Preliminary study of high mobility group box chromosomal protein 1(HMGB1) in ankylosing spondylitis patients

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Abstract

Objective

To compare the serum levels of high mobility group box chromosomal protein 1 (HMGB1) between patients with AS and healthy controls, and evaluate its association with disease activities and functional abilities; to investigate the cell surface receptors related to HMGB1 in AS patients.

Methods

The HMGB1 serum levels from71 previously untreated AS patients and 40 healthy controls were detected by ELISA method. Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), Ankylosing Spondylitis Disease Activity Score (ASDAS), Bath Ankylosing Spondylitis Functional Index (BASFI), erythrocytesedimentationrate (ESR), and C-reactive protein (CRP) levels were assessed on these participants. The mRNA expression of HMGB1 and its relevant cell surface receptors RAGE, TLR2, TLR4, and IL-1Racp complex were analysed by RT-PCR.

Results

The HMGB1 serum levels from AS patients were significantly higher than those from healthy controls and remarkably positive correlated with BASDAI, ASDAS, BASFI, CRP, and ESR. ASDAS showed more correlated to HMGB1 serum levels than BASDAI. Besides, the expression of TLR2, TLR4, and IL-1Racp from PBMCs revealed significant correlations with the expression of HMGB1.

Conclusion

HMGB1 might be a good laboratory index for the evaluation of disease activities and disease severity in AS patients. Further, extracellular HMGB1 play its inflammatory role mainly via the expression of cell surface receptors TLR2, TLR4 and IL-1RAcP complex.

Key words

high mobility group box chromosomal protein 1 (HMGB1), ankylosing spondylitis (AS), disease activities, functional abilities with quality of life, cell surface receptors

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Introduction

Ankylosing spondylitis (AS) is a chronical inflammatory disease characterised by major involvement of axialsjoints, bilateral sacroiliitis, and sometimes, peripheral arthritis. The typical pathological manifestation is the inflammation of synovium, joint capsule, tendon and ligament attachment points. It involves extra-articular manifestations occasionally, such as iridocyclitis, psoriasis, osteoporosis, cardiovascular disease, bowel disease, and pulmonary fibrosis (1). The main clinical feature of AS manifests as the pain of the waist, back, neck, hip and joints of the body, and serious spinal deformity and joint stiffness can occur in the later stages. AS occurs predominantly in male adolescents. The prevalence of AS is 0.2–0.54% in the general population of China, which is similar to the Europe and America (1-2). In recent years, biological agents directed against tumour necrosis factor (TNF) represent notably effect for patients with AS in reducing signs and symptoms, delaying the structural destruction in the spine, as well as improving physical function and quality of life (3-4). The exact pathogenesis of AS disease is still an enigma. Etiology studies showed an excessive association between AS and human leukocyte antigen (HLA)-B27, results in family aggregation (5-6). According to statistics, the percentage of HLA-B27 positive in patients with AS in China is approximately 78.4% (7-8). Beyond this, researchers have found that exogenous pathogen-associated molecular patterns (PAMPs) from microbes and endogenous damage-associated molecular patterns (DAMPs) formed by endogenous alarmins contribute to the development of syndesmophytes and bamboo spine in AS (9). High mobility group box chromosomal protein 1 (HMGB1) is a highly conserved histone chromosome binding protein which is wide spread in the mammal eukaryotic cell nucleus (10). When it was passively released into the extracellular matrix during cell necrosis or damage, or actively secreted by activated immune cells such as monocytes, macrophages, NK cells, dendritic cells, synovial fibroblasts in response to appropriate signal stimulation, it acts as an endogenous DAMP and plays pro-inflammatory and inflammatory roles in the progress of chronical inflammatory and autoimmune diseases through binding to various cell signal transduction receptors on the surface of responding cells (11). These receptors including receptor for advanced glycation end products (RAGE), Toll-like receptors (TLRs), Interleukin-1 receptor accessory protein (IL-1Racp) complex (12-14). Extracellular HMGB1 combined with these receptors leads to activation of the NF-κB pathway, thus leading to inflammatory cytokines such as TNF-α, IL-1β, and IL-6 release and forming an inflammatory reaction cascade (15). Studies have found that HMGB1 was high expressed in the synovium of RA patients, along with the high expression of cell surface receptors RAGE, TLR2, TLR4, and IL-1RAcP complex (16). Moreover, HMGB1 in complex with LPS, IL-1α or IL-1β could increase the production of inflammatory factors and tissue-destructive enzymes, which were mediated through receptors TLR4 or IL-1RAcP complex, respectively from cultured synovial fibroblasts of RA (17). No or minimal HMGB1 was released during apoptosis (18).

