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ORIGINAL ARTICLE

Evaluation of *DNMT1* gene expression profile and methylation of its promoter region in patients with ankylosing spondylitis

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Abstract Ankylosing spondylitis (AS) is an autoimmune disease with a chronic inflammatory arthritis. The critical role of methylation in the biology of immunocytes has increasingly been surveyed to discover disease etiology. DNA methyltransferase 1 (DNMT1) is an enzyme, which establishes and regulates patterns of methylated cytosine residues. The aim of the current investigation was to unveil if methylation circumstances of CpG sites in *DNMT1* promoter could affect the mRNA expression level of this gene in peripheral blood mononuclear cells (PBMCs) from AS patients. PBMCs were isolated from whole blood of 40 AS patients and 40 healthy individuals. Total RNA and DNA contents of leukocytes were extracted. Afterward, quantitative analysis was carried out by real-time PCR using the SYBR Green PCR Master Mix. Finally, to determine the methylation level, PCR products of bisulfite-treated DNA from patients and controls were sequenced. Compared with healthy controls, expression level of *DNMT1* in AS patients was significantly downregulated. Methylation of *DNMT1* promoter was significantly higher in AS patients in comparison to controls. While a negative correlation between methylation and expression level of *DNMT1* was observed in AS patients, both methylation and expression level of *DNMT1* did not correlate with clinical manifestations. Considering the observation that decreased expression level of *DNMT1* was associated with hypermethylation of *DNMT1* promoter in PBMCs from AS patients, this survey suggests that dysregulation of *DNMT1* expression through altered methylation level of other target genes would probably contribute to AS development.

Keywords Ankylosing spondylitis · CpG sites · DNA methylation · DNMT1 · Gene expression

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Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory disorder in the spine and sacroiliac joints and mainly characterized by arthritis and enthesitis at the spine and other peripheral joints [1, 2]. AS is a multifactorial disease, in which both environmental factors and genetics are involved [3, 4]. Nonetheless, the precise mechanisms underlying the pathogenesis of AS remain unclear. Human leukocyte antigen (HLA)-B27 is documented to be strongly associated with AS risk but accounts for only a part of the overall risk for AS. This implies to the involvement of other genes as well other factors in the development of disease [5–7].

Autoimmune diseases are usually known as multifactorial diseases. Genetic predisposition impresses susceptibility to or protection from disease onset but cannot completely explain disease initiation and development [8]. Although recent genome-wide association studies (GWAS) linked strong genetic background with disease, these studies almost failed to identify the presence of a specific genetic mechanism for immune tolerance breakdown. Furthermore, the identified

Table 1 Baseline and demographic features of AS patients and healthy subjects

Property	AS patients ($N = 40$)	Healthy subjects ($N = 40$)
Gender		
Male	31 (77.5 %)	31 (77.5 %)
Female	9 (22.5 %)	9 (22.5 %)
HLA-B27 status		
Positive	33 (82.5 %)	–
Negative	7 (17.5 %)	–
Age	40.7 ± 6.9	38.9 ± 5.3
BASDAI	3.4 ± 1.90	–
BASFI	3.1 ± 2.18	–
BASMI	3.6 ± 2.74	–
ESR (mm/h)	24.5 ± 5.23	4.5 ± 2.3
Disease duration	10.4 ± 7.67	–

AS Ankylosing Spondylitis, *HLA* human leukocyte antigen, *ESR* erythrocyte sedimentation rate, *BASDAI* Bath Ankylosing Spondylitis Disease Activity Index, *BASFI* Bath Ankylosing Spondylitis Functional Index, *BASMI* Bath Ankylosing Spondylitis Metrology Index

significant genetic associations are found only in a few numbers of patients [9].

According to definitions, epigenetics is known as constant and heritable changes in gene expression without alterations in DNA sequence. It seems that the epigenetic modifications are important for controlling the gene expression profile during the cell cycle, development, and even in response to environmental or biological stimuli. In other words, epigenetics has come to explain how cells with distinct and limited number of genes can differentiate into different cell types and how a certain phenotype can be passed to daughter cells [10].

Nowadays, it is a consensus that epigenetic modifications like DNA methylation are involved in regulating the gene expression [11]. DNA methylation, which occurs at the 5′ position of cytosine of a CpG dinucleotide and leads to the formation of 5-methylcytosine, is a well-defined epigenetic modification [12]. Alternately, aberrant gene promoter hypermethylation, alongside with gene silencing, has been related to a number of human disorders [13, 14]. The etiology of AS is only partially explained by the current genetic risk map. Considering the fact that aberrant DNA hypermethylation plays an important role in human disease, it is of interest to evaluate if DNA methylation status is also involved in the pathogenesis of AS. In this survey, it is aimed to evaluate methylation status of CpG sites in the promoter of DNA methyltransferase 1 (*DNMT1*) gene and assess the effect of methylation status on the transcript level of this gene. Moreover, the impression of methylation and mRNA expression level of *DNMT1* on disease activity in AS patients was determined.

