PERIODICUM BIOLOGORUM
VOL. 125, No 3–4, 213–222, 2023
DOI: 10.18054/pb.v125i3-4.25616

UDC 57:61
CODEN PDBIAD
ISSN 0031-5362

Original Research Article

Increased mRNA expression of ADAMTS1 and ADAMTS4 in peripheral blood mononuclear cells of psoriasis patients developed psoriatic arthritis

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Keywords: psoriatic arthritis; psoriasis; ADAMTS1; ADAMTS4; ADAMTS5

Abbreviations

PsA	– psoriatic arthritis
PsO	– psoriasis
ADAMTS	– A disintegrin-like and metalloproteinase domain with thrombospondin-1 repeats
PBMCs	– peripheral blood mononuclear cells
RA	– rheumatoid arthritis
OA	– osteoarthritis
ECM	– extracellular matrix
MMPs	– matrix metalloproteases
RT-qPCR	– real-time quantitative PCR
IGF-1	– insulin-like growth factor-1

Received February 28, 2023
Revised July 13, 2023
Accepted July 13, 2023

Abstract

Background and purpose: *Psoriatic arthritis (PsA) is a chronic inflammatory disease associated with psoriasis (PsO) affecting both skin and joint. ADAMTS (A disintegrin-like and metalloproteinase domain with thrombospondin-1 repeats) is a large family of proteoglycanase enzymes and the expression levels of ADAMTS proteases are upregulated in arthritis. In this study, we aimed to determine mRNA expression levels of ADAMTS1, ADAMTS4 and ADAMTS5 and identifying the key signaling pathways involved in the regulation of these proteases in peripheral blood mononuclear cells (PBMCs) of patients with PsO who later developed PsA.*

Materials and methods: *25 PsA patients, 25 PsO patients and 25 healthy individuals were included in this study. PBMCs were isolated from venous blood and mRNA expression levels of ADAMTS1, ADAMTS4 and ADAMTS5 were measured through Real-time quantitative PCR (RT-qPCR).*

Results: *mRNA expression levels of ADAMTS1 and ADAMTS4 were found to be increased in PsA patients compared with control and PsO patients. In response to TNF-α stimulation, the expression of ADAMTS1 in PsA patients was determined to be reduced in a Erk1/2 activity dependent manner, whereas p38 and JNK activities were shown to induce the expression of ADAMTS4 in PsA patients. The reduced ADAMTS1 expression in PsA patients induced by IL-1β stimulation was revealed to be dependent on NFkB activity.*

Conclusions: *mRNA expressions of ADAMTS1 and ADAMTS4 regulated by MAPKs and NFkB were increased in PBMCs of PsA patients. This study supports the hypothesis that ADAMTS1 and ADAMTS4 mRNA level might be diagnostic markers for identifying psoriatic patients who are more likely to develop PsA and a future drug target for PsA treatment.*

INTRODUCTION

Psoriatic arthritis (PsA) is an immune-mediated chronic inflammatory arthritis associated with psoriasis (PsO). PsA is the second most common form of inflammatory arthritis after rheumatoid arthritis (RA) *(1)*. The articular disease is characterized by systemic inflammation, synovitis, enthesitis, dactylitis and spondylitis which leads to severe bone and cartilage destruction, functional impairment and a significant reduction in quality of life *(2)*. About 30 % of patients with PsO develop PSA. The exact cause of PsA still remains unclear because of a complex interaction of genetic, immunological, and environmental factors *(3)*.

Nowadays, there is not a definitive cure for PsA and specific biomarkers accurately detecting disease progression and therapeutic response are not available, highlighting the need for better understanding of cellular and molecular mechanisms playing roles in the pathogenesis of PsA.

Inflammatory factors and cytokine pathways are involved in PsA pathogenesis. Aberrant cytokines production is associated with disease progression. TNF-α and IL-1β induce inflammatory response contributing to destruction of articular cartilage *(4)*. TNF-α level was reported to be elevated in PBMCs of PsA patient, which intensifies expression of proteases including matrix metalloproteases (MMPs) *(5)*. Pro-inflammatory cytokines including IL-1, IL-6 and IL-8 were shown to be upregulated in correlation with systemic inflammation markers (eryrosit sedimentation rate, C-reactive protein) in synovial fluid of PsA patients *(6)*. In addition, IL-6 level was demonstrated to be increased and correlated with disease severity in PsA patients compared to PsO patients *(7)*. Recent evidence showed that IL-18 and IL-20 also contribute to pathogenesis of PsA *(8)*.

ADAMTS (A disintegrin-like and metalloproteinase domain with thrombospondin-1 repeats) is a large family of proteolytic enzymes which is involved in extracellular matrix (ECM) deposition in various physiological and disease processes. ADAMTS proteases are key players of tissue development and maintenance process *(9)*. These proteases degrade ECM components associated with the regulation of cellular signals of inflammation, cell adhesion, migration, and angiogenesis *(10)*. ADAMTS proteases consist of 19 family members having structural and functional domains containing an N-terminal catalytic domain and C-terminal region composed of thrombospondin type 1-like repeats *(11)*. ADAMTS family members are differentially expressed in various human tissues. Dysregulation or mutation of these proteases contribute to onset and/or progression of numerous diseases including, arthritis, cancer, and atherosclerosis, and they are involved in the pathogenesis of many inflammatory and thrombotic disorders through induction of pro-inflammatory stimuli, impairment of cellular signaling and the degradation structural proteins *(12)*. ADAMTS1, ADAMTS4 and ADAMTS5, known as aggrecanases are key players of aggrecan and versican turnover during development and homeostasis. ADAMTS4 and ADAMTS5 mediate destruction of aggrecan contributing to cartilage destruction in osteoarthritis (OA) *(13)*.