Since the importance of HMGB1 as endogenous DAMP in the pathogenesis of immune response, HMGB1 has been proven to be critical in the pathogenesis of rheumatoid arthritis, osteoarthritis (OA), systemic lupus erythematosus (SLE), Sjögren's syndrome (SS), polymyositis (PM) and dermatomyositis (DM). According to our knowledge, there are little reports about the relationship between HMGB1 and AS disease, and the association of HMGB1 with disease activity and quality of life is obscure because of its small sample size of the participants (19). In the present report, we detailed analysed the serum levels of HMGB1 in 71 previously untreated AS patients compared to levels in 40 healthy controls, as well as its association to disease activity and functional ability. We found the results were quite different from previous study (19). Furthermore, we investigated the expression of HMGB1 and its correlations with corresponding cell signal transduction receptors which

might play main roles in peripheral blood mononuclear cells.

Materials and methods

Patients and samples

A total of 71 previously untreated (never treated before or not treated with NSAIDs or DMARDs at least in six months, and never treated with biologics before) AS patients, who fulfilled the 1984 modified New York criteria, were consecutively enrolled from the outpatient department and inpatient department of Rheumatology and Immunology, Tongji Hospital (Wuhan, China). Patients who had a history of recurrent infections or cancer or serious liver, renal, haematological or neurological diseases have been excluded. A structural questionnaire was used to define the age, sex, disease severity indices including Bath Ankylosing Spondylitis Disease Activity Index(BASDAI), Ankylosing Spondylitis Disease Activity Score(ASDAS), Bath Ankylosing-Spondylitis Functional Index (BASFI), C-reactive protein (CRP), erythrocytesedimentationrate (ESR). The scores for each scoring system ranged from 0 to 10. The measurement of CRP adopted immunonephelometry by CRP Reagents (BioSystems S.A, Span), and the ESR was detected by Westergren method. The blood samples of healthy controls were obtained from 40 age- and sex-matched healthy volunteers, who have no AS history, AS family history, or other diseases. This research was approved by the ethics committee of Tongji Hospital (Tongji Hospital, Huazhong University of Science and Technology institutional review broad approval, IRB ID 20121001). Before the study, informed consents were obtained from all participants.

Samples of peripheral blood were allowed to clot and then centrifuged at 2000 ×g for 10 minutes. Serum samples in aliquot were collected and stored at -80°C immediately. Beyond this, whole blood in EDTA was collected from 29 AS patients with good compliance and 17 healthy volunteers. The peripheral blood mononuclear cells (PBMCs) were isolated by standard Ficoll-Hypaquedensity centrifugation, and then RNAiso PLus (Takara, USA) was added to the PBMCs immediately for total RNA.

HMGB1 ELISA detection

The estimation of serum levels of HMGB1 were performed with commercial enzyme-linked immunosorbent assay (ELISA) kits (USCNLife Science Inc, Wuhan) according to instruction manual. All samples were tested in duplicated.