Materials and methods

Patients and control subjects

The patients were recruited from Rheumatology Research Center (RRC) clinic, Shariati Hospital, Tehran, Iran, and diagnosed as having AS according to the modified New York criteria [15]. Disease severity and functional disabilities were deliberated through Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) [16], Bath Ankylosing Spondylitis Functional Index (BASFI) [17], and Bath Ankylosing Spondylitis Metrology Index (BASMI) [18]. Additionally, erythrocyte sedimentation rate (ESR) and HLA-B27 status were measured for each AS patient and healthy control. Forty AS patients and 40 healthy age- (40.7 ± 6.9 vs. 38.9 ± 5.3, respectively) and sex (31 (77.5 %) males and 9 (22.5 %) females in both groups)-matched healthy volunteers as control group were included in the study. None of the healthy controls had autoimmune disease in neither themselves nor their immediate family members. Patients had taken non-steroidal anti-inflammatory drugs (NSAIDs) including indomethacin (28 cases), naproxen (8 cases), and celecoxib (4 cases) before 3 months at the time of sampling. However, patients did not receive immunomodulatory therapy for 3 months before inclusion in the study. Demographic and

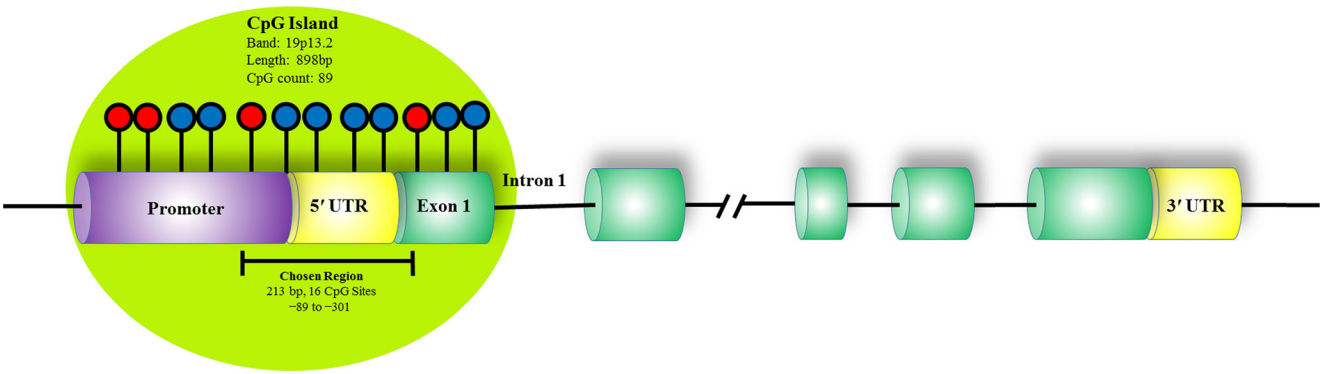


Fig. 1 Schematic illustration of the chosen CpG sites in the promoter region of the DNMT1 gene. The DNA target for PCR amplification was composed of a 213-base pair segment at the 19p13.2 band, which covered a CpG island from promoter till exon 1 with 898 bp. Sixteen CpG sites were found in the selected region from −89 to −301 in the promoter of DNMT1

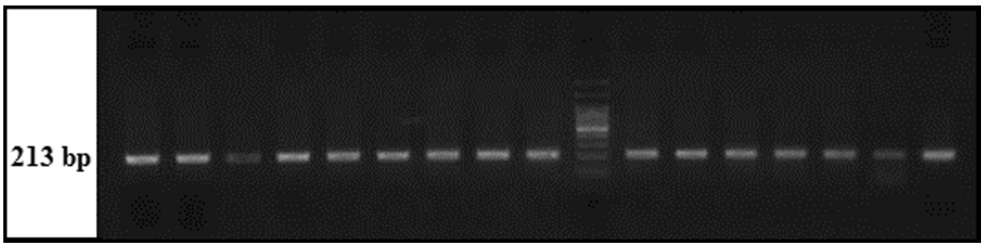


Fig. 2 Representative gel electrophoresis image of amplified 213-bp target after bisulfite treatment of DNA samples from PBMCs from AS patients and normal controls

clinical specification of AS patients and control group are listed in Table 1 with more details. HLA-B27-positive AS patients were major compartment of the case group (33 (82.5 %) cases). AS patients had increased ESR level compared to the healthy individuals (24.5 ± 5.23 vs. 4.5 ± 2.3). In AS patients, the BASDAI, BASFI, and BASMI scores were measured to be 3.4 ± 1.90, 3.1 ± 2.18, and 3.6 ± 2.74, respectively.