The activity of MAPK family members including Erk1/2, JNK and p38 was reported to be increased in the inflammatory response *(14)*. NFkB and STAT3 are transcription factors that control the expression of many genes involved in inflammation and immunity. Pro-inflammatory cytokines exert their biological activities using MAPK, NFkB and STAT3 signaling pathways. TNF-α and IL-β regulate ADAMTS4 expression via induction of NFk-B and MAPK signaling pathways *(15)*. In addition, IL-6 upregulates the expression of ADAMTS4 and ADAMTS5 in a STAT3 dependent manner *(16)*.

The expression levels of ADAMTS proteases are upregulated in arthritis, such as OA and RA *(17,18)*. It is well established that pro-inflammatory cytokines induce expression of ADAMTS leading to the destruction of articular cartilage in OA and RA *(16,19)*. Although intensive studies have been done on immunological factors in PsA, whether ADAMTS proteases associated with inflammatory process play roles in the pathogenesis of PsA remains unknown. The objective of this study was to evaluate mRNA expression pattern of ADAMTS1, ADAMTS4 and ADAMTS5 and the signaling pathways involved in the regulation of these proteases in PsA.

MATERIALS AND METHODS

Study subjects

Twenty-five PsO patients followed in dermatology clinics of Dicle University hospital (14 females, 11 males, the mean age 35.93±17.46) were recruited. Psoriasis's clinical evaluation was performed by a dermatologist. Twenty-five PsA patients with PsO (14 female, 11 males, the mean age 43.26±14.77) followed in the rheumatology clinics of Dicle University hospital were recruited. The rheumatologist was evaluated all patients according to the recommendations by GRAPPA (Group for Research and Assessment of Psoriasis and Psoriatic Arthritis) and PsA patients were diagnosed according to CASPAR criteria *(20)*. Twenty-five healthy individuals (14 females, 11 males, 40.33 ± 10.79) were included in the study as controls (Table 1). The study was approved by the ethics committee of Dicle University Faculty of Medicine. All participants were informed about the study and their written consent was obtained. Patients did not receive medication at least three months before enrolment. Patients with other chronic diseases including rheumatic diseases, cardiovascular diseases and autoimmune diseases and individuals under 18 years and pregnant women were excluded from this study.

Sample preparation and cell culture

10 ml peripheral blood was drawn into heparin tubes and the blood samples were transported to the laboratory within 1 h at ambient temperature. PBMCs were isolated from fresh blood samples by Ficoll-Paque Plus density gradient centrifugation (GE Healthcare Biosciences AB, Uppsala, Sweden). After PBMCs isolation, the cell pellets were suspended in serum free RPMI 1640 growth medium (Life Technologies, UK) (Hyclone, UK) supplemented with 100 units/ml penicillin/streptomycin (Life Technologies, UK) (Figure 1). To measure the basic mRNA level of ADAMTS, 0.5x 10^6 cells for each sample were subjected to RNA isolation. For culture condition, the 0.5x 10^6 cells were plated in twenty-four well plate and