RNA extraction and cDNA synthesis

Briefly, total RNA was extracted from PBMCs using the RNAisoPLus (Takara, USA) solution according to the manufacturer's instructions. Reverse transcription (RT) was performed using the RevertAid First Strand cDNA Synthesis Kit (Fermentas, Canada). All RNA samples (2μg/11μL) were incubate with Oligo (dT) primer (1μL) at 65°C for 5 min to denature the secondary structure then put in ice, and the following components were added up to 20μL: 5 × Reaction buffer 4μL, dNTP (10mM) 2μL, RNase Inhibitor 1 μL, Reverse Transcriptase 1 μL. The total reagents were heated at 42°C for 60 min, and terminated the reactions by heating at 70°C for 5min. The cDNA was stored at -80°C.

Quantitative real-time PCR (qRT-PCR)

The expressions of HMGB1, RAGE, TLR2, TLR4, IL-1RacP were measured by quantitative real-time polymerase chain reaction (qRT-PCR) using Lightcycler 480 II RT-PCR Systerm (Roche, Switzerland) in a total volume of 20μl, containing 10μL SYBER Green real-time PCR master mix (ToYoBo, Japan), 1μL forward and reverse primer and 2μL cDNA templates. The recommended cycling conditions for qRT-PCR was: pre-denaturation 95°C 30 sec followed by 40 cycles of denaturation 95°C 5 sec, Annealing 60°C 10 sec, extension 72°C 15 sec. The melting curve analysis was used for controlling the specificity of the amplification products. Differences in primer expression between groups were expressed as cycle time (Ct) values normalised with β-actin. The primers were designed according to Genbank sequences and synthesised by Shanghai Sangon Biological Engineering Technology and Service Company. All sequences of primers were shown as follows: HMGB1:5'-TGCAGTTCCCACATTAAAGAAGACC-3'(sense), 5'-AGTCTCGTTTCCTGAGCAGTCCA-3'(anti-sense);RAGE:5'-CTACCGAGTCCGTGTCTACCA-3'(sense), 5'-CATCCAAGTGCCAGCTAAGAG-3'(anti-sense); TLR2: 5'-GACTTCTCCCATTTCCGTCT-3'(sense), 5'-CAGGTAGGTCTTGGTGTTCA-3'(anti-sense); TLR4:5'-GACCTTTCCAGCAACAAGATTC-3'(sense), 5'-GAGAGATTGAGTAGGGGCATTT-3'(anti-sense); IL-1RAcP: 5'-AGTCTTCATTCGCAGTTTATGG-3'(sense), 5'-AATAGTCAGCCACACAGTCACC-3'(anti-sense); β-actin: 5'-TGGAATCCTGTGGCATCCATGAAAC-3'(sense), 5'-TAAAACGCAGCTCAGTAACAGTCCG-3' (anti-sense).

Relative differences between groups were calculated and expressed as the percent difference relative to healthy controls.

Statistical analysis

Statistical analysis was performed using SPSS17.0 statistical analysis software (IBM Corp., New York, NY, USA). Comparisons between paired or unpaired groups were performed using the independent Student's *t*-test. The Mann-Whitney U-test was applied in comparison for non-parametric analysis. Correlations between variables were determined by Spearman's rank correlation test. A *p*-value of less than 0.05 was considered to be statistically significant difference.

Results

Serum levels of HMGB1 in AS patients and healthy controls and their correlations with disease parameters

This study enrolled 71 previously untreated AS patients and 40 healthy controls (35 men and 5 women, mean age 27.43 years) who matched for age and sex (Table I). In our study, the serum HMGB1 levels in AS patients were significantly higher than levels in the healthy controls (HMGB1:1056.10 ±

Table I. Demographic and disease characteristics of the 71 ankylosing spondylitis patients.

Characteristics	AS patients (n=71)	Healthy controls (n=40)
Age (y)*	28.37 ± 9.27	27.43 ± 1.95
Male/Female*	62/9	35/5
BASDAI	3.85 ± 1.97	undetected
ASDAS	3.06 ± 1.32	undetected
BASFI	2.43 ± 1.96	undetected
CRP (mg/L)	34.6 ± 37.9	undetected
ESR(mm/h)	34.45 ± 31.0	undetected
Serum HMGB1(ng/ml)*	1056.10 ± 1033.05	27.05 ± 21.50

Values are shown as mean±SD. *indicates $p>0.05$. ** $p<0.0001$.
BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; BASFI: Bath Ankylosing Spondylitis Functional Index; ASDAS: Ankylosing Spondylitis Disease Activity Score; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate.