The Human Research Ethics Committee from the Tehran University of Medical Sciences approved this study. Written informed consent was taken by all the participants. Blood samples from AS patients were obtained during clinical diagnosis; as such, samples were obtained from healthy controls. Blood sampling was done in the early morning. About 10 ml of blood from each subject was collected in EDTA-anticoagulated tubes and ESR blood collection test tubes using venipuncture.

PBMC preparation and RNA and DNA extraction

In order to isolate PBMCs from peripheral blood of the subjects, the Ficoll-Hypaque density gradient centrifugation approach was exploited. Total cellular RNA was extracted using High Pure RNA Isolation Kit (Roche, Germany) according to the manufacturer's manual. DNA extraction was performed through the phenol–chloroform approach. Yield and purity of RNA and DNA were determined using a NanoDrop spectrophotometer at 260/280 nm (NanoDrop ND-2000C Spectrophotometer, Thermo Fisher Scientific, USA).

Reverse transcription and complementary DNA synthesis

Complementary DNA (cDNA) was synthesized from the RNA of cells using the Transcriptor First Strand cDNA Synthesis Kit (Roche, Germany) according to the manufacturer's instructions. Concisely, first, 1 μg of isolated RNA from the previous procedure was mixed with 2 μl of random hexamer primer and corresponding amount of RNase-free H_2O to a total volume of 13 μl and then incubated at 65 °C for 10 min. Afterward, the microtubes were chilled on ice, and a mixture of 4 μl reaction buffer, 0.5 μl RNase inhibitor, 2 μl dNTP mix, and 0.5 μl reverse transcriptase was added to each sample. Samples were immediately incubated at 25 °C for 10 min followed by 50 °C for 60 min; the reaction was finally terminated by heating at 85 °C for 5 min. Reverse transcription was performed with the final volume of 20 μl per tube.

Real-time quantitative polymerase chain reaction

Primers (DNMT1 and β2M as a housekeeping gene) were designed using Primer Express 3.0 (Applied Biosystems, Foster City, CA, USA). For accuracy and specificity, primers were checked using the Basic Local Alignment Search Tool

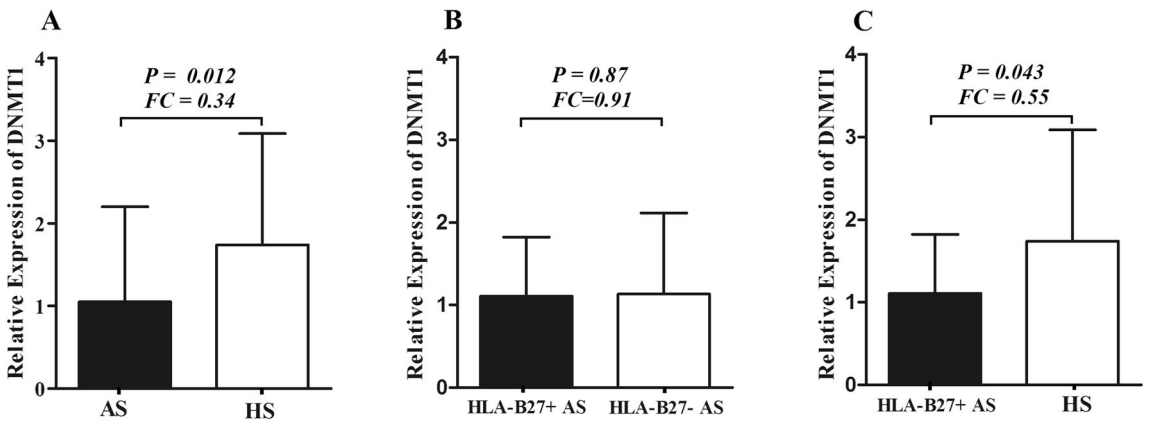


Fig. 3 Relative expression level of DNMT1 mRNA in three categories of AS vs. healthy subjects, HLAB27+ AS patients vs. HLA-B27− AS patients and HLA-B27+ AS patients vs. healthy subjects. The Kolmogorov–Smirnov test did not comply with the normal distribution of data, and therefore, the Mann–Whitney U test calculated the represented *P* values (*AS* ankylosing spondylitis, *HS* healthy subjects, *FC* fold change)

