

## Systemic sclerosis-related myopathy

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Systemic sclerosis (SSc) is an autoimmune disorder which can affect nearly every body organ. Muscle involvement is one of the most serious manifestations of SSc. It can present itself in a wide range of pathologies. It can be as indolent as a subclinical myopathy which manifests simply as a mild muscle enzyme level elevation, or it can present itself in a similar manner to other SSc organ involvements in the form of fibrosing myopathy without any existing evidence of inflammation. It can also present itself aggressively as an idiopathic inflammatory myopathy, the overlap syndrome of SSc-myositis. Due to the wide range of witnessed pathologies and the different diagnostic criteria that are used, opinions vary on the estimated prevalence of myopathy in SSc with estimates ranging from 3.3% to 14%. The severity and distribution of clinical manifestations differ among SSc-myopathy and SSc patients. These manifestations have different effects on the survival rates of patients, which will be discussed in this review. This paper will also focus on the existing treatment methods for SSc patients suffering from myopathy and their challenges.

**Keywords:** fibrosing myopathy, idiopathic inflammatory myopathy, muscle histopathology, systemic sclerosis.

### Introduction

Systemic sclerosis (SSc) is a member of the family of disorders known collectively as collagen-vascular disorders. Diseases in this category are sometimes accompanied by various auto-immune disorders. Myopathies are disorders that are sometimes associated with SSc [1]. The presentation of these disorders differs greatly in patients, with some suffering from idiopathic inflammatory myopathies (IIM), such as polymyositis (PM), and others showing signs of non-inflammatory myopathies (NIM) which share a common pathogenesis with SSc itself, such as fibrosing myopathy (FM). Comparing the severity of extra-muscular manifestations and their prevalence among SSc patients with those who have a concurrent myopathy is a top area of research in the field. A subsequent challenge that arises relates to the treatment of these patients. While high-dose corticosteroids are the main treatment method for patients with IIM, renal crisis development is observed in SSc patients on whom this same treatment is applied, creating a challenge for rheumatologists. The impact of myopathies on the survival rate of SSc patients is a highly debated issue, but it seems that it may play a role in reducing life expectancy. To date, many articles have been published in this field since the first

documented case of SSc-IIM in 1969. The current study has tried to review the most recent research carried out in this area [2]. This article reviews the epidemiology, clinical manifestation, diagnosis, therapy, and survival rate of SSc patients with concurrent muscle involvement.

### Epidemiology

Due to the differing criteria used to diagnose IIM and the varying sizes of the cohorts involved, heterogeneity is seen in reports regarding the prevalence of SSc-myopathy. Based on a study performed on the Nijmegen Systemic Sclerosis cohort with a total of 422 patients, the prevalence of SSc-PM overlap was 5.9%. All of these patients fulfilled the Bohan and Peter diagnostic criteria for IIM [3]. In another study performed on 302 Japanese patients, the prevalence was estimated to be 14% with a total of 43 cases of SSc-IIM [4]. Patients with proximal muscle weakness in addition to abnormal results in at least one of either enzyme levels, electromyogram (EMG), or muscle biopsies were considered to have IIM. The South Australian SSc registry, which made use of similar criteria, reported 20 patients with myositis from among 374 living and 234 deceased patients (a total of 608 cases), showing an estimated prevalence of 3.3% [5].

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The Canadian Scleroderma Research Group reported a prevalence of 5.6% of patients with creatine kinase (CK) elevation ( $>200 \mu\text{L}$  for women and  $>250 \mu\text{L}$  for men) from among 1145 patients [6]. They also mentioned a prevalence of nearly 10% for myopathies including IIM based on physicians' reports.

### Diagnosis and Muscle histopathology

The histopathologic difference between concurrent SSc-IIM and isolated IIM cases is a field of great interest. Based on the Nijmegen Systemic Sclerosis cohort, the prevalence of necrotic muscle fibers is the sole difference between these groups, being present in 96% of SSc-PM and 67% of PM patients [3]. The major histopathologic characteristics observed in the muscle biopsies of 35 patients from a multicentric French study showed varying results as opposed to those obtained from the previous study [7]. Inflammation was the most common finding, observed in 63% of patients, followed by atrophy and necrosis which were present in 60% and 59% of patients, respectively. Fibrosis was present in 24% of the samples, and angiopathy was detected in 27%. Small vessel vasculitis was seen in 3 cases (9%), and one case (3%) was reported to have mitochondrial abnormality. In a recently published investigation, Paik *et al.* analyzed 42 muscle biopsies obtained from SSc patients showing signs of weakness [8]. Similar to the aforementioned study, necrosis was the most prevalent finding, seen in 66.7% of cases, followed by inflammation and acute neurogenic atrophy which were both observed in nearly half of the obtained samples. Fibrosis was detected in one third of the obtained biopsies. Overall, non-specific myositis and necrotizing myositis were diagnosed in 15 and 9 patients, respectively. Surprisingly, magnetic resonance imaging (MRI) results, EMG, and serum CK levels were normal in 30%, 10%, and 26% of patients, respectively, even though these patients showed signs of proximal muscle weakness. In their investigation, Corallo *et al.* compared the histopathologic features of SSc myopathy, IIM, and NIM to elucidate a possible pathognomonic histologic characteristic of SSc myopathy [9]. They obtained muscle biopsies from 35 patients with clinical, biologic, and EMG findings suggestive of myopathy. They reported that fibrosis was significantly higher in SSc cases (81%) compared to patients with IIM (32%) and NIM (18%). Surprisingly, the pro-angiogenic factor (vascular endothelial growth factor A (VEGF-A)) was significantly downregulated in SSc cases as opposed to

the anti-angiogenic factor VEGF-A165b which was significantly upregulated. Transmission electron microscopy showed endothelial cell swelling, significant collagen deposition, and thickened sub-endothelial basement membranes in the muscle biopsies of SSc patients. These findings propose that the physiopathology underlying vasculopathies in SSc (e.g., Raynaud's phenomenon (RP)) may also provoke muscle involvement.