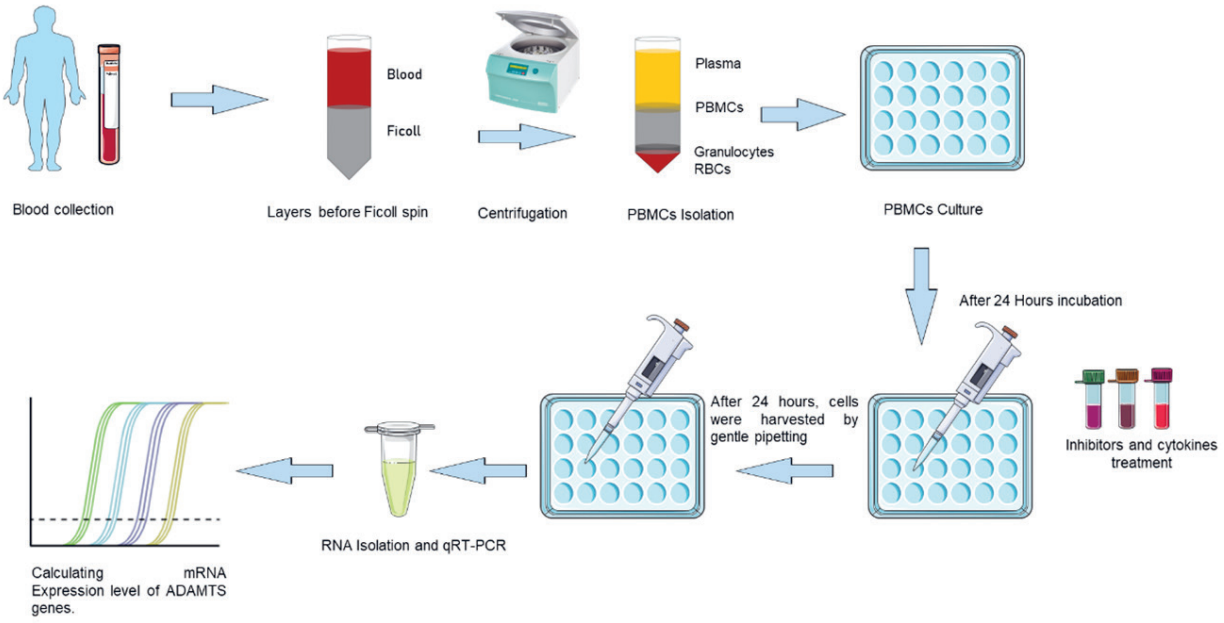

Figure 1: *Schematic illustration of the experimental design.*

incubated for 24 h at 37 ºC in a humidified atmosphere containing 5 % CO_2. After serum starvation, the cells treated with 10 µM specific inhibitors of Erk1/2, p38, JNK, STAT3 and NFkB (PD98059, SB203580, SP600125, parthenolide, sm7368, respectively) for 2h. As the inhibitors were dissolved in DMSO, the same amount of DMSO (0,1 %), which was used for the preparation of 10 µM inhibitors concentration was added to the well not treated with the specific inhibitors. After the incubation time, the cells were stimulated with TNF-α (100 ng/ml), IL-6 (100 ng/ml) and IL-1β (20 ng/ml) and incubated for 24 h at 37 ºC in a humidified atmosphere containing 5 % CO_2. Control cells received water as the stimulators was dissolved in the water. After the treatments, the cells were detached by pipetting and the detached cells were collected and centrifuged. After centrifugation, the cell pellet was washed with PBS and subjected to total RNA isolation.

Real-time quantitative PCR

The mRNA expression of ADAMTS genes (ADAMTS1, ADAMTS4, ADAMTS5) were quantified by RT-qPCR. High Pure RNA Isolation kit (Roche) was used to extract total RNAs from PBMCs. RNA quality and purity were analyzed by spectrophotometry using the BioDrop µ LITE. Complementary DNA (cDNA) was synthesized from total RNA using Transcriptor First Strand cDNA Synthesis Kit (Roche) according to the manufacturer's instructions. RT-qPCR primers and probes which were commercially available as Real-Time Ready assays (Roche) were obtained from Roche. GAPDH was used as a reference gene for normalization of the target genes. RT-qPCR reactions were performed using Light Cycler 480 Probes Master (Roche) in Light Cycler 480 (Roche) instrument. All reactions were run in triplicate. The CT values obtained for each sample were analyzed by the $2^{-\Delta\Delta Ct}$ method.

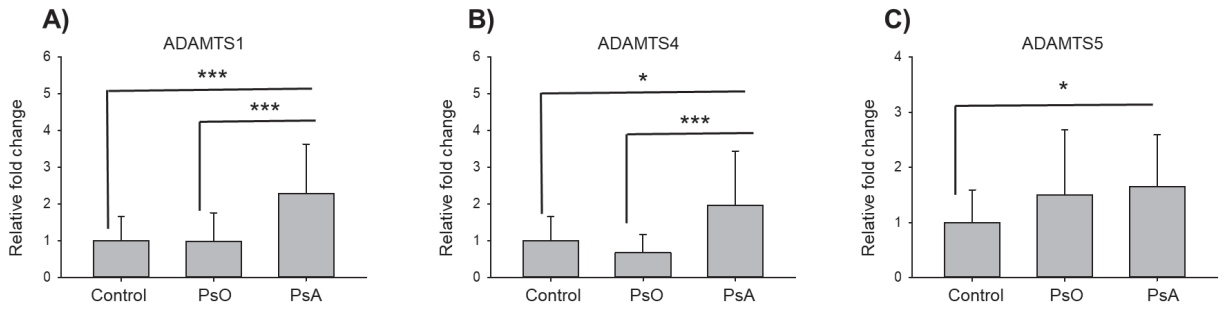

Figure 2. *Increased mRNA levels of ADAMTS1 and ADAMTS4 in PsO patients developed PsA. The Relative gene expression of the ADAMTS1, ADAMTS4 and ADAMTS5 in PBMCs from PsO and PsA patients (n=25) and individuals not affected by psoriasis (control n=25) was measured by RT-qPCR. The results of these experiments are presented as an expression relative to GAPDH. *= p< 0.05 ***= p< 0.001.*

Statistical analysis

The significance of the differences between controls and patients was calculated by One Way ANOVA using the Sigmaplot 14 software package (Systat). A p-value of < 0.05 was considered as statistically significant.

RESULTS

The mRNA expression levels of ADAMTS1 and ADAMTS4 were found to be significantly increased in PBMCs of PsA patients compared to the control group and PsO patients (Figure 2A, 2B). While there was a small but statistically significant increase in the mRNA expression level of ADAMTS5 in PBMCs of PsA patients compared to the control group, but there was no significant difference between PsA and PsO patients in terms of mRNA expression of ADAMTS5 (Figure 2C). In addition, DMSO treatment of the PBMCs which has been used as control for the cultured PBMCs did not affect the basal mRNA expression of ADAMTS1 and ADAMT4 in patients and healthy subjects (Figure 3A, 3B).

Next, to investigate whether MAPK signaling pathways have an effect on the mRNA expression of ADAMTS1 and ADAMTS4, PBMCs obtained from healthy individuals and patients (PsO and PsA) were treated with Erk1/2, p38 and JNK specific inhibitors before TNF-α stimulation. In the control group treated with DMSO, TNF-α stimulation and Erk1/2 and p38 inhibitions did not cause a significant difference in ADAMTS1 mRNA expression, however; TNF-α stimulation resulted in a statistically significant increase in ADAMTS1 mRNA expression in the presence of JNK inhibition (Figure 4A). TNF-α stimulation induced a small but not statistically significant increase in ADAMTS4 mRNA expression, whereas the inhibition of Erk1/2 and p38 activities were observed to block the TNF-α-induced increase in ADAMTS4 mRNA expression (Figure 4B). In addition, JNK inhibition was found to have no effect on ADAMTS4 mRNA expression (Figure 4B). In the PsO group, the mRNA expression of ADAMTS1 was significantly elevated in response to TNF-α stimulation. Whereas the inducing effect of TNF-α stimulation on the mRNA expression of ADAMTS1 was abolished as a result of the inhibitions of Erk1/2, p38 and JNK activities (Figure 4C). While TNF-α induced ADAMTS4 mRNA expression was not changed by the inhibition of Erk1/2 activity, p38 and JNK inhibitions blocked the TNF-α-induced ADAMTS4 expression (Figure 4 D). In the PsA group, TNF-α stimulation resulted in a significant reduction in ADAMTS1 mRNA expression. It was observed that Erk1/2 inhibition abolished the suppressive effect of TNF-α on ADAMTS1 mRNA expression, however; the suppressive effect of TNF-α on the ADAMTS1 mRNA expression was not affected by p38 and JNK inhibitions (Figure 4E). Although ADAMTS4 mRNA expression was not affected by TNF-α stimulation and Erk1/2 inhibition, p38 and JNK inhibitions led to a decrease in the basal mRNA expression of ADAMTS4 (Figure 4E).