Table 2 Methylation status of each 16 CpG sites in AS and healthy groups

CpG sites	AS patients $N = 40$ (%)	Healthy controls $N = 40$ (%)	P value
CpG1			
Methylated	35 (87.5 %)	27 (67.5 %)	0.59
Unmethylated	5 (12.5 %)	13 (32.5 %)	
CpG2			
Methylated	25 (62.5 %)	15 (37.5 %)	*0.044*
Unmethylated	15 (37.5 %)	25 (62.5 %)	
CpG3			
Methylated	30 (75 %)	26 (65 %)	0.465
Unmethylated	10 (35 %)	14 (35 %)	
CpG4			
Methylated	32 (80 %)	24 (60 %)	0.087
Unmethylated	8 (20 %)	16 (40 %)	
CpG5			
Methylated	38 (95 %)	31 (77.5 %)	*0.048*
Unmethylated	2 (5 %)	9 (22.5 %)	
CpG6			
Methylated	25 (62.5 %)	17 (42.5 %)	0.117
Unmethylated	15 (37.5 %)	23 (57.5 %)	
CpG7			
Methylated	36 (90 %)	31 (77.5 %)	0.225
Unmethylated	4 (10 %)	9 (22.5 %)	
CpG8			
Methylated	35 (87.5 %)	28 (70 %)	0.099
Unmethylated	5 (12.5 %)	12 (30 %)	
CpG9			
Methylated	36 (90 %)	29 (72.5 %)	0.083
Unmethylated	4 (10 %)	11 (27.5 %)	
CpG10			
Methylated	19 (47.5 %)	27 (67.5 %)	0.113
Unmethylated	21 (52.5 %)	13 (32.5 %)	
CpG11			
Methylated	36 (90 %)	24 (60 %)	*0.004*
Unmethylated	4 (10 %)	16 (40 %)	
CpG12			
Methylated	31 (77.5 %)	19 (47.5 %)	*0.010*
Unmethylated	9 (22.5 %)	11 (52.5 %)	
CpG13			
Methylated	21 (52.5 %)	30 (75 %)	0.062
Unmethylated	19 (47.5 %)	10 (35 %)	
CpG14			
Methylated	25 (62.5 %)	13 (32.5 %)	*0.013*
Unmethylated	15 (37.5 %)	27 (67.5 %)	
CpG15			
Methylated	27 (67.5 %)	21 (52.5 %)	0.254
Unmethylated	13 (32.5 %)	9 (47.5 %)	
CpG16			
Methylated	33 (82.5 %)	21 (52.5 %)	*0.008*
Unmethylated	7 (17.5 %)	9 (47.5 %)	

Italicized values denote significant P values

on the US National Center for Biotechnology Information website (http:// www.ncbi.nlm.nih.gov/tools/primer-blast/). Primers were produced by the custom oligonucleotide synthesis service Metabion (Martinsried, Germany). Quantitative analysis was performed by real-time PCR using the SYBR Green PCR Master Mix and StepOne real-time PCR System (both Applied Biosystems). Each reaction mixture contained a total volume of 20 μl (master mix 10 μl, cDNA 2 μl, forward primer 0.8 μL, reverse primer 0.8 μL and H_2O 6.4 μl). The quantitative real-time PCR conditions were 95 °C for 10 min, then 40 cycles of 95 °C for 15 s and 60 °C 60 s. A widely used approach to represent relative gene expression, the comparative C_T method, was exploited to evaluate expression as previously described by Schmittgen and Livak [19]. Relative amount of target mRNA in test sample was calculated and normalized to the corresponding β2M mRNA transcript level as a housekeeping gene. Then, relative expression for each sample was calculated using the following equation: relative mRNA expression = $(2^{-\Delta Ct}) \times 10^3$.

DNA treatment through bisulfite conversion

Bisulfite conversion principles were carried out according to a manual described by Kate Patterson et al. [20]; first, 10 μl of sample DNA with the concentration of 1 μg/μl and 35 μl of distilled water were added into the tube. Then, 5 μl of NaOH 2 M was added into each tube and mixed by pipetting. After that, each tube was taped and incubated for 20 min in 37 °C. Then, 30 μl of 100 mM hydroquinone (C6H6O2) was added into each tube and pipetted until the color of the tube content was converted to light yellow. Afterward, 520 μl of 3 M sodium bisulfite (NaHSO3) was added into each tube and sealed. Each tube was incubated for 16 h in 50 °C water bath. After that, the DNA content of the tubes was extracted by a DNA extraction kit (Roche, Germany). After DNA sedimentation preparation, 16.5 μl of 3 M NaOH was added into each tube and pipetted. Afterward, the tubes were incubated in room temperature for 5 min. Then, 3 μl of glycogen, 51 μl of ammonium acetate (4 °C), and 600 μl of absolute ethanol (4 °C) were added into the tubes and mixed by tapping. Then, the tubes were incubated for at least 2 h in a −20 °C refrigerator. The procedures were followed by centrifugation at 14,000 rpm, for 30 min, in 4 °C. After that, the supernatant was removed, and then 400 μl of 70 % ethanol (4 °C) was added into the tubes. The centrifugation was repeated again at 14,000 rpm, for 30 min, in 4 °C. After removing the supernatant, the tubes were air dried. Ultimately, 30 μl of distilled water was added into each tube and mixed. The DNA was used for subsequent PCR for target DNA amplification.