Table II. Spearman's correlation between clinical parameters and serum HMGB1 levels in the 71 ankylosing spondylitis patients.

Clinical parameters	BASDAI	ASDAS	BASFI	CRP (mg/L)	ESR(mm/h)
Serum HMGB1	0.542**	0.78**	0.55**	0.832**	0.76**
ESR	0.475**	0.793**	0.537**	0.839**	–
CRP	0.540**	0.903**	0.533**	–	0.839**

Values are shown as r. r is determined by Spearman's rank correlation test. ** $p<0.0001$.
BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; BASFI: Bath Ankylosing Spondylitis Functional Index; ASDAS: Ankylosing Spondylitis Disease Activity Score; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate.

1033.05ng/mL *vs*. 27.05 ± 21.50 ng/mL, $p<0.0001$). Of the 71 AS patients, we evaluated the correlation between the serum levels of HMGB1 and clinical parameters. As showed in Table II and Fig. 1A-E, the serum levels of HMGB1 significantly positive correlated with BASDAI (r=0.542, $p<0.0001$), ASDAS (r=0.78, $p<0.0001$), BASFI (r=0.55, $p<0.0001$), CRP (r=0.832, $p<0.0001$) and ESR (r=0.76, $p<0.0001$). In our study, ASDAS C version was adopted to evaluate the disease activity, and the formulae was ASDAS C = 0.121 × Back pain + 0.058 × Duration morning stiffness + 0.110 × Patient global + 0.073 × Peripheral pain/swelling + 0.579 × log e (CRP+1) (Back pain: BASDAI Q2; duration of morning stiffness: BASDAI Q6; fatigue: BASDAI Q1; peripheral pain/swelling: BASDAI Q3) (20). Obviously, from the correlation coefficient in the table we concluded that the ASDAS scores for disease activity assessment showed a stronger correlation with serum HMGB1 levels than BASDAI, and that the correlation of ASDAS and clinical laboratory indices ESR/CRP, which were used to reflect disease activities, was much stronger than the correlation of BASDAI and ESR/CRP. These results suggested that ASDAS scoring system might be a better index for AS disease activities. Therefore, we divided the 71 AS patients into low and moderate active groups (n=19), high active groups (n=24) and very high active groups (n=28) according to ASDAS <2.1, 2.1≤ASDAS≤3.5 and ASDAS>3.5 (21). A comparison among these different disease activity groups and healthy controls revealed that there were significant differences between each groups, and the very high active AS groups had the highest levels of serum HMGB1, followed by the high active group, low and moderate active groups, while healthy controls had the lowest levels (Fig. 2). Besides these, we have demonstrated that both ESR and CRP showed a remarkable positive correlation with BASDAI and ASDAS, and the latter was more pronounced (Table II). HMGB1 serum levels also had a significant correlation with ESR and CRP in our data, and comparing to BASDAI and ASDAS, CRP had the strongest correlation with the serm levels of HMGB1.

The mRNA levels of HMGB1, TLR2, TLR4, IL-1RAcP and RAGE in 29 AS patients and 17 healthy volunteers