Next, we addressed whether IL-6 induced STAT3 activity is required for the regulation of the ADAMTS1 and ADAMTS4 mRNA expressions in PBMCs of healthy subjects and patients (PsO and PsA). In both control and PsO groups, a significant increase in ADAMTS1 mRNA expression was observed in response to IL-6 stimulation, but the inhibition of STAT3 activity did not significantly change IL-6-induced ADAMTS1 mRNA expression level (Figure 5A, 5B). In the PsA group, IL-6 stimulation led to a significant reduction in ADAMTS1 mRNA expression, whereas the suppressive effect of IL-6 on ADAMTS1 mRNA expression was not affected by STAT3 inhibition (Figure 5C). It was observed that there was no significant change in ADAMTS4 mRNA expression as a result of IL-6 stimulation in the control and PsO groups (Figure 3D, 3E). In the presence of STAT3 inhibition, IL-6 stimulation was found to have an inducing effect on ADAMTS4 mRNA expression, but this inducing effect was not statistically significant when compared to the control group (Figure 5D, 5E). In the PsA group, IL-6 stimulation resulted in a significant decrease in AD-

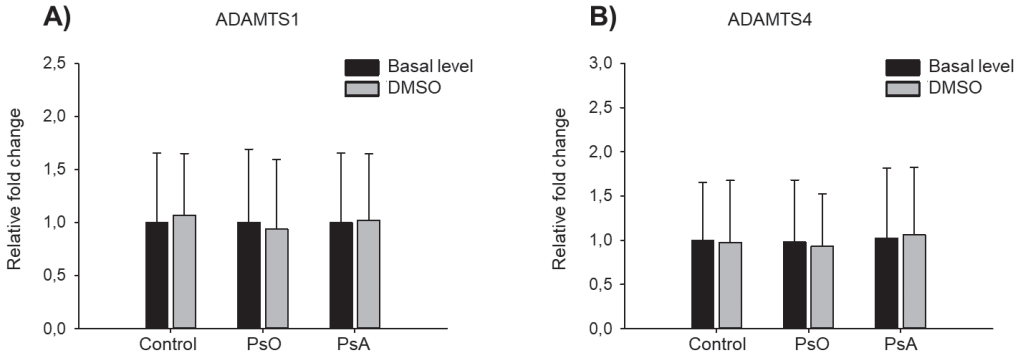

Figure 3. *DMSO treatment did not affect the basal mRNA expression level of ADAMTS1 and ADAMTS4 in patients and healthy subjects. The Relative gene expressions of the ADAMTS1, ADAMTS4 PBMCs isolated from the subjects and treated with DMSO (0,1 %) in culture condition were measured by RT-qPCR. The results of these experiments are presented as the expression relative to GAPDH.*