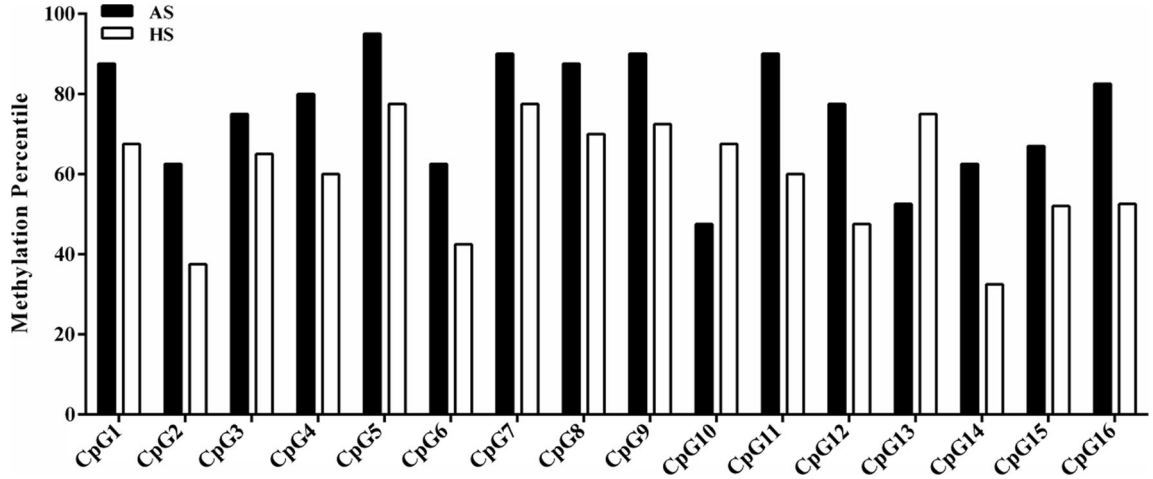


Fig. 4 Distribution of methylation percentile of 16 CpG sites in AS patients and normal subjects. As a point, CpGs 10 and 13 were hypomethylated in patients than in controls. (*AS* ankylosing spondylitis, *HS* healthy subjects)

PCR target DNA amplification

The target DNA for PCR amplification was a 213 base pair segment at 19p13.2 band, which was included in a CpG island from promoter to exon 1 with 898-bp length. The number of CpG sites in the target DNA by the primer was 16 sites (Fig. 1). Primer, which covered from −89 to −301, was designed by Methprimer website (http://www.urogene.org/cgi-bin/methprimer/methprimer.cgi) with the forward sequence of 5′-GGGAAAGGTATTGAGGGATTTT-3′ and reverse of 5′-CCCCAAATACAACAAACTTTAAATT-3′. For PCR amplification, each tube contained 1.5 μl of forward and reverse primers, 4 μl of bisulfite-treated DNA, 10 μl of PCR master mix (containing PCR buffer and Taq polymerase) and H2O to a total volume of 25 μl. PCR was performed under the following conditions: 35 PCR cycles of denaturation at 95 °C for 30 s, primer annealing at an optimized temperature of 60 °C for 30 s, and an extension at 72 °C for 45 s. A final extension at 72 °C for 7 min was carried out. After amplification, each sample was gel electrophoresed for amplification validation (Fig. 2) and, afterward, sequenced through difficult sequencing method (Macrogen, Seoul, Korea). The DNA sequencing results were analyzed by CodonCode Aligner version 2 software.

Statistical analysis

Data analysis was fulfilled via SPSS software version 21 (SPSS, Chicago, IL, USA). Scale variables were evaluated for normality using the Kolmogorov–Smirnov test. Through the independent sample *t* test, group comparisons of continuous variables were carried out. Pearson correlation was applied to assess the relationship between scale variables. In order to draw the graphs, the GraphPad Prism version 6.00 for Windows (GraphPad Software, La Jolla California USA, www.graphpad.com) was applied. All results were expressed as mean ± standard deviation (SD) with statistical significance set at 0.05.

Results

mRNA expression level of DNMT1 in PBMCs

The expression level of DNMT1 mRNA in AS patients was downregulated compared with healthy controls (AS vs. healthy controls, fold change = 0.34; $P = 0.012$; Fig. 3a). In addition, HLA-B27-positive AS patients expressed the DNMT1 mRNA slightly lower than did the healthy subjects (0.55 times downregulated; $P = 0.043$; Fig. 3c). On the other side, HLA-B27-positive AS patients expressed the DNMT1 mRNA almost equal to HLA-B27-negative patients (0.91 times downregulated; $P = 0.87$; Fig. 3b).

Methylation level of DNMT1 promoter in PBMCs

The methylation percentile distribution among AS patients and healthy controls has been illustrated in Table 2 and Fig. 4. All the CpG sites, except CpG 10 and 13, were highly methylated in patients compared with the control group. The differences in methylation level of CpG 2, 5, 11, 12, 14, and 16 in the promoter region of the DNMT1 gene in PBMCs between AS patients and healthy subjects were significant ($P = 0.044$, 0.048, 0.004, 0.01, 0.013, and 0.008, respectively).