### Clinical manifestation and Serologic features

Several studies have compared the clinical manifestations and serologic features of SSc-IIM patients with those of SSc and IIM patients. According to the Nijmegen Systemic Sclerosis cohort, SSc-PM patients had a male to female ratio of 1:1 compared to the 2:1 ratio seen in SSc cases [3]. Lung fibrosis was more prevalent in patients with SSc-IIM (83%) compared with those patients with either SSc or PM (49% and 53%, respectively). Pulmonary arterial hypertension confirmed by right heart catheterization was significantly lower in overlap cases compared to SSc patients (0% vs. 31%). Levels of anti-topoisomerase 1 antibody were not detected in SSc-IIM patients, and levels of anti-Jo antibody were significantly lower in this group compared with PM patients (8% vs. 42%). Another finding was that 12% of SSc patients showed elevated serum CK levels, and 5% had proximal muscle weakness but failed to fulfill the Bohan and Peter diagnostic criteria. In a French multicentric study conducted on 40 SSc-IIM cases, the findings were compared with those of 80 SSc cases matched for sex, age at onset, disease duration, and subtype [10]. Based on multivariate analysis results, reduced forced vital capacity (FVC) and heart involvement (including congestive heart failure, left ventricular ejection fraction (LVEF)  $<60\%$ , arrhythmia, and conduction abnormality) had significant correlations with myopathy, and the presence of the anti-centromere antibody was negatively associated. Interestingly, among SSc-IIM patients, individuals without histologic evidence of inflammation did not have a significantly reduced FVC compared to the control group. In a Japanese study that compared 259 SSc and 43 SSc-IIM cases, it was reported that the male to female ratio, being a member of the diffuse subset, pulmonary fibrosis (diagnosed by chest x-ray (CXR) or high resolution computed tomography (HRCT)), heart involvement (including symptomatic pericarditis/left ventricular heart failure

and treatment-requiring arrhythmia), and skin hyperpigmentation were significantly higher in patients with IIM [4]. In addition, the presence of anti-centromere antibodies and mean age were significantly lower in this group. Remarkably, the erythrocyte sedimentation rate (ESR) and serum C-reactive protein (CRP) levels were similar in both groups. The association of myocardial involvement and skeletal muscle involvement in SSc patients can be screened and differentiated using cardiac troponin tests [11]. Cardiac troponin T is also expressed in regenerative skeletal muscles, which is highly evident in IIM. Testing for troponin T is more sensitive than measuring CK levels, but cardiac troponin I is specific to myocardial cells. Therefore, skeletal muscle involvement can be differentiated from myocardial involvement, and subclinical myocarditis can be detected using these tests. A Canadian cohort of SSc with 1145 patients compared the clinical and serologic data of patients with normal and abnormal serum CK levels [6]. Findings, compatible with those of previous studies, showed that the male to female ratio, diffuse cutaneous SSc (dcSSc), tendon friction rub, and FVC<70% were more prevalent in cases with abnormal serum CK levels. In addition, the mean age and disease duration were lower in this group. Among the various serologic markers, anti-topoisomerase and anti-ribonucleoprotein antibodies were also more prevalent in these cases. In another study carried out on 306 members of the aforementioned cohort, 4 patients produced autoantibodies against the hydroxymethylglutaryl coenzyme A reductase (HMGCR) antibody [12]. Although this event did not occur often enough to be of use in statistical analyses, none of the reported cases had a history or evidence of myopathy, nor did they report current or past use of statins. Anti-HMGCR antibody was significantly associated with a higher percentage of pulmonary arterial hypertension and a higher cardiac severity score. Also, SSc-related nailfold capillaroscopic changes were significantly more common among patients with genetic myopathies (without RP) compared to primary RP cases [13].

## Treatment

Treating patients with SSc-IIM overlap is tremendously challenging for rheumatologists because of the beneficial effects of corticosteroids in IIM patients and their dangerous effects on SSc patients. To date, no unified accepted protocol for IIM treatment in SSc patients has been developed, and due to the rarity

of these patients, there is limited data on this matter. A French multicentric study on 35 SSc-IIM patients reported a correlation between the severity of histopathologic findings and treatment outcome [7]. According to multivariate analyses, findings suggestive of inflammation and necrosis in muscle biopsy samples were associated with a better response to treatment. In addition, abnormal MRI results also appeared to be related to a better prognosis. High doses of corticosteroids (1 mg/kg per day) were prescribed for the majority of cases, and multivariate and univariate analyses were carried out. Multivariate analysis showed that histologic inflammation was correlated with a better response to treatment, while muscle necrosis and elevated CK levels (>5 upper normal limit) showed the same association in univariate analysis. Two patients (6%) experienced renal crises after corticosteroid treatment. Allano et al. conducted a study to elucidate the effects of high-dose corticosteroids and immunosuppressive medications for IIM in 4 naïve-to-treatment cases of SSc with myocarditis that had been confirmed by cardiac magnetic resonance (CMR) [14]. Based on this study, all of the cases had early and delayed enhancement due to myocarditis and wall hypokinesia before treatment. Each received 1 g methylprednisolone for 3 days followed by 1 mg/kg prednisone in addition to an immunosuppressive drug (3 cases were treated with cyclophosphamide and 1 with azathioprine). After 6 months of treatment, the hypokinesia disappeared and the area of enhancement was successfully reduced. A recently published study on the EULAR scleroderma trial and research (EUSTAR) network of SSc patients evaluated the clinical outcome of abatacept on SSc cases with refractory myopathy compared to conventional disease-modifying antirheumatic drugs (DMARDs) and cyclophosphamide [15]. In that study, 10 mg/kg/month of abatacept was administered for a mean duration of 18 months in 7 patients with SSc-related refractory myopathy. The treatment was not effective in increasing muscle force or reducing serum CK levels, and it had no visible effect on FVC or modified Rodnan skin scores (mRSSs). No clinical trials have been conducted on the efficacy of Rituximab (RTX) in treating SSc-IIM, although a case report showed the beneficial effect of RTX on SSc-related resistant IIM. However, this was accompanied by late onset neutropenia (without serious infection), which gradually resolved [16].

## Survival

Apart from the muscular involvement of IIM, this disorder can also severely affect various internal organs, and therefore influence patient survival. With the aim of better diagnosing and treating patients, the survival rate and mortality causes of patients suffering from SSc-IIM overlap were assessed. There is much difference of opinion regarding the mortality rate of these patients and its causes, and therefore, the results of different studies will be referred to individually. According to the Nijmegen Systemic Sclerosis cohort, the mortality rate of SSc-PM patients was significantly higher than that of SSc patients [17]. In this cohort, 24 SSc-PM and 396 SSc cases were followed for 10 years. The 10-year survival rates were 68% for SSc-PM cases and 87% for SSc cases. Multivariate survival analysis gave a hazard ratio of 2.34 for SSc-PM compared to SSc after adjusting for sex, age at diagnosis, mRSS, and SSc cutaneous subtype. Causes of mortality were not statistically compared; however, in both groups, cardiopulmonary involvement was the major culprit. In a French multicentric case-control study, the survival rate of SSc-IIM patients was similar to that of SSc cases: 7.5% and 16.3%, respectively, during a similar time span [10]. In a Canadian SSc cohort of SSc with 1145 cases, individuals with abnormal serum CK levels and patients with a prior history of myopathy (including IIM) had significantly lower survival rates compared with other SSc patients [6]. The major causes of death were reported to be interstitial lung disease, pulmonary hypertension, and cardiac involvement. In an abstract recently presented at the 2016 ACR/ARHP annual meeting, Paik *et al.* compared the survival rate and clinical features of 28

SSc patients with IIM and 8 patients with FM that had been histopathologically confirmed [18]. Individuals with FM had a significantly higher death rate compared to patients with IIM (62.5% versus 14.3%). Patients with SSc-FM also had lower serum CK levels, higher anti-topoisomerase antibody levels, shorter disease duration, a lower FVC, and more cases in the diffuse cutaneous subtype. However, none of these differences were statistically significant.