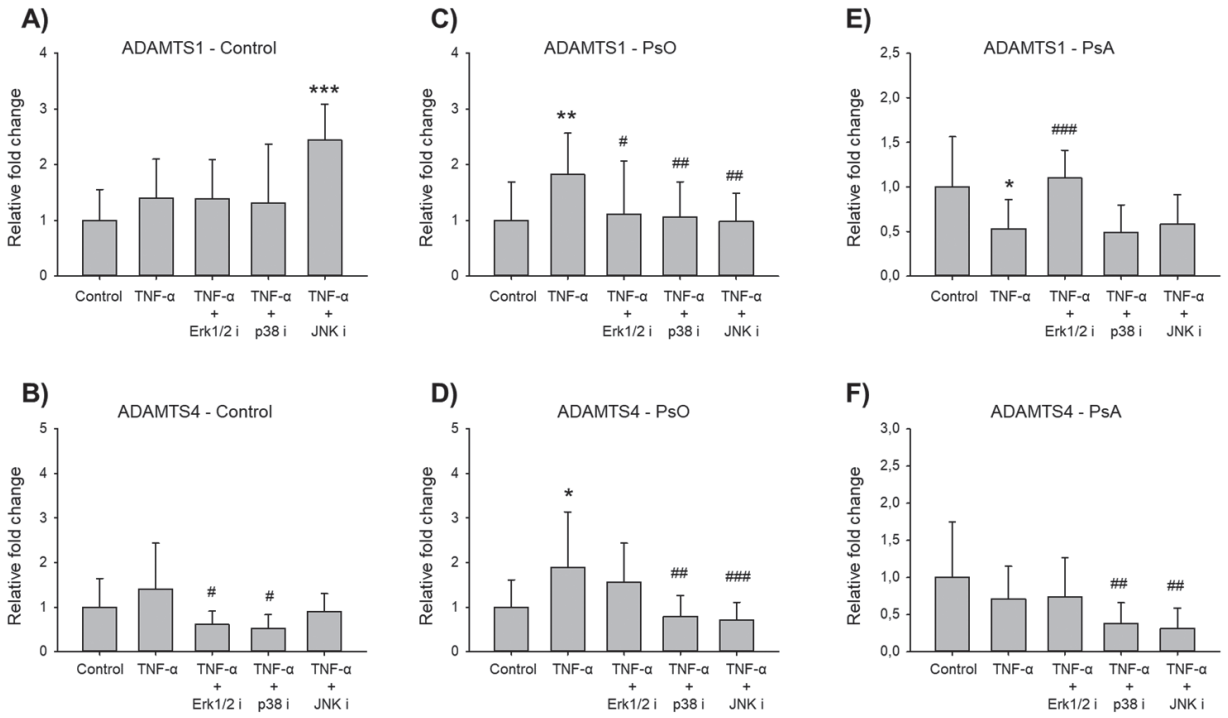

Figure 4. *mRNA Expression of ADAMTS1 and ADAMTS4 was regulated by MAPK. PBMCs were treated with 10 μM Erk1/2, p38 and JNK specific inhibitors (Erk1/2 i: PD98059, p38 i: SB203580 and JNK i: SP600125) for 2 hours before stimulation with TNF-α (100 ng/ml) for 24 hours. After the treatments, the relative gene expression of ADAMTS1 and ADAMTS4 in PBMCs from PsO and PsA patients (n=25) and healthy controls (n=25) was measured by RT-qPCR. The results of these experiments are presented as the expression relative to GAPDH. *= p< 0.05 **= p< 0.01 ***= p< 0.001 compared to control cells. #= p< 0.05 ##= p< 0.01 ###= p< 0.001 compared to TNF-α treated cells.*

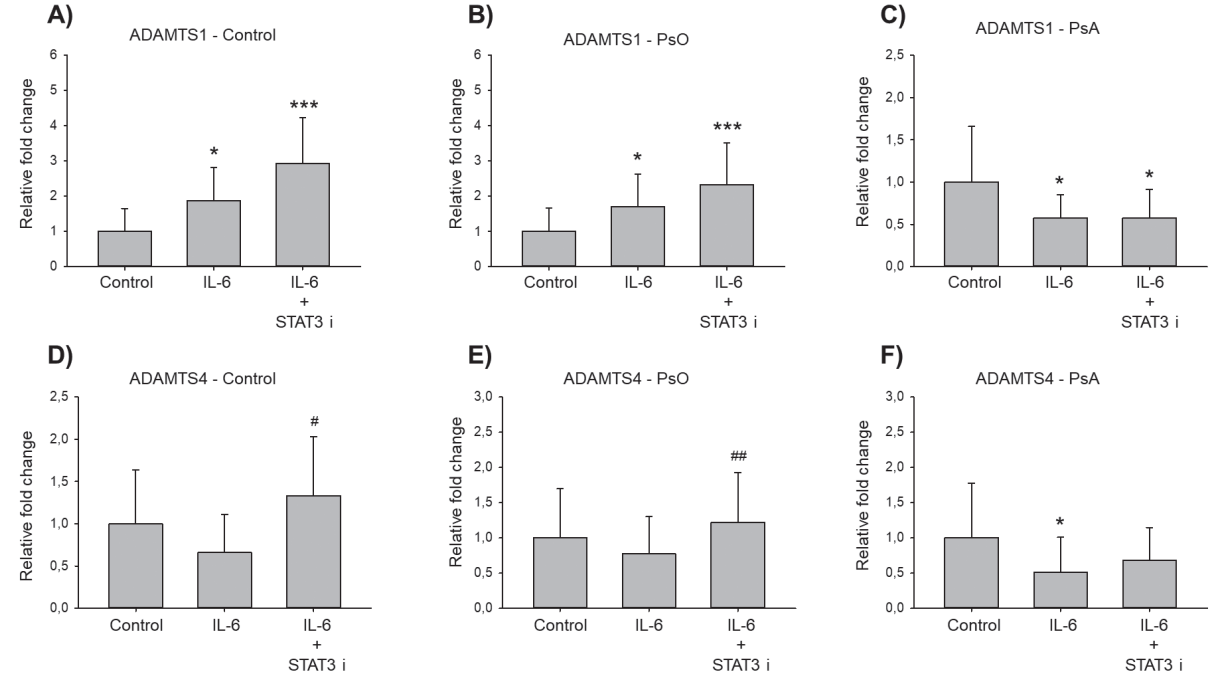

Figure 5. *mRNA expression of ADAMTS1 and ADAMTS4 was regulated by STAT3. PBMCs were treated with 10 μM STAT3 specific inhibitor (parthenolide) for 2 hours before stimulation with IL-6 (100 ng/ml) for 24 hours. After the treatments, the relative gene expression of ADAMTS1 and ADAMTS4 in PBMCs from PsO and PsA patients (n=25) and healthy controls (n=25) was measured by RT-qPCR. The results of these experiments are presented as the expression relative to GAPDH. *= p< 0.05 ***= p< 0.001 compared to control cells. #= p< 0.05 ##= p< 0.01 compared to IL-6 treated cells.*

AMTS4 mRNA expression, but the suppressive effect of IL-6 on ADAMTS4 mRNA expression were not altered in the presence of STAT3 inhibition (Figure 5F).