We further examined the levels of mRNA HMGB1 in PBMCs. We found the transcriptional levels of HMGB1 were failed to demonstrate marked difference between AS patients and healthy controls. Besides, the extracellular HMGB1 participates in the pathogenesis of inflammatory through binding to various receptors on the surface of responding cells, such as RAGE, TLR2, TLR4 and IL-1RAcP, therefore the expression of these specific receptors were also discussed. In our research, the expression of mRNA TLR2, TLR4, IL-1RAcP and RAGE in AS patients were also not showed significant higher than healthy controls, and there was no significant correlation between diseases activities and the mRNA expression of HMGB1 and these cell surface receptors *(data was not shown)*. At the same time, we analysed the correlationship of the mRNA expression between HMGB1 and these cell surface receptors in the 29 AS patients and 17 healthy controls. As show in Table III, in AS patients, the mRNA level of HMGB1 did show positive correlation with the expression of mRNA TLR2 (r=0.374, $p<0.05$), mRNA TLR4 (r=0.441, $p<0.05$) and mRNA IL-1RAcP (r=0.455, $p<0.05$), and the mRNA levels of TLR2, TLR4, and IL-1RAcP also showed significant correlations between each other, The correlation between mRNA TLR2 and mRNA TLR4 was 0.539, $p=0.003$; The correlation between mRNA TLR2 and mRNA IL-1RAcP was 0.647, $p<0.0001$; The correlation between mRNA TLR4 and mRNA IL-1RAcP was 0.579, $p<0.001$. However, these positive correlations were not seen in health controls. The association between mRNA HMGB1 and mRNA RAGE was not significant neither in AS patients or in health controls.

Discussion

The role of high mobility group box protein 1 (HMGB1) in the pathogenesis of proinflammatory and inflammatory diseases had been investigated by numerous researches, especially in the field of chronic, inflammatory autoimmune diseases, such as OA, RA, SLE,

