et al. [37] found that there was not a statistically significant correlation between BUSES and ESR, CRP, ASDAS, BASDAI, and BASFI.

In the study conducted by Miguel et al. [36], they studied the extent of gray scale and power Doppler entheseal involvement in the Achilles tendon. A favorable association was observed between the ultrasonography parameters, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and Ankylosing Spondylitis Disease Activity Score (ASDAS) values, persisting even at the 6- and 12-month follow-up assessments subsequent to the original examination.

This proves the discrepancy of the conclusions from the studies that were performed on patients with AS for entheseal assessment either clinically or ultrasonographically.

Limitation of the study

First, a larger sample from different locations and with participants of different races was required due to the relatively small sample size. Furthermore, we did not assess the efficacy of the long non-coding RNA (lncRNA) to differentiate between AS and other rheumatic diseases. Moreover, the expression level of long non-coding RNA H19 was not reassessed after follow-up period.

Conclusion

Both lncRNA H19 and Belgrade score have good discriminative ability between patients with axSpA and normal population; this suggests a possible role in early diagnosis for patients with axial spondyloarthritis who do not fulfill ASAS classification criteria. Their role to monitor the disease activity still needs further studies to be established.

Musculoskeletal ultrasound is much more accurate in the assessment of enthesis than clinical examination. Further studies are needed to study the correlation between ultrasonographic entheseal scores and activity markers either clinically or laboratory.

Abbreviations

ASDAS	Ankylosing Spondylitis Disease Activity Score
BASDAI	Bath Ankylosing Spondylitis Disease Activity Index
BASFI	Bath Ankylosing Spondylitis Functional Index
BASMI	Bath Ankylosing Spondylitis Metrology Index
BUSES	Belgrade Ultrasound Enthesitis Score
CRP	C-reactive protein
ESR	Erythrocyte sedimentation rate
HLA	Human leukocyte antigen
lncRNA H19	Long non-coding RNA H19
mSASSS	Modified Stoke Ankylosing Spondylitis Spine Score.
SPARCC	The Spondyloarthritis Research Consortium of Canada

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Authors' contributions
All authors have read and approved the manuscript, and all authors have contributed significantly and agree with the content of the manuscript. A.I. EL-Zwawy contributed to the idea, study design, and writing and editing of the manuscript. E. Soliman contributed to the idea, study design, and editing of the manuscript and she was in charge of ultrasound examination of all the patients. Eman T. Elsayed was responsible for calculation of relative expression of lncRNA H19 in blood samples of patients and controls. Mai M. Morsy handled the clinical examination of the patient, calculation of different disease indexes, clinical examination of enthesis, and wrote and edited the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on request.

Declarations

Ethics approval and consent to participate
The research followed the principles of the Declaration of Helsinki. A written consent was signed from all candidates before the beginning of the study; the research was approved by the Ethics Committee of Faculty of Medicine of Alexandria University (EC serial protocol number 0201488) IRB NO: 00012098 -FWA NO: 00018699.

Consent for publication
Not applicable.

Competing interests
The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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