Lastly, we investigated the effect of IL-1β induced NFkB activity on the mRNA expression of ADAMTS1 and ADAMTS4 in PBMCs of healthy subjects and patients (PsO and PsA). In the control group, it was observed that both IL-1β stimulation and NFkB inhibition had no effect on ADAMTS1 mRNA expression (Figure 6A). In the PsO group, it was found that IL-1β stimulation did not change ADAMTS1 mRNA expression, but ADAMTS1 mRNA expression was upregulated in the presence of NFkB inhibition (Figure 6B). In the PsA group, it was revealed that IL-1β stimulation caused a decrease in ADAMTS1 mRNA expression, while NFkB inhibition abolished the suppressive effect of IL-1β on ADAMTS1 mRNA expression (Figure 6C). In the control group, it was observed that IL-1β stimulation did not cause a change in ADAMTS4 mRNA expression, but IL-1β stimulation downregulated ADAMTS4 mRNA expression in the presence of NFkB inhibition (Figure 6D). In the PsO group, both IL-1β stimulation and NFkB inhibition were found to have no effect on ADAMTS4 mRNA expression (Figure 6E). In the PsA group, ADAMTS4 mRNA expression was decreased as a result of IL-1β stimulation, but NFkB inhibition had no effect on ADAMTS4 mRNA expression (Figure 6F).

There was not a statistically significant change among groups in term of mean age and sex difference. The effect of sex difference on the mRNA expression levels of ADAMTS1 and ADAMTS4 in PsA and PsO patients could not be analyzed because of the small sample size (Table 1).

Table 1. *Demographic features of study subjects: F: female; M: male. There was not a statistically significant change among groups in term of mean age and sex difference.*

Subjects (N=25)	The mean age (years)	Sex (F)	Sex (M)
Control	43.26±14.77	14	11
PsO	35.93±17.46	14	11
PsA	40.33 ± 10.79	14	11

DISCUSSION

In this study, mRNA expression profile of ADAMTS1, ADAMTS4 and ADAMTS5 in PsA patients have been revealed for the first time. It has been shown that the mRNA expression of ADAMTS1 and ADAMTS4 in PBMCs of PsO and PsA patients is differentially regulated by TNF-α/MAPK, IL 6/STAT3 and IL-1β/NFkB signaling pathways (Table 2). Previous studies have reported that

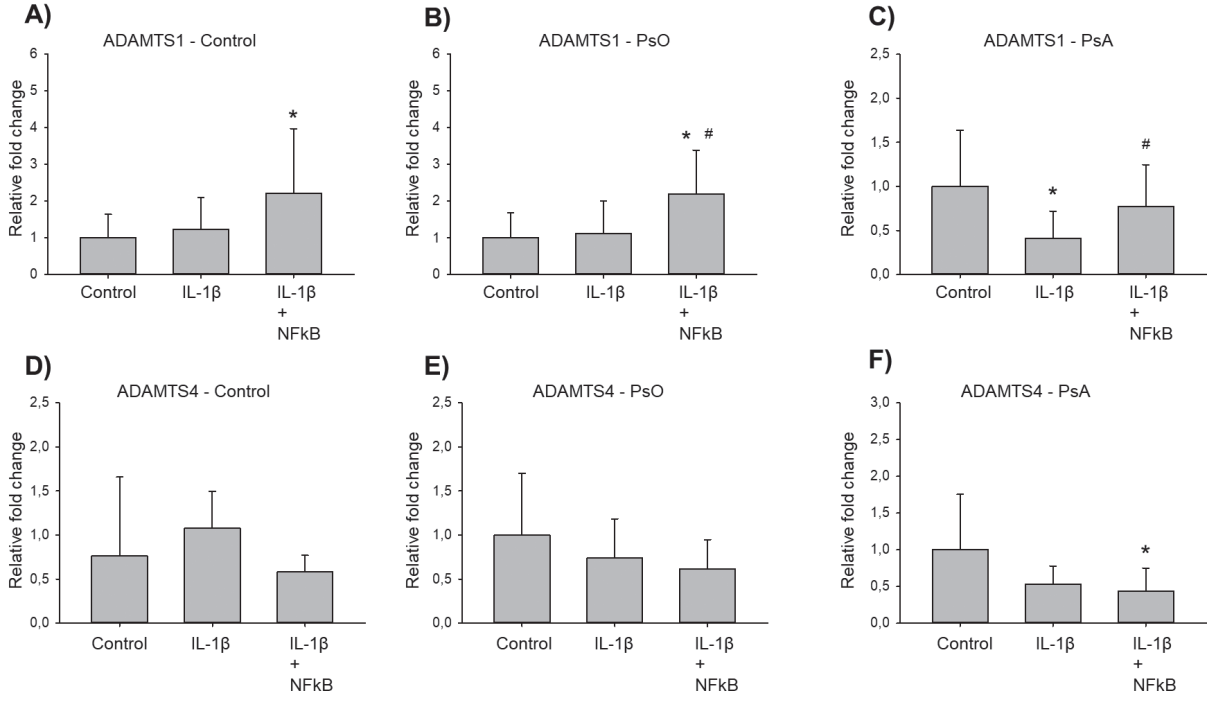

Figure 6. *mRNA expression of ADAMTS1 and ADAMTS4 was regulated by NFkB. PBMCs were treated with 10 μM NFkB specific inhibitor (sm7368) for 2 hours before stimulation with IL-1β (20 ng/ml) for 24 hours. After the treatments, the relative gene expression of ADAMTS1 and ADAMTS4 in PBMCs from PsO and PsA patients (n=25) and healthy controls (n=25) was measured by RT-qPCR. The results of these experiments are presented as the expression relative to GAPDH. *= p< 0.05 **= p< 0.01 compared to control cells. #= p< 0.05 ##= p< 0.01 compared to IL-1β treated cells.*

Table 2. *Summary of main findings.*

	PsO Patients			PsA patients		
mRNA level	TNFα/MAPK	IL6/STAT3	IL1β/NFkB	TNFα/MAPK	IL6/STAT3	IL1β/NFkB
ADAMTS1	↑	↑	↑	↓	↓	↓
ADAMTS4	↑	–	–	–	↓	↓

ADAMTS is associated with inflammatory diseases, such as RA and OA, but the role of ADAMTS in the pathogenesis of PsA is still not investigated. Overproduction of pro-inflammatory cytokines plays an important role in the pathogenesis and progression of inflammatory diseases. Understanding the pathophysiological, molecular and biochemical mechanisms associated with cartilage degeneration in PsA will enable the development of new potential therapeutics.