## Conclusion and Recommendation

Muscle involvement occurs mainly in two general forms among SSc patients: inflammatory and fibrosing myopathy. Histopathology can be used as a tool to differentiate between these two diagnoses. Early treatment of inflammatory myositis with high doses of corticosteroids is highly recommended, despite the risk of renal crises occurring in scleroderma patients. Treating fibrosing myopathy, which occurs in more severe cases of the disease, in which patients present with mild muscle enzyme elevation is adjusted according to the severity of involvement of other organs. Immunosuppressive treatment with cytotoxic drugs without the use of high-dose corticosteroids may, therefore, be a better option in these cases. In conclusion, the early diagnosis of skeletal muscle involvement by serum muscle enzymes level and histopathology is recommended to better distinguish inflammatory myopathies from non-inflammatory types. It is also recommended to start cytotoxic treatment early.

## Conflicts of interest

The authors declare no conflicts of interest.

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## Pentoxifylline as a new adjunctive therapy in ankylosing spondylitis: A randomized clinical trial

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Treatment with tumor necrosis factor alpha inhibitors has been increasingly implicated in the management of autoimmune diseases. In spite of their promising effects, they are commonly associated with side effects. This issue indicates the need for newer drugs with the same efficacy and fewer serious adverse effects. Pentoxifylline is a phosphodiesterase which inhibits TNF secretion and exerts to some degree an anti-inflammatory effect. The purpose of this randomized clinical trial was to evaluate the effect of pentoxifylline as an adjunctive therapy in the management of ankylosing spondylitis. Twenty-five patients suffering from ankylosing spondylitis were randomly assigned to treatment or placebo groups having been matched for age and gender. The treatment group received pentoxifylline (1200 mg daily), and the placebo group received a placebo in addition to the standard treatment of sulfasalazine 2-3 gram daily and indomethacin 50-75 mg per day that were given to all the patients in both groups. Serum levels of TNF- $\alpha$  were measured before and after the study intervention. Serum levels of TNF- $\alpha$  were reduced significantly in both groups with a p-value of  $< 0.001$ . However, the reduction was more prominent in the group receiving pentoxifylline than in the placebo group, although this between-group difference was not statistically significant. The results demonstrate the need for further studies on the use of pentoxifylline as a safe adjunctive therapy in controlling disease activity and reducing tumor necrosis factor-alpha levels in patients with ankylosing spondylitis.

**Keywords:** ankylosing spondylitis, NSAIDs, pentoxifylline, spondyloarthritis, TNF- $\alpha$

### Introduction

Ankylosing Spondylitis (AS) is a chronic inflammatory disease with an unknown etiology that mostly affects the sacroiliac joints and spine and, less commonly, the peripheral joints. It typically presents with low-back pain and gradually progresses to stiffness and fusion of the vertebrae, and it creates a great economic and social burden [1, 2].

An effective treatment for AS should control symptoms, decrease structural damage, and improve the patient's quality of life. Non-steroidal anti-inflammatory drugs (NSAID) are known as first-line drugs for the management of this condition [3]. In patients with peripheral arthritis, disease-modifying anti-rheumatic drugs (DMARD) such as sulfasalazine could also be considered as well as intra-articular injections of

glucocorticoids [4]. However, there is no evidence to support the administration of these drugs in patients with axial spondyloarthritis.

Recent studies have suggested the use of anti-tumor necrosis factor (anti-TNF) agents as another option for patients with elevated disease activity, especially for axial arthritis [5, 6]. Infliximab, etanercept, adalimumab, golimumab, and certolizumab are anti-TNF agents that have been used successfully in the treatment of AS in recent years [6-9]. On the other hand, inhibition of TNF- $\alpha$  with the monoclonal antibody infliximab [10] or the soluble TNF receptor etanercept has resulted in increased mortality and is associated with many side effects [11]. Consequently, it is necessary to find some other medications with anti-TNF- $\alpha$  properties and less serious side effects.

Pentoxifylline, an oral anti-TNF agent that inhibits

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phosphodiesterase [12], has been shown to be useful in decreasing serum levels of TNF- $\alpha$  in many disease conditions with an inflammatory pathogenesis, including AIDS [13], acute lung injury [14], alcoholic and non-alcoholic steatohepatitis [15], and refractory nephrotic syndrome secondary to lupus nephritis [16]. Some studies have found that pentoxifylline is effective in the treatment of patients with rheumatoid arthritis [17-19]. However, there is no study on the role of pentoxifylline in the treatment of patients with AS. Therefore, the current study was designed to evaluate the role of pentoxifylline as an adjunctive therapy in slowing disease progression in patients with ankylosing spondylitis.

### Patients and Methods

This randomized clinical trial was conducted on patients with active ankylosing spondylitis (AS) who referred to the Rheumatology Clinic at Ali-ebne-Abitaleb Hospital, Zahedan, Iran. The diagnosis was confirmed by a rheumatologist using the Modified New York Criteria for AS. All patients with no history of receiving anti TNF- $\alpha$  drugs were included to the study. Any patients with an infection, history of central nervous system bleeding or coagulopathy, or drug reaction during the period of study were excluded from the study.

Patients were informed about the study and told they had the right to leave the trial at any time during the study. After informed consent was obtained from each patient, a thorough medical history was taken and a complete physical examination was performed. The trial design and all ethical issues concerning the patients were confirmed by the Ethics Committee of Zahedan University of Medical Sciences. Disease activity was evaluated based on BASDAI (Bath Ankylosing Spondylitis Disease Activity Index). Disease duration, type, and duration of previous treatments were determined and recorded. Blood samples were taken to measure serum levels of TNF- $\alpha$  by the ELISA method, ESR, and CRP as a determinant factor of baseline disease activity before intervention. After matching for age, gender, and average disease activity, 25 patients were randomly assigned to treatment (n=12) and placebo (n=13) groups using the random classified blocking method. The duration of study was 12 weeks. Patients of both groups received similar standard treatments for AS, including sulfasalazine 2-3 g per day and indomethacin 50-75 mg per day. In case of failure to respond to sulfasalazine during the study, 10 mg of weekly methotrexate was added to the treatment regimen for both groups.

The treatment group received pentoxifylline (1200 mg daily) as an adjunct to the standard treatment in divided doses (400 mg TDS for 12 weeks), while patients of the placebo group received a placebo in addition to the routine regimen. Blinding was performed according to random classified blocking methods. Doses of all of the drugs received by the patients were documented precisely in the beginning, during, and at the end of the study, and any changes in doses were noted. Symptoms associated with serious side effects, including bleeding, were described for the patients, and they were asked to refer immediately to an emergency department in case of experiencing such symptoms. During treatment, patients were visited periodically and monitored for drug side effects. Twelve weeks after the trial was initiated, another complete physical exam was conducted and blood specimens were taken for the measurement of serum levels of TNF- $\alpha$ , ESR, CRP and the determination of disease activity using BASDAI. All samples were transferred to measure the serum level of TNF- $\alpha$  all at once and measurements were performed using the same brand of laboratory kits before and after intervention. This strategy was considered to minimize measurement bias. Changes in ESR and CRP were assessed after 12 weeks of follow-up in all patients.