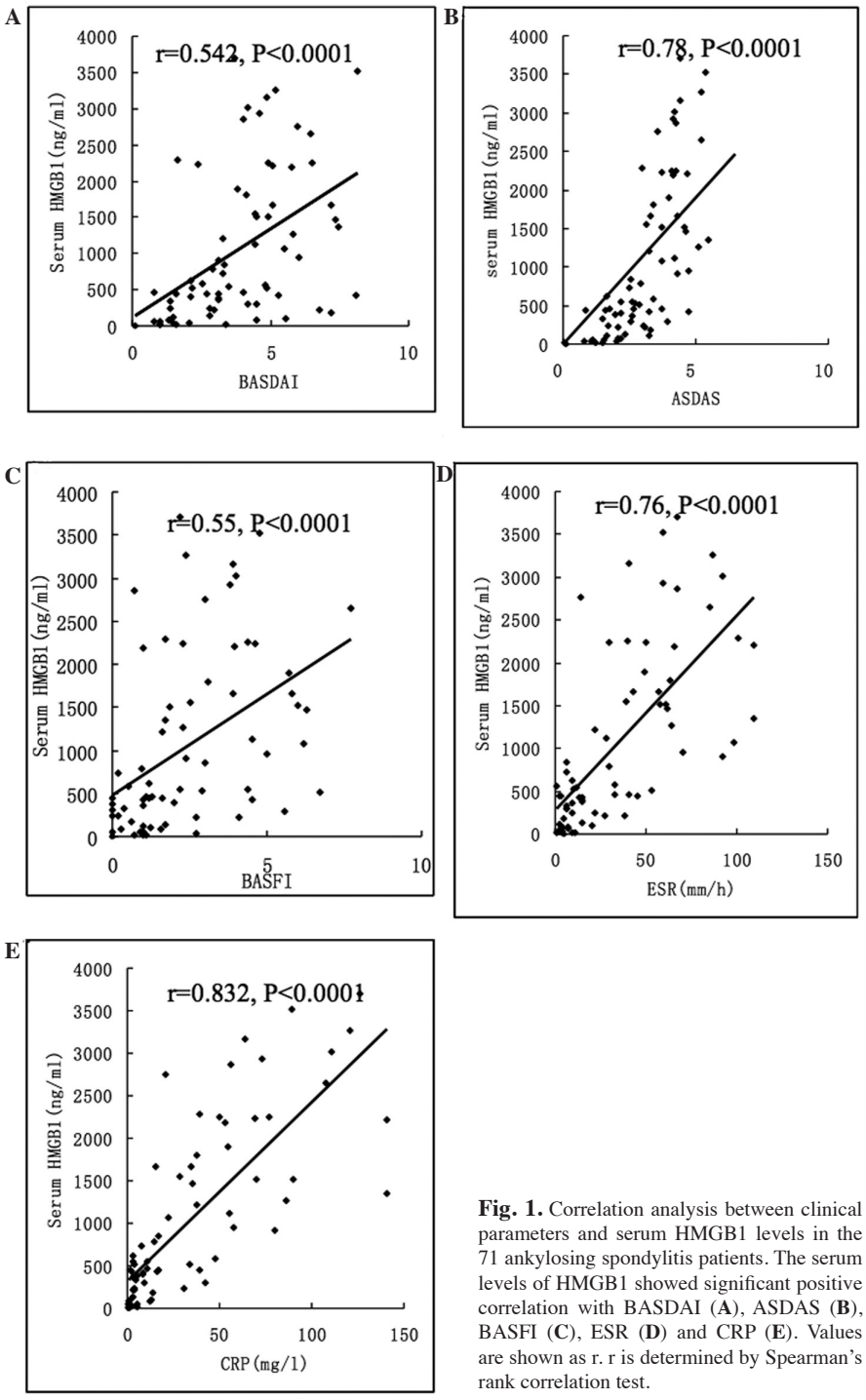

Fig. 1. Correlation analysis between clinical parameters and serum HMGB1 levels in the 71 ankylosing spondylitis patients. The serum levels of HMGB1 showed significant positive correlation with BASDAI (**A**), ASDAS (**B**), BASFI (**C**), ESR (**D**) and CRP (**E**). Values are shown as r. r is determined by Spearman's rank correlation test.

SS, PM and DM (23-27). The serum levels of HMGB1 in these diseases were not merely significantly higher than those in healthy controls, but also positively correlated to disease activities. So far there was only one clinical study concerning the association between AS and HMGB1 (19). In the study, they concluded that the HMGB1 serum levels display a marked difference between AS patients and healthy controls, while it was important to note that their study samples were fairly small, consisting of only 30 AS patients (treated or untreated were not separated, only the patients who received TNF-α inhibitor treatment were excluded) and 29 healthy controls. Therefore, their conclusion about the increased HMGB1 serum levels were not correlated with disease activity and quality of life seemed premature. In fact, the inflammatory status contributes to an accelerated progression of the AS disease. Patients with AS have a nearly two-fold increased risk of death compared to the general population, which is largely due to cardiovascular risk, and the inflammatory process seems to have an potential role in causing this excess risk, which contributes to all stages of atherosclerosis, from early atheroma formation to plaque instability and thrombus development (1). Besides, ESR is one of the best predictors for carotid plaques in AS patients (28). In the present study, serum samples of 71 previously untreated AS patients and 40 healthy volunteers were collected. By using the same HMGB1 ELISA kits (USCNLife Science Inc, Wuhan), we demonstrated the significantly increased serum levels of HMGB1 in AS patients, while the average values of HMGB1 in our results (1056.10±1033.05ng/mL in AS patients *vs*. 27.05±21.50ng/mL in healthy controls, $p<0.0001$) showed quite different from previous study. The values of HMGB1 were much higher than the previous report (19) may attribute to the quality control standards discrepancy of different ELISA kits. Even the properties of the same labelled kits may vary according to the manufacturing year, and version of the kits. The results were genuine and believable, provided that all the samples were tested by the same labelled ELISA kits of the same manufactures. We also found that the increased HMGB1 serum levels indicated positive correlation with BASDAI, ASDAS, BASFI, CRP and ESR. The ASDAS scoring system showed a stronger correlation with the serum levels of HMGB1 than BASDAI scoring system did in view of the correlation coefficient. It is well known that CRP and ESR are some of the most common laboratory markers used to reflect disease activity in AS patients in clinical practice (29-30). In our study, both BASDAI and ASDAS showed a remarkable positive correlation with ESR and CRP, and the latter was especially obvious. What's more, CRP showed a much better correlation