Taking all the CpG sites together, methylation analysis revealed that the promoter of DNMT1 in AS patients was hypermethylated in comparison to healthy individuals ($P = 0.0071$, Fig. 5a). On the other hand, there was no significant difference in the methylation level of the promoter region of DNMT1 between HLA-B27-positive and HLA-B27-negative AS patients ($P = 0.077$, Fig. 5b). However, it was observed that DNMT1 was highly methylated in PBMCs from HLA-B27-positive AS patients in comparison to healthy individuals ($P = 0.0012$, Fig. 5c).

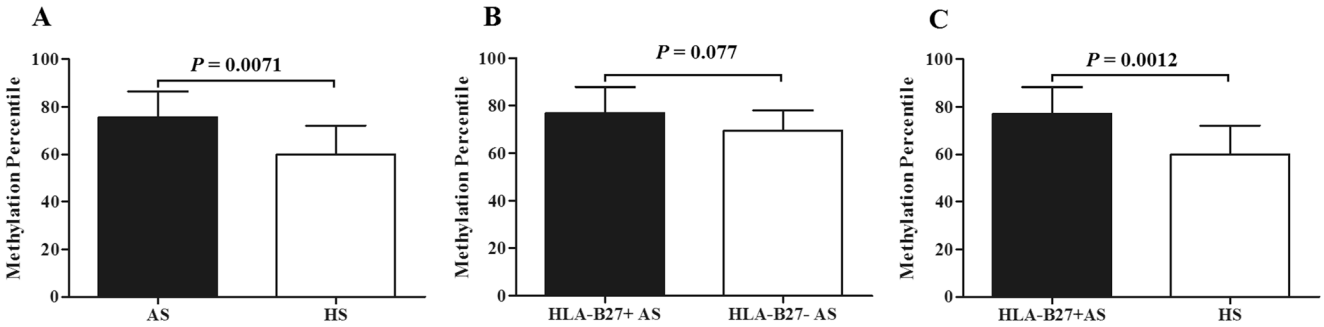


Fig. 5 Overall methylation level of DNMT1 promoter region in three categories of AS vs. healthy subjects, HLA-B27+ AS patients vs. HLA-B27− AS patients and HLA-B27+ AS patients vs. healthy subjects. The Kolmogorov–Smirnov test confirmed the normal distribution of data, and therefore, the Independent sample *t* test calculated the represented *P* values (*AS* ankylosing spondylitis, *HS* healthy subjects)

Correlation of DNMT1 expression and methylation with clinical manifestations

It was seen that mRNA expression of DNMT1 in PBMCs from AS patients negatively correlated with methylation level of promoter of this gene ($P = 0.037$, $r = -0.315$; Fig. 6). However, no significant correlation was observed between mRNA expression level of DNMT1 and clinical manifestations of AS patients with regard to the ESR level and BASDAI, BASFI, and BASMI scores (Fig. 7a). Moreover, the methylation level of DNMT1 promoter region did not reveal significant correlation with the manifestations of patients, namely, ESR level and BASDAI, BASFI, and BASMI scores (Fig. 7b).

Discussion

In this survey, it was aimed to exhibit if aberrantly methylated promoter of DNMT1 could possibly play a role in the pathogenesis of AS. Over the course of the past few decades, epigenetic concept has been a fast-expanding field of research, attempting to shed a new light on the etiopathogenesis of various diseases. A bulk of investigations has focused on the role of 5-methylcytosine (5-mC) in CpG-rich regions, known as CpG islands, on transcription modulation in autoimmune diseases [21]. CpG islands are commonly located in promoter of genes in the vicinity to transcriptional start sites. Methylation of cytosines has been associated with long-term gene silencing such as X-chromosome inactivation. DNA methyltransferase (DNMT) enzymes, with S-adenosylmethionine (SAM) as the methyl donor, mediate DNA methylation. The methyl group (CH3) is transferred onto the C5 position of cytosines to form the 5-mC [22].