### Statistical analysis

All data was analyzed using STATA 10 software. The normality of the BASDAI scores and the serum levels of TNF- $\alpha$  were examined with the Shapiro-Wilk test. In case of normality of data, all comparisons were performed by parametric tests, *i.e.* t-test and paired t-test. In case the data was not distributed normally, non-parametric tests including the Wilcoxon Signed Ranks Test were used to evaluate changes in ESR and CRP. A p-value of 0.05 was considered significant.

### Results

Twenty-five patients with the diagnosis of ankylosing spondylitis participated in this study and were randomly divided into treatment (n=12) and placebo (n=13) groups. Serum TNF- $\alpha$  levels of one patient in the placebo group were very high and differed significantly from that of the other patients. In order to prevent bias in analyzing, that data was omitted. Two individuals in the placebo group left the trial because of a lack of interest in continuing the study protocol. All data related to those two individuals was also omitted from statistical analyses. Data from the 10 individuals remaining in the placebo group were analyzed.

During the study period, pentoxifylline was well



tolerated by patients. None of the patients in the treatment or placebo group complained of any symptoms indicating allergies or other drug side effects.

There was no significant difference in BASDAI scores between the treatment and placebo groups before intervention ( $P=0.12$ ). The mean BASDAI scores in both groups were significantly lower after the trial ( $P<0.001$ ); however, the decrease of the score in the treatment group following the trial was more prominent. Moreover, the mean level of this score was significantly lower in the treatment group after trial than in the placebo group ( $P=0.004$ ).

Results of the current study indicated no significant baseline difference in the median amounts of serum levels of TNF- $\alpha$  between treatment and placebo groups ( $P=0.151$ ). The levels of TNF- $\alpha$  significantly decreased after intervention in both groups ( $P<0.01$ ). This decrement was more prominent in the treatment group in comparison with the placebo group, although this prominence was not statistically significant ( $P=0.091$ ). The level of TNF- $\alpha$  was significantly lower in the treatment group than in the placebo group after intervention (0.016). The serum levels of ESR and CRP was not significantly different after 12 weeks of treatment in the placebo group. ESR decreased 5 mm/hr ( $P=0.25$ ), and CRP decreased 1.70 mg/l ( $P=0.72$ ). However, in the treatment group, the reductions in ESR and CRP serum levels were significant. ESR decreased 37.10 mm/hr ( $P=0.01$ ), and CRP decreased 16.00 mg/l ( $P=0.02$ ).

**Table 1.** BASDAI scores of treatment and placebo groups at the beginning and at the end of the study protocol

Group	Before intervention	After intervention	After-before difference	P-value
Treatment (median; IR*)	46.65; 62.95	19.55; 36.78	-26.20; 26.52	<b>0.002</b>
95% CI	(21.96 - 87.81)	(11.10 - 49.72)	(-38.71 - -12.03)	
Placebo (Median; IR)	104.00; 85.90	89.00; 75.30	12.50; 18.50-	<b>0.003</b>
P-value	0.151	0.016	0.091	

**Table 2.** Serum levels of TNF- $\alpha$  of treatment and placebo groups at the beginning and at the end of the study protocol

Group	Before intervention	After intervention	P-value
Treatment (mean $\pm$ SD)	4.58 $\pm$ 0.83	2.36 $\pm$ 0.48	<0.001
(95% CI)	(4.05-5.11)	(2.05-2.66)	
Placebo (mean $\pm$ SD)	3.95 $\pm$ 1.04	3.25 $\pm$ 0.81	0.001
(95% CI)	(3.25-4.65)	(2.71-3.80)	

IR: Interquartile range

## Discussion

The mainstay of treatment for ankylosing spondylitis is currently based on a combination of education, exercise, physical therapy, rehabilitation, patient associations, self-help groups, and pharmacotherapy which is aimed at improving joint pain and function, and consequently enhancing the quality of life. NSAIDs are the first-line pharmacological agents used in the management of this condition in combination with intra-articular corticosteroids and sulfasalazine and other DMARDs with more prominent peripheral features. However, anti-tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) drugs have been shown to have promising outcomes regarding their impact on joint pain and function [20].

This randomized clinical trial demonstrated the considerable benefit of pentoxifylline as an add-on therapy in the treatment of ankylosing spondylitis (AS). The BASDAI scores reflecting the level of disease activity in the patients improved significantly following treatment with pentoxifylline. In addition, significantly lower levels of TNF- $\alpha$  were seen in patients treated with pentoxifylline compared to the placebo individuals. TNF blockers have shown significant efficacy in symptomatic relief and improvement of quality of life among AS patients; however, these agents have not been associated with decreased radiographic progression of disease. Despite the significant role of TNF blockade in preventing erosive bone damage in RA and psoriatic arthritis, new bone formation, which is the mainstay of radiologic findings in AS, has not been shown to be suppressed by TNF blockers [5, 6, 21, 22].

Given the possible role of TNF- $\alpha$  in the pathogenesis of many inflammatory disorders, TNF blockade has been increasingly proposed for the treatment of such disorders in recent years [23].

Nevertheless, the wide variety of roles TNF plays makes the use of these biologic agents a concerning issue, as their impact is not limited to the role of TNF in the pathologic process, but also alters the physiologic roles of this important agent causing a significant range

of side effects. One such effect is its important role in the mediation of TH1 response to intracellular bacteria and viruses which is an especially important mechanism in limiting infectious pathogens like mycobacterium tuberculosis [23].

After more than a decade of developing TNF blockers, their safety profiles have been largely revealed. Opportunistic infections such as those caused by *Listeria*, *Cryptococcus*, *Pneumocystis*, and *Aspergillus* as well as *Mycobacterium tuberculosis* have been described. Risks of the reactivation of Hepatitis B and latent tuberculosis (TB) and the exacerbation of Hepatitis C have also been associated with the use of infliximab. Among these, tuberculosis reactivation is especially significant in TB-prevalent areas as applies to the southeast of Iran. In addition to infectious adverse effects, cholecystitis, gall stones, non-infectious hepatitis, and jaundice have also been mentioned. The increased risk of malignancies such as lymphoma and cutaneous malignancies is another safety concern of TNF blockers [24]. Furthermore, a lupus-like syndrome has been reported following treatment with infliximab. Seizure, optic neuritis, and multiple sclerosis are among the neurologic disorders associated with the use of TNF blockers. Despite some proposed benefits of TNF blockers in congestive heart failure, it is known that TNF blockers can worsen a patient's underlying heart condition, especially in the elderly [24, 25]. In addition to the mentioned clinical consequences of biologic anti-TNF agents, there are other pitfalls that complicate the use of these drugs. Among these, one important aspect is their cost which is especially significant for patients of the lower socioeconomic classes and may result in non-compliance. Other economic issues associated with the use of biologic anti-TNF agents are imposed by the costs of their adverse effects. According to the available data, none of the current anti-TNF agents appear to be cost-effective for the management of AS with infliximab showing the poorest results in short-term models [21].