We found that the mRNA expression of ADAMTS1 and ADAMTS4 in PsA patients showed a significant increase compared to the PsO patients and healthy individuals. In addition, there was a small increase in expression level of ADAMTS5 in PsA patients compared to the control group. To the best of our knowledge, this is the first study that revealed elevated mRNA expression of ADAMTS1, ADAMTS4 and ADAMTS5 in PBMCs of PsA patients. A very recent study demonstrated altered expression of ADAMTS1 and ADAMTS5 in n the knee joints of osteoarthritic mice suggesting critical roles of ADAMTS in destruction of the articular cartilage *(21)*. ADAMTS1 expression was also reported to be increased in dystrophin-deficient *mdx* mouse and anti-ADAMTS1 treatment improved muscle strength and functions *(22)*.

In our study, ADAMTS1 and ADAMTS4 appeared to be major agrecanases in PBMCs of PsA patients. Our results are consistent with the literature suggesting that ADAMTS1 and ADAMTS4 may be involved in articular cartilage degradation in PsA disease. In the present study we used PsO patients without PsA as control as well as healthy subjects to address whether altered mRNA levels of ADAMTS resulted from PsA disease associated with PsO. The mRNA levels of ADAMTS1 and ADAMTS4 were significantly elevated in PsO patients with PsA compared to those without PsA and healthy subjects, suggesting that these molecules may be involved in the pathogenesis of PsA. These findings also suggest that increased mRNA expression levels of ADAMTS1 and ADAMTS4 could be used as diagnostic biomarkers for PsA and that could be also used for monitoring disease progression for PsO patients. Evidence showed that pro-inflammatory cytokines, including TNF-α, IL-6, IL-17 are overexpressed in PsA patients and involved in the pathogenesis of PsA by affecting joint structure *(23)*. A possible cause of elevated mRNA expression of ADAMTS1 and ADAMT4 in PBMCs of PsA patients is that increased proinflammatory cytokines might induce gene expression, including ADAMTS1 and ADAMTS4, which has been previously shown to be regulated by inflammatory cytokines.

Previous studies reported that ADAMTS5 was a main agrecanase in OA and it is a promising molecular target for disease-modifying OA drugs *(24)*. Emerging evidence suggested ADAMTS5 inhibitor GLPG1972/S201086 for the treatment of OA *(25)*. Another study also revealed that ADAMTS4 and ADAMTS5 levels were elevated in serum of RA patients *(26)*. In addition, mRNA levels of ADAMTS4 and ADAMTS5 were also reported to be increased in diabetic OA cartilage compared to the normal OA cartilage, suggesting that inflammatory process triggered by diabetes mellitus accelerates cartilage degradation in OA patients *(27)*. In the present study, we postulate that the reason for the small but significant increase in ADAMTS5 mRNA expression may be due to our study at the PBMCs level, unlike materials such as synovial fluid and cartilage used in other studies.

TNF-α is a pro-inflammatory cytokine that is increased in most inflammatory and autoimmune diseases. Anti TNF-α drugs including, etanercept, infliximab, adalimumab are commonly used in the treatment of inflammatory diseases, including RA, PsO and PsA, but it does not show efficacy in some patients *(28,29)*. Interestingly, the present study demonstrated that TNF-α stimulation suppressed mRNA expression of ADMTS1 in PBMCs of PsA patients and had the opposite effect in PBMCs of PsO patients. One possible explanation of this result is that as mRNA expression ADAMTS1 was increased in PBMCs of PsA patients compared to PsO patients, sustained TNF-α stimulation might activate protein degradation pathways leading to suppressed mRNA expression of ADAMTS1. Indeed, a previous study showed that in the presence of TNFα, proteasome activity was elevated in RA synovial fibroblasts but not in control cells (30).

In a study investigating the effects of IL-1β and insulin-like growth factor-1 (IGF-1) on ADAMTS1 expression levels in healthy and OA articular cartilage tissue, it was stated that ADAMTS1 mRNA expression level was decreased in response to IL-1β stimulation, while no significant changes occurred in ADAMTS1 mRNA expression level by IGF-1 treatment *(31)*. In another study, IL-1β stimulation resulted in an increase in both ADAMTS4 and ADAMTS5 mRNA levels in human chondrocytes obtained from OA joints, whereas ADAMTS1 mRNA level remained unchanged upon IL-1β stimulation *(32)*. According to our results, upon TNF-α stimulation

mRNA expression of ADAMTS1 and ADAMTS4 were observed to be increased in the PsO group but decreased in the PsA group and mRNA level of ADAMTS5 was not changed between the groups (data not shown). These results suggest that increased ADAMTS1 and ADAMTS4 mRNA expressions induced by TNF-α may have a role in the pathogenesis of inflammatory disease, such as PsO. Another possible explanation for the decrease in mRNA level of ADAMTS1 induced by TNF-α in PsA patients is that PBMCs of PsA patients might have already overproduced TNF-α and the response of the cells was dysregulated by further TNF-α stimulation.