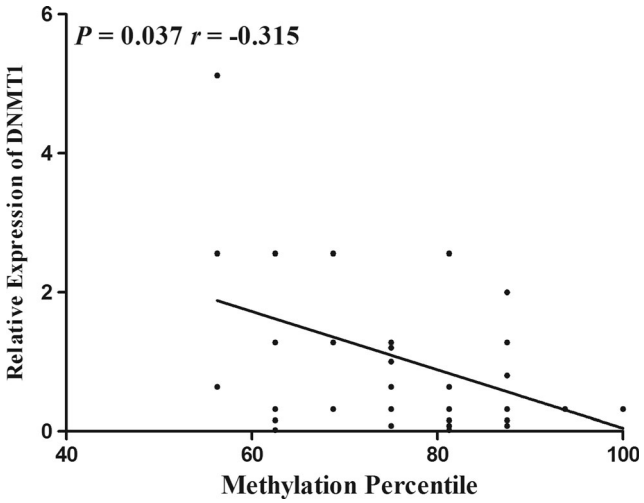
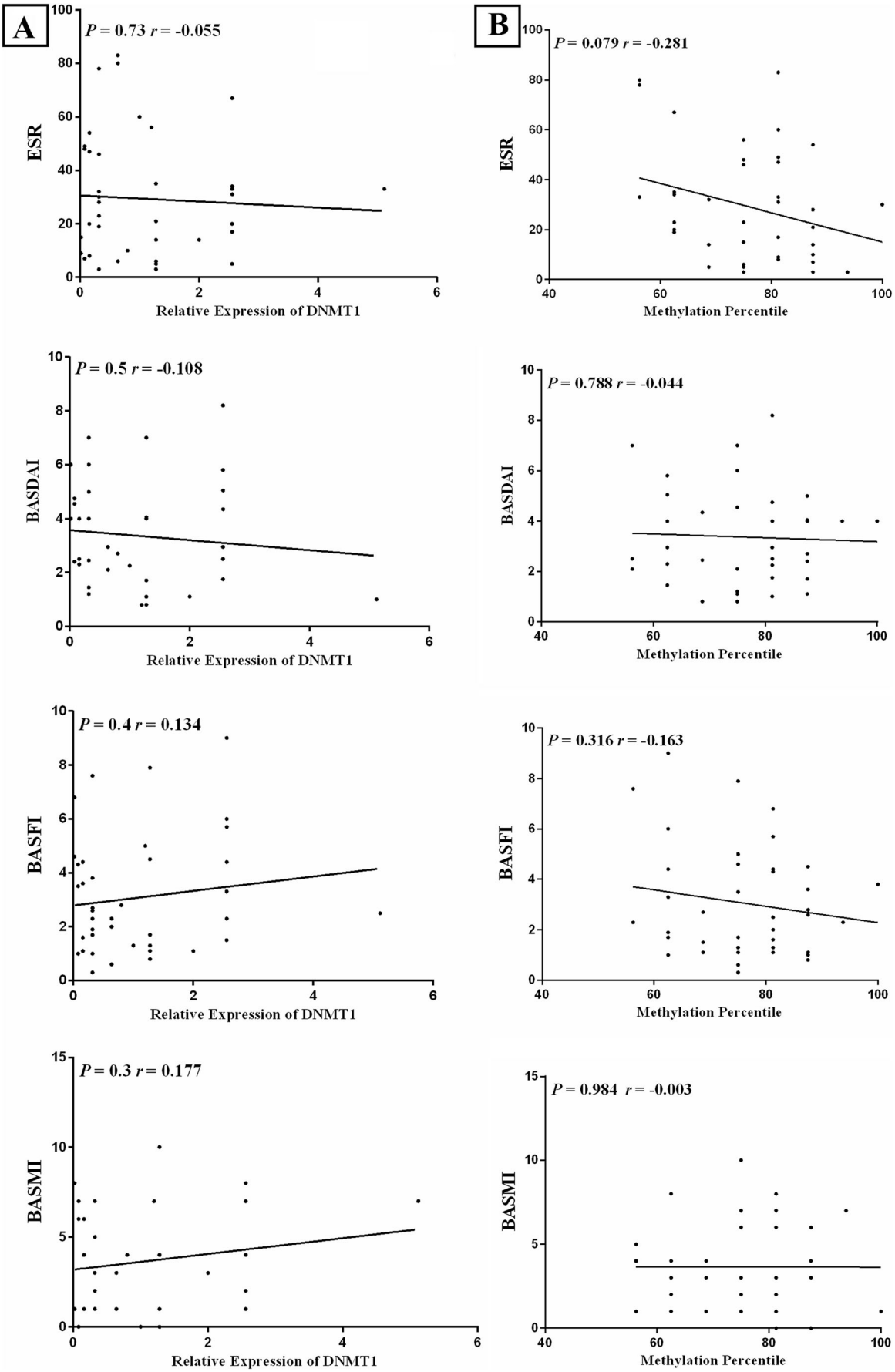


Fig. 6 Scatter plot of mRNA expression level of DNMT1 and methylation level of DNMT1 promoter region in AS patients. The expression level correlates with methylation level ($P = 0.037$, $r = -0.315$). The regression line is also illustrated (Pearson correlation coefficient (*r*) was calculated)