Pentoxifylline, however, is a phosphodiesterase inhibitor which has anti-TNF $\alpha$  properties with many advantages over biologic agents such as infliximab. The side effects described for pentoxifylline are very limited, presenting it as a safer medication. No severe infections have been reported following the administration of this drug. Furthermore, pentoxifylline

is not expensive and is available to all patients of different socio-economic classes. Pentoxifylline is not only compatible with other drugs used for the management of AS, but it is also postulated to act synergistically to reduce TNF- $\alpha$  levels through independent mechanisms with sulfasalazine. Their effectiveness has been shown in other autoimmune disorders such as pemphigus vulgaris, entero-Behcet's disease, and psoriasis [26-28]. This is particularly important in the geographical area where the authors of the current study practice because of the higher prevalence of infectious diseases such as TB and the relatively lower socioeconomic status of the population, both of which make the prescription of anti-TNF agents an even more challenging issue and the search for alternatives a necessary area of research. The results of this trial suggest that drugs like pentoxifylline are effective in reducing disease activity among patients with progressive ankylosing spondylitis. The authors recommend further clinical trials that compare the effects of pentoxifylline with biologic agents such as infliximab, etanercept, or adalimumab on reducing disease activity and improving the quality of life of AS patients, to further assess the usefulness and effects of the drug in clinical practice, and to determine whether this drug can be considered for addition to the list of routine drugs used in line with or as an alternative agent to biologic anti-TNF agents in the management of AS. It also recommended that long-term studies on a higher number of patients and with different doses of pentoxifylline be conducted to discover the most efficient doses. The BASDAI scoring system was used in this trial to evaluate disease activity; more measurable indices are recommended for the evaluation of disease activity in future trials so as to improve the fidelity of the results.

## Conclusion

According to the results of this study, the vast variety of side effects mentioned for anti-TNF- $\alpha$  agents indicate the need for research on the efficacy of drugs like pentoxifylline with similar activity and fewer side effects as an adjunctive therapy in the management of patients with ankylosing spondylitis

## Conflict of interest

The authors declare no conflicts of interest.

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## Role of anti-CCP in arthritis in patients with systemic lupus erythematosus

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Systemic lupus erythematosus (SLE) is an autoimmune disease with multi-organ involvement. Patients with SLE feature a lower tendency to develop erosive arthritis in comparison with rheumatoid arthritis (RA); however, in some arthritis cases it may be difficult to differentiate SLE from RA. Anti-cyclic citrullinated peptide (Anti-CCP) antibodies are highly-specific for RA. The current study evaluated the relationship between anti-CCP and arthritis in SLE patients. In this study, anti-CCP antibodies were tested in 300 patients with SLE. The INOVA Diagnostics QUANTA Lite™ CCP IgG ELISA and the Axis-Shield Diagnostics Diastat™ anti-CCP ELISA test were applied. Patients were divided into two groups: those with and those without arthritis. Patients with chronic arthritis (>6 weeks) had radiography done on the involved joints. Chi square and Fisher's exact tests were applied to compare the two subsets. Anti-CCP antibodies were detected in 4.7% of all patients (CI: 2.6-7.8). Anti-CCP was positive in 6.4% of patients with arthritis and 2.3% of patients without arthritis ( $P=0.09$ ). From seven patients with chronic arthritis, one had both positive anti-CCP and erosions. In the studied Iranian SLE patients, anti-CCP levels were higher in patients with arthritis than in those without arthritis. This study did not show any association of anti-CCP with erosion in SLE patients with arthritis. Ethnic and geographical variance may have influenced the results. More studies on chronic arthritis in SLE are needed to confirm this hypothesis.

**Keywords:** anti-CCP, arthritis, arthropathy, systemic lupus erythematosus.

### Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease with multi-organ involvement. Arthritis is one of the major clinical findings in SLE reported in up to 90% of patients [1-4]. Similar to other diseases such as rheumatoid arthritis (RA), arthritis has a considerable effect on disease burden and imperils quality of life [5, 6]. The majority of arthritis lesions in SLE are non-erosive and non-deforming [7, 8]; however, there is a tendency to develop erosion in RA arthritis [9]. In less than 5% of SLE patients, erosive arthritis develops; this is known as rhupus [10-12]. Erosive arthritis in SLE has a prognosis and clinical course similar to that of RA [13]. The risk factors for the development of erosive arthritis are not fully understood. Recent studies have challenged the concept of non-erosive arthropathy featured in SLE. In some cases with erosive lesions, it may be difficult to differentiate SLE from RA, and many SLE cases are initially misdiagnosed as having RA [14, 15]. Erosive lesions more strongly debilitate and affect the quality of life in SLE patients [16]. Regarding the difference in particular outcomes, it is

helpful to use a serological marker to distinguish them at the onset of disease.

Anti-cyclic citrullinated peptide (anti-CCP) antibodies are highly specific and sensitive measures in RA diagnosis and predict the prognosis of disease [17]. Moreover, in a number of non-RA inflammatory conditions such as SLE, positive Anti-CCP is detectable, which demands careful interpretation [18, 19]. A number of previous studies have proposed an association between anti-CCP and erosive or deforming arthritis in SLE and related complications [20-22]. However, the association is not adequately addressed in the literature. The current study evaluated the prevalence of anti-CCP various subsets in SLE patients.

### Materials and Methods

The clinical records of 300 patients visited between January 2006 and February 2007 were studied. The studied population comprised 300 SLE patients (271 females and 29 males). This demographic study was conducted in the connective tissue diseases unit of the Rheumatology Research Center, Tehran University of

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Medical Sciences (TUMS). It was approved by the TUMS Ethical Committee. This study was conducted in accordance with the ethical principles outlined in the World Medical Association Declaration of Helsinki.

Patients who fulfilled the American College of Rheumatology (ACR) criteria for SLE were eligible to be enrolled in the current study. The availability of clinical records and radiological exams were other inclusion criteria. Patients with concurrent comorbidities which warranted additional treatment were not eligible to participate in the study.

Data regarding recent complete blood count (CBC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), rheumatoid factor (RF), anti-dsDNA, fluorescent antinuclear antibody (FANA), complement component 3 (C3), complement component 4 (C4), complement total hemolytic (CH50), anti-cardiolipin (IgG, IgM), creatinine, urine analysis U/A, and urine protein were extracted from the participants' files.

Ten milliliters (ml) of whole blood was collected from each participant, and anti-CCP antibodies were measured using enzyme-linked immunosorbent assays (ELISA) (first generation anti-CCP1-test; Euroimmun, Lübeck, Germany). An anti-CCP level > 5 RU/mL was considered positive. Recent clinical and laboratory findings such as alopecia, leucopenia, photosensitivity, discoid rash, anemia, thrombocytopenia, and raised creatinine (>1 mg/dL) from the patients' files were studied.