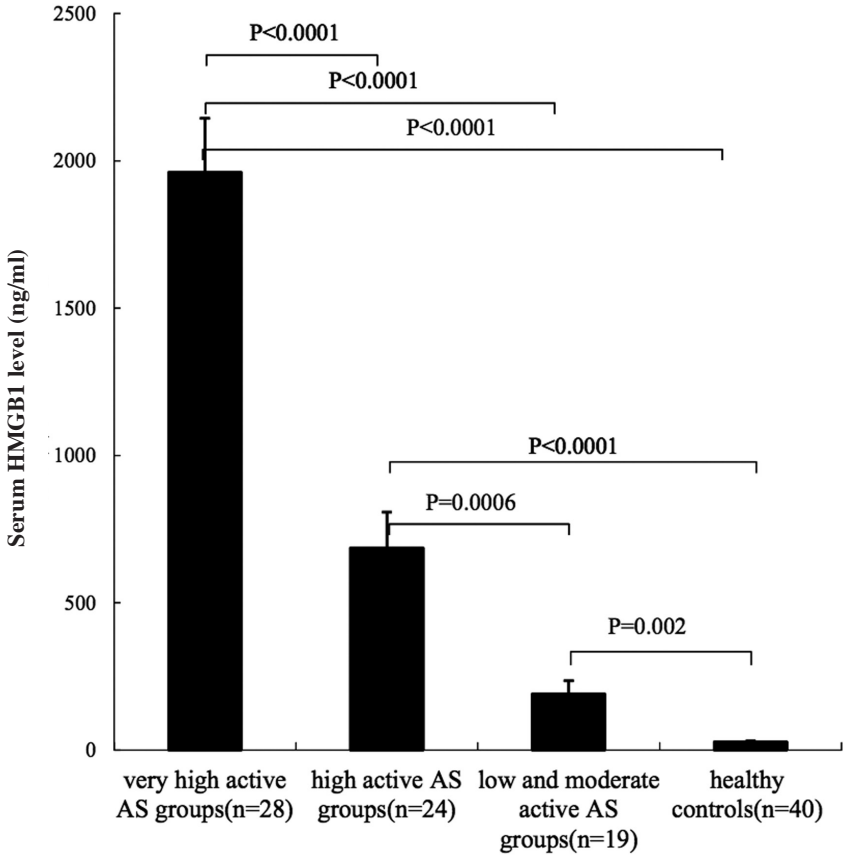

Fig. 2. The comparison of serum levels of different AS disease activities group and healthy controls AS patients were divided into extreme active, highly active, low and moderate active groups according to ASDAS scoring system. The mean HMGB1 serum levels of these groups were showed by histogram. Patients with very high active AS groups (n=28) had significantly highest serum HMGB1 levels, followed by high active AS groups (n=24), low and moderate active AS groups (n=19) and healthy controls (n=40) [mean±SD =1960.36±972.57ng/ml *vs*. 686.63±591.79 *vs*. 190.21±196.17ng/ml *vs*. 27.05±21.50ng/ml]. The independent Student's t-test results were showed in the bar graph. Mann-Whitney U-test was applied in comparing the serum levels of four groups, and the result showed $p<0.001$.

with BASDAI and ASDAS than ESR did, which suggested a superiority in reflecting disease activity in AS. It is of interest to note that HMGB1 serum levels also had a significant correlation to the ESR and CRP in our data, and CRP had a much better correlation comparing to BASDAI and ASDAS. The limitation of this research is that the study is only prospective one centre preliminary study, and multicentre randomised study is necessary.

As is known to all, HMGB1 was first discovered to participate in DNA replication, transcription, recombination, repair, and in regulation of the transcriptional activity of steroid hormone receptors and glucocorticoid receptors as a nuclear DNA chaperone inside the cell (31-32). Our study revealed no significantly increase of mRNA HMGB1 in AS peripheral blood mononuclear cells compared to healthy controls, which was consistent with the previous studies (33-34), This result might indicate that the total mRNA expression level of HMGB1 in the nuclear was not changed in the condition of inflammatory. HMGB1 participates in the pathogenesis of inflammatory only when it is actively or passively released into cytoplasm and extracellular matrix (11).