A survey, performing the whole-blood transcriptional profiling in AS, demonstrated that expression level of *DNMT1* was downregulated [23]. Our findings, in accordance with this investigation, disclosed that *DNMT1* mRNA was downregulated in PBMCs from AS patients compared with healthy subjects. Regarding the role of DNMT1 enzyme in maintaining the methylation pattern of DNA, it is expected that downregulation of *DNMT1* can impress DNA methylation level of downstream genes. Lai et al. observed that methylation of *suppressor of cytokine signaling 1* (*SOCS-1*) gene could be detected in serum of HLA-B27-positive AS patients but not in B27-positive healthy controls. The methylation level of *SOCS-1* was shown to be correlated with the degree of inflammation with respect to sacroiliitis, acute phase reactant, ESR, and C-reactive protein, as well as cytokine level of interleukin (IL)-6 and tumor necrosis factor (TNF)-α [24]. In mammals, there are three known DNMT enzymes. DNMT1 copies the pattern of CpG methylation from the parental DNA strand to the daughter strand during S phase and thereby maintains

Fig. 7 Scatter plot exhibiting the correlation between mRNA expression level of DNMT1 (**a**) and methylation of promoter region of the DNMT1 gene (**b**) with erythrocyte sedimentation rate (ESR), Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), Bath Ankylosing Spondylitis Functional Index (BASFI), and Bath Ankylosing Spondylitis Metrology Index (BASMI). The regression line is also illustrated (Pearson correlation coefficient (*r*) was calculated)


A
B
P = 0.73 r = -0.055
P = 0.079 r = -0.281
ESR
Relative Expression of DNMT1
Methylation Percentile
P = 0.5 r = -0.108
P = 0.788 r = -0.044
BASDAI
P = 0.4 r = 0.134
P = 0.316 r = -0.163
BASFI
P = 0.3 r = 0.177
P = 0.984 r = -0.003
BASMI

patterns of DNA methylation [25]. It was believed for some time that DNMT1 could, by itself, maintain patterns of DNA methylation during cell replication. However, it is now reported that collaborations from DNMT3a and DNMT3b are also required for methylation pattern maintenance [26]. DNA methylation may suppress gene expression directly by impeding the binding of transacting factors and indirectly by recruiting the histone deacetylases (HDACs) through DNMT and methylated CpG binding proteins [25]. Consequently, the methylation level of genes and the expression suppression depend on several pathways and molecules except the DNMT1 function only.

On the other hand, our investigation demonstrated that the expression level of *DNMT1* in AS patients was significantly downregulated compared with healthy participants. Overall, the methylation of *DNMT1* promoter was significantly higher in AS patients compared with that in controls. Moreover, statistical analysis divulged a negative correlation between methylation and expression level of *DNMT1* in AS patients. Epigenetic mechanisms that disturb expression of immune system-related genes, particularly those involved in the biology of T cells such as thymic education, differentiation, and effector function, depend on methylation events, which can profoundly affect self-tolerance and contribute to the occurrence of autoimmunity [27]. As a result, it is not beyond expectations that downregulation of *DNMT1*, as a key player in methylation modification, could impress methylation pattern of numerous genes, which in turn could lead to several malfunctions in the immune system. Meanwhile, we detected that both methylation and expression level of *DNMT1* did not correlate with clinical manifestations of AS patients.

Over the course of the past few years, microRNAs (miRNAs) have been focused on more than the other epigenetic regulations in AS. Upregulation of miR-16, miR-221, and let-7i has been observed in T cells from AS patients, and increased expression of let-7i in AS T cells contributes to the immunopathogenesis of AS via enhancing the Th1-associated inflammatory response [28]. It was also found that miR-29, which directly targets the Dickkopf homolog 1 (Dkk-1) mRNA, had upregulated expression in the PBMCs of AS patients in comparison to rheumatoid arthritis patients and healthy individuals. [29]. Reduced miRNA-130a and increased HDAC3 levels were observed in PBMCs from AS patients. HDAC3, by constituting a negative feedback loop with miR-130a as well as by promotion of TNF-α expression, played a role in the pathogenesis of AS [30]. Finally, Huang et al. observed a significantly increased level of miR-21 in AS patients [31]. Up to now, little is explored with regard to the involvement of epigenetic modifications in AS; however, the data considering the role of epigenetics in the etiopathology of AS have started to accumulate.

Conclusion

It is believed that the epigenetic modifications could be regarded as a useful diagnostic marker for complications and manifestations in AS and might provide promising therapeutic tools in the future. However, improving our knowledge of the role that epigenetic modifications play in the development of autoimmunity is likely to increase the prospects for controlling or preventing autoimmune disease. To this end, we aimed to assess the role of DNA methylation in development of AS. We found for the first time, to our best knowledge, that methylation of *DNMT1* promoter was significantly higher in AS patients compared with healthy controls. Relative to healthy controls, expression level of *DNMT1* in AS patients was significantly downregulated. Moreover, a negative correlation was observed between promoter methylation level and mRNA expression of *DNMT1*.

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Compliance with ethical standards

Ethical statement The Human Research Ethics Committee from the Tehran University of Medical Sciences approved this study. Written informed consent was taken by all the participants.

Disclosures None.

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