Patients with chronic arthritis (more than 6 weeks) had radiography performed on the involved joints to detect the presence of erosive arthritis.

To address the correlation between anti-CCP and arthritis, patients were divided into two groups, those with and those without arthritis. Patient serum levels of anti-CCP and other data were compared in the two subgroups.

### Statistical methods

Chi square and Fisher's exact test were applied to compare the two subsets. A *p-value* less than 0.05 was considered statistically significant. All statistical analyses were carried out with the SPSS software, version 21 (Chicago, IL, USA).

### Results

The demographic and clinical characteristics of patients with SLE are shown in Table 1. Positive anti-CCP was detected in 14 out of 300 patients (4.7%, CI: 2.6-7.8). All anti-CCP positive patients were female. In patients with positive anti-CCP, the mean level was 33.96 RU/mL. There was no statistically significant difference

in age, gender, or disease duration between the anti-CCP positive and negative subgroups (Table 2).

Arthritis was present in 170 SLE patients (56.7%). From SLE patients with arthritis, 163 patients (95.9%) had transient arthritis, and 7 (4.1%) had chronic arthritis.

**Table 1.** Demographic, clinical and paraclinical characteristics of studied patients

	Anti-CCP positive subgroup (N=14)	All Patients (N=300)
Duration of disease (years)	5.7	6.34
Female	14 (100)	271(90.3)
Mean age (years)	35.1	31.59
Arthritis	10 (71.5)	170(56.7)
Chronic Arthritis (6 weeks)	1 (7)	7 (2.5)
CNS Involvement (history)	1 (7)	38 (12.7)
Renal Involvement (history)	3 (21.4)	124(41.3)
Photosensitivity (Recent)	4 (28.6)	105 (35)
Malar Rash (Recent)	0	29 (9.7)
Oral Ulcer (Recent)	2 (14.3)	23 (7.7)
Discoid Rash (Recent)	0	12 (4)
Alopecia (Recent)	3 (21.4)	49 (16.3)
Arthritis (Recent)	0	25 (8.3)
Leukopenia (WBC<4000)	1 (7)	51 (17)
Anemia (Hb <10mg/dl)	3 (21.4)	40 (13.3)
Thrombocytopenia	0	16 (5.4)
Creatinine > 1	1 (7)	15 (5)
Increased ESR	8 (57.1)	148(49.3)
Positive CRP	2 (14.3)	50 (16.6)
Positive RF	2 (14.3)	18 (6)
Positive FANA	7 (50)	120 (40)
Positive Anti-ds DNA	6 (42.9)	115(38.3)
Positive anti-Cardiolipin (Ig G)	1 (7)	24 (8)
Positive anti-Cardiolipin (Ig M)	1 (7)	21 (7)
Low C3	2 (14.3)	62 (20.7)
Low C4	1 (7)	50 (16.7)
Low CH50	1 (7)	24 (8)
Proteinuria <3500 mg/24hours	3 (21.4)	44 (14.6)
Proteinuria >3500 mg/24hours	0	5 (16)
Low dose prednisolone <15mg/day	198 (66)	12 (85.7)
Moderate dose prednisolone 15<<30 mg/day	47 (15.7)	1 (7)
High dose prednisolone 30<mg/day	25 (8.3)	1 (7)
Hydroxychloroquin	210 (70)	9 (64.3)
Methotrexate	16 (5.3)	4 (28.6)
Azathioprine	49 (16.3)	4 (28.6)
Cyclophosphamide	35 (11.7)	0
Cellcept	10 (3.3)	0
Cyclosporine	2 (0.7)	0
Overlap with Scleroderma	4 (1.4)	1 (7)
Overlap with Polymyositis/ Dermatomyositis	4 (1.4)	0

**Table 2.** The relation of anti-CCP with sex, age, and duration of disease

	Positive anti-CCP (N=14)	Negative anti-CCP (N=286)	P value
Female	14 (100%)	257 (89.9%)	0.34
Male	0	29 (10.1)	
Age (year)	35.07±13.02	31.41±10.42	0.207
Duration of disease	5.71±3.38	6.36±6.57	0.516

Positive anti-CCP was noted in 11 patients with arthritis (6.5%) and 3 patients of the non-arthritis subset (2.3%). The difference in anti-CCP positivity between the two subgroups was not statistically significant ( $P$ -value= 0.09) (Table 3). Mean anti-CCP titer was 39.35±15.05 RU/mL (mean±S.E) in patients with arthritis and 14.23±4.72 RU/mL (mean±S.E) in those without arthritis ( $P$ -value=0.41).

**Table 3.** The relation of Anti CCP with Arthritis and its characteristics

		Positive anti-CCP N=14 Number (%)	P value
Arthritis	Yes (n=170)	11 (6.4)	0.090
	No (n=130)	3 (2.3)	
Chronic Arthritis	Yes (n=7)	1 (14.3)	0.391
	No (n=163)	10 (6.1)	
Erosion	Yes (n=1)	1 (100)	0.143
	No (n=6)	0	

Eleven arthritis cases with positive anti-CCP comprised 10 cases of transient arthritis and one of chronic arthritis. The patient with chronic arthritis had erosive joint damage confirmed by x-ray imaging (Table 3). The prevalence rate of anti-CCP positivity among chronic arthritis cases was (14.3%), while 6.1% of transient arthritis cases were anti-CCP positive (Table 3).

Positive RF was reported in two patients with positive anti-CCP (14.3%). RF-positive cases included one patient with transient arthritis and one with chronic arthritis. There was a significant relationship between anti-CCP and RF in this study ( $P$ =0.004).

The anti-CCP-positive subgroup had a higher rate of anti-ds DNA and FANA. However, in comparison with the total studied group, low complement levels were less frequent in anti-CCP-positive cases.

## Discussion

The concept of non-erosive lupus arthropathy has recently been challenged by innovative radiological

techniques. Some studies have postulated that erosive arthritis develops in a higher percentage of SLE patients [23, 24]. However, this debilitating complication has not been amply discussed in the literature. The underlying pathogenesis of erosive arthritis is not fully understood [11]. The predictive value of serological markers in the development of specific lupus complications such as erosive arthritis has been the subject of an ongoing dispute [20]. A review article by Budhram et al. revealed anti-CCP as a predictor of erosive arthritis in SLE [25]. There is growing evidence that suggests a higher prevalence of anti-CCP expression in lupus in comparison with SLE patients [26]. However, SLE patients with deforming arthropathy demonstrate clinical features comparable to cases of lupus [27]. Lower levels of complement components (C3, C4, and CH50) were less common among anti-CCP-positive cases in comparison with the whole cohort. This notion may be partly due to the limited number of anti-CCP-positive cases in this study.

The prevalence rate of anti-CCP positivity in sera in this study was similar to that in some previous reports [28, 29]. The prevalence rate of positive anti-CCP and the level of antibody expression were higher in the arthritis subset than in the non-arthritis subset; however, the difference was not statistically significant. This might result from the small number of patients with positive anti-CCP.