Extracellular HMGB1 closely associated with certain cell signal transduction receptors such as RAGE, TLRs, IL-1RAcP, thus activating the NF-κB pathway, and leading to inflammatory mediators released (11). In contrast, the activation and expression of HMGB1 can be suppressed by high expression of heat shock protein 72 (HSP72), binding to thrombospondin and Siglec-10 (human) / G(mouse) through CD24, which played a negative regulatory role in the MAPKs and NF-κB pathway (35-38). Researchers have confirmed high expression of TLR2, TLR4 IL-1RAcP, and RAGE in the synovial fluid macrophages of RA patients (39-40). Increased expression of TLR2, TLR4, and RAGE were found in the renal tissues of lupus nephritis patients (41). However, whether or not these functional cell surface receptors of HMGB1 were also high expressed in AS peripheral blood mononuclear cells were still not reported before. The current study suggested that none of these cell surface signal transduction receptors revealed significantly increase on total PBMCs from AS patients. In previous studies, both TLR2 and TLR4 were observed high expressed in SpA synovium (42), which is one of the reasons caused local inflammatory of AS. A new finding of our study was that the relationship between mRNA HMGB1 and the mRNA levels of cell surface receptors TLR2, TLR4 and IL-1RAcP complex showed remarkable positive correlations in AS patients. What's more, the correlation among mRNA TLR2, mRNA TLR4, mRNA IL-1RAcP complex also exist significantly positive correlations. Therefore, we speculated that the extracellular HMGB1 participates in the inflammatory mechanisms of ankylosing spondylitis mainly through binding to the cell surface receptors of TLR2, TLR4 and IL-1RAcP complex, but the receptor of RAGE could not be independently verified to have correlation with HMGB1 from our study. Further studies needed to clarify the exact mechanism of the signal transduction of HMGB1 in AS patients.

In conclusion, the results presented here suggested that HMGB1 might play a role in the inflammatory pathogenesis of AS. Increased HMGB1 showed significantly positive correlation with disease activities measured by BASDAI, ASDAS, ESR, CRP and functional abilities with quality of life measured by BASFI. HMGB1 might be a good laboratory index for the evaluation of disease activity and disease severity in AS patients. Extracellular HMGB1 play its inflammatory role mainly via the expression of cell surface receptors TLR2, TLR4 and IL-1RAcP complex.

Table III. Spearman's correlation analysis of the mRNA expression between HMGB1 and its cell surface receptors in the 29 ankylosing spondylitis patients.

		HMGB1	TLR2	TLR4	IL-1 RacP
AS patients	HMGB1	1	0.374 (0.045)*	0.441 (.017)*	0.455 (0.013)*
	TLR2	0.374 (0.045)*	1	0.539 (0.003)**	0.647 (p<0.0001)**
	TLR4	0.441 (0.017)*	0.539 (0.003)**	1	0.579 (p<0.001)**
	IL-1 RacP	0.455 (0.013)*	0.647 (p<0.0001)**	0.579 (p<0.001)**	1
	RAGE	0.143 (0.476)	0.197 (0.324)	0.094 (0.641)	0.277 (0.162)
Health controls	HMGB1	1	0.028 (0.914)	0.269 (0.297)	0.34 (0.182)
	TLR2	0.028 (0.914)	1	-0.516 (0.034)*	0.06 (0.819)
	TLR4	0.269 (0.297)	-0.516 (0.034)*	1	0.458 (0.064)
	IL-1 RacP	0.34 (0.182)	0.06 (0.819)	0.458 (0.064)	1
	RAGE	0.091 (0.729)	0.081 (0.757)	-0.081 (0.757)	-0.292 (0.256)

Values are shown as r (p-value). r is determined by Spearman's rank correlation test. * p<0.05, ** p<0.01.

Therefore, it seems reasonable to conclude that the progression of AS disease could be delayed by therapeutic administration of HMGB1 specific inhibitors, though further studies are needed to substantiate it.

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