As demonstrated in previous studies, MAPK family proteins are involved in the induction of pro-inflammatory cytokines and matrix metalloproteinase enzymes. MAPK activation has been shown to play a role in the initiation and maintenance of TNF-α signaling. Since TNF-α is involved in the onset and development of many inflammatory diseases, it has been reported that MAPK activation may also have an effect on inflammation *(33,34)*. Studies have defined that MAPK signal activation has a role in RA synovial and PsO skin lesions *(35,36)*. Erk1/2, which is one of three well-defined MAPK pathways is an important signaling pathway that increases the efficacy of pro-inflammatory cytokines, such as TNF-α *(37)*. Studies have shown that Erk1/2 pathway activation is important for inflammation in RA synovial fluids, and it has been emphasized that TNF-α stimulation also increases Erk1/2 activity *(35,38)*. In addition, p38, which is another MAPK pathway, is involved in post-transcriptional regulation of cytokine production. Pamapimod, a p38 MAP kinase inhibitor has been reported to have weak effects in the treatment of RA *(39)*. The importance of JNK activity, another well-defined MAPK pathway, in inflammatory diseases has also been investigated. In a study conducted with mice with JNK epidermal deletion, it was stated that there were structures like skin lesions and increased arthritis in patients with PsO. It was reported that mice with JNK and TNF-α deletion had a decrease in skin lesions and did not develop arthritis *(40)*.

In our study, the inducing effect of TNF-α in mRNA expression of ADAMTS1 in PsO patients was suppressed by the inhibition of Erk1/2, p38 and JNK activities. Moreover, TNF-α induced mRNA expression of ADAMTS4 was specifically blocked by p38 and JNK inhibitors. These results indicate that mRNA expression of ADAMTS1 and ADAMTS4 in PBMCs of PsO patients is regulated by Erk1/2, p38 and JNK activity induced by TNF-α. In addition, we found that the inhibition of Erk1/2 activity abolished the suppressive effect of TNF-α on the mRNA expression of ADAMTS1 in PBMCs of PsA patients, however; p38 and JNK inhibitors did not affect mRNA expression of ADMTS1 after TNF-α treatment. This is consistent with a previous study that demonstrated that modulation of ADAMTS5 expression in chondrocytes is dependent on Erk1/2 activity, but not p38 and JNK *(42)*. Furthermore, Erk1/2 activity is essential for priming inflammatory processess and proteosomal functions *(43)*, which supports our hypothesis that Erk1/2 inhibition may protect ADAMTS1 aganist proteosomal degradation in PBMCs of PsA patients upon TNF-α stimulation. Altogether, our results are consistent with literature showing the importance of MAPK signaling pathways in regulation of inflammatory process and mRNA expression of ADAMTS1 and ADAMTS4 genes in both PsO and PsA patients.

In a study, it was shown that the pro-inflammatory cytokine IL-6 increased the expression of ADAMTS4 and ADAMTS5 genes in a STAT3 dependent manner and in the presence of STAT3 inhibitor, the inducing effect of IL-6 on ADAMTS4 and ADAMTS5 mRNA expression levels was abolished *(16)*. In our study, it was observed that inducing effect of IL-6 on ADAMTS1 mRNA expression was further increased in the control and PsO groups by inhibition of STAT3 activity. However, the suppressive effect of IL-6 on ADAMTS1 expression in PsA appeared to be independent from STAT3 activity. Interestingly, IL-6 stimulation resulted in a decrease in the mRNA expression of ADAMTS4 in PsA group, but the inhibition of STAT3 activity did not significantly affect ADAMTS4 mRNA expression. Our results suggest that both IL-6 and STAT3 have differential effects on the mRNA expression of ADAMTS1 and ADAMTS4 in PsO and PsA patients.

In a study, it was stated that IL-1β caused an increase in the mRNA levels of ADAMTS4 and ADAMTS5 in chondrosarcoma cells, but no increase was observed in ADAMTS1 mRNA levels. TNF-α has also been shown to have a synergistic effect with IL-1β *(32)*. These findings suggest that IL-1β may contribute to the development of arthritis by inducing mRNA expression of ADAMTS4 and ADAMTS5. High NFkB activation in some inflammatory diseases, such as RA reveals that NFkB plays a role in the pathogenesis of inflammation *(41)*. In consistent with literature our results demonstrated that IL-1β stimulation led to a decrease in the mRNA expression of ADAMTS1 and ADAMTS4 in PBMCs of PsA patients but did not affect the mRNA expression of ADAMTS5 (data not shown). In addition, the suppressive effect of IL-1β on the mRNA expression of ADAMTS1 in PBMCs of PsA patients was demolished by the inhibition of NFkB activity, suggesting that NFkB activity suppresses IL-1β-induced inflammation in PsA patients.

In this study, the effects of the sex difference on the mRNA expression of ADAMTS1 and ADAMTS4 in PsA and PSA patients were not analyzed because of the limited sample size. Therefore, further studies with a larger sample size are required to examine the effect of the sex difference on the mRNA expression of the ADAMTS1 and ADAMTS4 in these immunological disorders.

In conclusion, our results suggest that ADAMTSs are differentially regulated by MAPK, STAT3 and NFkB

signaling pathways induced by inflammatory cytokines and may have important roles in the formation of inflammatory response and cartilage destruction in PsA. In addition, since there is no laboratory finding specific to PsA, it indicates that determining the mRNA levels of ADAMTS1 and ADAMTS4 may be a diagnostic biomarker for PsO patients developing PsA. As a result, ADAMTS1 and ADAMTS4 may be potential drug targets for the treatment of PsA disease.

Acknowledgements: *We thank Bilal Sula for helping with some of the patient samples. This study was supported by the TUBITAK (Scientific and Technical Research Council of Turkey) under the grant 214S024.*

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