Radiographic evaluation of patients with chronic arthritis showed erosive arthritis in only one patient. This patient was the only case with positive anti-CCP among all chronic arthritis cases. The current study did not demonstrate any significant association between anti-CCP positivity and the development of erosive arthritis in the SLE population. This result was in contrast with those of a number of previous studies which have indicated a meaningful association between anti-CCP and erosive arthritis in SLE patients [13, 29]. This lack of association must be cautiously interpreted. The limited number of enrolled patients, the small number of cases with chronic arthritis, and specifically the single case of erosive arthritis may have partially affected the results. Qing et al. have proposed the role of ethnic and geographical variance in the expression of anti-CCP antibodies in SLE patients [21]. Similarly, ethnic and geographical variance may have influenced the results of the current study.

A significant association between serum RF levels and anti-CCP positivity was observed in SLE patients. This finding was similar to previously-described findings in patients with rheumatoid arthritis [10, 11,

12, 13, 21-23]. In contrast, another study of SLE, rhus, and RA has negated the significant association between anti-CCP levels and erosive or non-erosive arthropathy [27].

Overall, erosive arthritis was confirmed in only one patient of the current study population. Larger studies on SLE patients are warranted to show a possible correlation between anti-CCP and erosive arthritis.

The current study had a number of limitations, namely, the small sample size and the lack of therapeutic information on arthritis.

## Conclusion

This study did not show any association of anti-CCP with erosion in SLE patients with arthritis. Future longitudinal studies are needed to further investigate the correlation between erosive arthritis and anti-CCP positivity in SLE.

## Conflict of interest

The authors declare no conflicts of interest.

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## Upregulation of *transforming growth factor-B1* gene in ankylosing spondylitis patients

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Ankylosing spondylitis is a chronic inflammatory disorder of the axial skeleton. The transforming growth factor-beta (TGF- $\beta$ ) is a cytokine that has the dual action of suppressing inflammatory cytokines and augmenting inflammation. The role of this cytokine in ankylosing spondylitis is still unknown. The current study purposed to determine *TGF-B1* gene expression in ankylosing spondylitis. A case-control study of 48 ankylosing spondylitis patients and 47 age- and gender-matched healthy controls was conducted. Quantitative polymerase chain reaction with specific primers was used to measure the expression of *TGF-B1* gene in participants. Clinical indices of the disease, including the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), Metrology Index (BASMI), Functional Index (BASFI), and AS quality of life (ASQoL) were determined. The expression of *TGF-B1* was compared between cases and controls. Correlations between gene expression and clinical indices were assessed. The expression of *TGF-B1* was significantly higher in AS patients than in the control group ( $P$ -value < 0.0001). The change was 1.32-fold. There was no significant correlation between gene expression and AS clinical indices. The increase in *TGF-B1* expression possibly demonstrates its activity in AS disease either in a regulatory role as a response to inflammation in the body or as the augmentation of inflammation which exacerbates the disease. Further research needs to be done on this issue to resolve this uncertainty.

**Keywords:** ankylosing spondylitis, clinical manifestations, expression, transforming growth factor-beta.

### Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory rheumatic disorder of the axial skeleton. It can also cause peripheral joint and enthesitis inflammation and extraarticular involvements. Initial symptoms are usually inflammatory back and gluteal pain caused by sacroiliitis. Chronic inflammation in the spine and axial joints leads to ankylosis, formation of syndesmophytes, and osteoporosis. Therefore, most patients suffer from loss of spinal mobility [1]. AS is usually first noticed under 30 years of age [2]. The prevalence of the disease is 0.12% in urban areas of Iran [3]. Genetic factors play an important role in AS, and among these factors human leukocyte antigen-B27 (HLA-B27) has the strongest association with this disease [4].

Transforming growth factor- $\beta$  (TGF- $\beta$ ) is a pleiotropic cytokine which has an important role in the formation and repair of cartilage and bone tissues. In

fact, injection of this cytokine into the periosteal sheath of rat bones causes cartilage formation, which then leads to bone formation after injections are ceased [5]. It also seems that, in the presence of TGF- $\beta$ , B-cells switch to the production of IgA [6], which has a higher concentration in AS patients [7]. Therefore, TGF- $\beta$  can possibly be a key cytokine in the pathogenesis of AS disease, in which inflammation, ankylosis, and bone formation are the main phenomena.

TGF- $\beta$  can act as either a regulatory or an inflammatory agent. This cytokine is mostly known for its regulatory role, which is done through inhibiting the proliferation and differentiation of self-reactive T cells. Other mechanisms of immune regulation include helping regulatory T cells (Treg cells) to survive and to differentiate. This cytokine induces peripheral tolerance and can have a protective effect on autoimmune diseases. The inflammatory role of TGF- $\beta$

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takes place in the presence of interleukin (IL-) 6, which drives the differentiation of T helper 17 (Th17) cells and can cause further inflammation in the body [8]. Interleukin (IL-) 17 is one of the Th17 cell cytokines. IL-17 and other Th17 cytokines play a role in many inflammatory and rheumatic diseases. In the presence of IL-1, IL-6, and TGF- $\beta$ , Th17 cells are differentiated from naive CD4 cells [9].

Due to the dual role of TGF- $\beta$  and its association with both Th17 and Treg cells, abnormal *TGF-BI* gene expression (the gene which encodes TGF- $\beta$ , located on chromosome 19q13 [10]) can be the cause of an auto-inflammation in the body. However, abnormality in this expression can also be a defensive response of the immune system to preexisting inflammation in the body. The objective of the current study was to detect possible abnormalities in *TGF-BI* gene expression. Determining the rates of *TGF-BI* expression in AS patients can be a step towards understanding its role in AS pathogenesis so as to use the treatments that affect its pathway more efficiently.

## Materials and Methods

### *Study participants*

In this case-control study, 48 ankylosing spondylitis patients were selected conveniently from the outpatient clinic of the Rheumatology Research Center (RRC), Shariati Hospital, Tehran University of Medical Sciences (TUMS). Inclusion criteria were a BASDAI score  $\geq 4$  (which indicates more disease activity) and a diagnosis based on the modified New York Criteria 1987 (mNYC) [11]. The exclusion criterion for cases was any previous usage of biologic drugs. The control group included 47 healthy individuals. The exclusion criteria for the control group were a personal or family history of rheumatologic, autoimmune or inflammatory diseases, and being younger than 18 years of age. The Human Ethics Committee of the Tehran University of Medical Science approved this study, and written informed consent was obtained from all participants. Age and gender variants were matched in the two groups.

Demographic, medical, familial, and pharmacological histories of the study subjects were recorded by a questionnaire. Indices including BASDAI (Bath Ankylosing Spondylitis Disease Activity Index), ASQoL (AS quality of life), BASMI (Bath Ankylosing Spondylitis Metrology Index), and BASFI (Bath Ankylosing Spondylitis Functional Index); were used to assess disease activity, quality of life, spinal mobility, and functional disability for each

patient, respectively. Disease duration, presence of aphthous, family relationship, and ESR were also assessed.

### *RNA extraction and Real time PCR*

A peripheral blood sample was collected from each participant. Mononuclear cells were immediately isolated by Ficoll-Hypaque. Total RNA was extracted from mononuclear cells using a High Pure RNA Isolation Kit (Roche life science product). The extracted RNA was then transcribed to complementary DNA (cDNA) using a Transcriptor First Strand cDNA Synthesis Kit (Roche life science product). SYBR Green Real Time PCR was performed on cDNA to assess *TGF-BI* gene expression. The relative *TGF-BI* gene expression was compared with *Beta-2-microglobulin* expression as the housekeeping gene. The comparative Ct method was used to analyze gene expression data. Relative gene expression for each sample was calculated using the equation:  $2^{-\Delta Ct} \times 10^3$ .

### *Statistical analysis*

Quantitative variables were tested for normality with the Kolmogorov-Smirnov test and are expressed as mean $\pm$ SD if normally distributed or as median $\pm$ IQR (interquartile ranges) if not normally distributed. If the data did not have a normal distribution, the Mann-Whitney nonparametric test was performed to compare means of relative gene expression in the case and control groups. Correlations between *TGF-BI* gene expression and ESR, BASDAI, BASFI, BASMI and ASQoL were tested by Spearman's Rank-Order. Significance level was defined by a *P*-value of less than 0.05. All statistical analyses were done using SPSS version 23 and GraphPad Prism 6.

## Results

### *Characteristics of study participants*

Forty-eight ankylosing spondylitis patients (34 males and 14 females) with a disease duration of 12.29 $\pm$ 9.4 years and 47 healthy individuals (36 males and 11 females) as the control group participated in this study. The demographic data of the participants is demonstrated in Table 1. There were no significant differences between the two groups in the distribution of age and gender; ESR was significantly higher in cases (*P*-value  $< 0.001$ ). Table 2 shows disease indices for ankylosing spondylitis patients. The pharmacological histories of the patients are shown in Table 3.

**Table 1.** Comparison of demographic data between case and control groups

Characteristics	Descriptive statistics cases (n=48)	Descriptive statistics controls (n=47)	P-value
Age (Year) <sup>1</sup>	40.0±11.21	37.8±9.40	0.30
Smoking <sup>2</sup>	Smoker	30 (62.5%)	16 (34%)
	Non-Smoker	18 (37.5%)	31 (66%)
Sex <sup>2</sup>	Male	34 (70%)	36 (76%)
	Female	14 (30%)	11 (24%)

<sup>1</sup>Mean± SD for continuous variables<sup>2</sup>Number (Relative Frequency%) for the categorical variables**Table 2.** Clinical data of ankylosing spondylitis patients

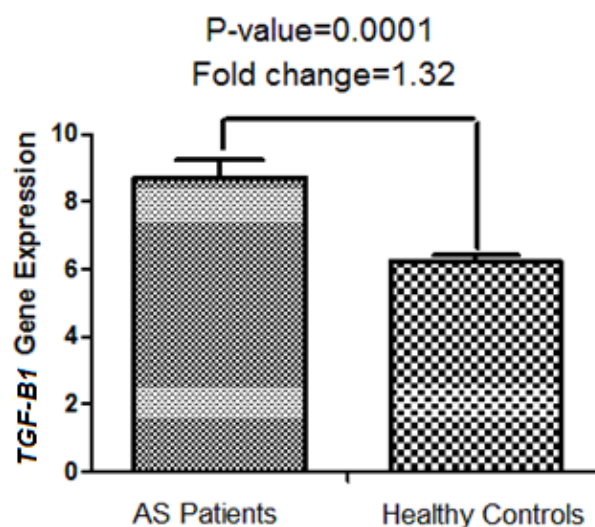
Characteristics	Descriptive statistics	
Disease duration (Year) <sup>1</sup>	12.29±9.4	
BASDAI score <sup>1</sup>	4.26±2.2	
BASFI score <sup>1</sup>	3.58±2.4	
BASMI score <sup>1</sup>	4.86±1.9	
ESR <sup>1,3</sup>	21±18.5	
Aphthous <sup>2</sup>	Presence	31 (64%)
	Absence	17 (36%)

<sup>1</sup>Mean± SD for continuous variables<sup>2</sup>Number (Relative Frequency%) for the categorical variables<sup>3</sup>Erythrocyte Sedimentation Rate (mm/hr)**Table 3.** Descriptive statistics for pharmacological history of patients (Number (%)) for all pharmacological drug consumption)

Drug Name	Categories	N (%)
NSAIDs	Using	32 (67%)
	Not Using	16 (33%)
Sulfasalazine	Using	24 (50%)
	Not Using	24 (50%)
Corticosteroid (prednisolone)	Using	3 (6.5%)
	Not Using	45 (93.5%)
Intra-articular injection (methyl prednisolone)	Using	8 (16.6%)
	Not Using	40 (83.4%)
Methotrexate	Using	7 (14.5%)
	Not Using	41 (85.5%)

### Gene expression results

The relative *TGF-β1* gene expression level was significantly higher in AS patients than in the control group ( $P$ -value < 0.0001). The change between cases and controls was 1.32-fold (Fig. 1).

**Fig. 1.** Comparison of relative gene expression of *TGF-β1* in ankylosing spondylitis cases and controls

### Correlations between gene expression and clinical indices

The correlations between *TGF-β1* gene expression and AS clinical indices are demonstrated in Table 4. There was no significant correlation between gene expression and clinical progression of the disease, however, there was a strong positive correlation between ASQoL and BASFI scores ( $r = 0.596$ ,  $P$ -value < 0.0001; data not shown).

**Table 4.** Correlation between *TGF-β1* gene expression and AS clinical indices

	Index	Correlation coefficient	P-value
TGF-β1 gene expression	BASDAI	0.138	0.35
	BASFI	0.092	0.53
	BASMI	-0.06	0.69
	ASQoL	0.14	0.33
	ESR	0.09	0.55

### Discussion

The role of proinflammatory cytokines in inflammatory diseases such as ankylosing spondylitis is well known. TGF-β is a cytokine with potential regulatory and inflammatory activities [8]. The inflammatory role of this cytokine takes place in the presence of IL-6, causing the differentiation of Th17 cells and thus causing further inflammation and augmentation of disease severity in autoimmune and inflammatory conditions. However, the most important and well-known activity of TGF-β is its immune regulatory

activity [8]. The purpose of the current study was to compare *TGF-*BI** gene expression in Iranian AS patients with healthy controls to learn its effects on the activity of AS. Other related surveys have mostly studied serum levels of cytokines or cytokine RNA production from immune cells and have attained controversial results about the cytokine effects on AS. The reason is likely to be the difficulty of measuring cytokine serum levels due to short cytokine half-lives and variations in measuring methods [12].

In the current study, the relative *TGF-*BI** gene expression was significantly higher in patients than in the control group. Based on the 1.32-fold change determined in this study, *TGF-*BI** gene expression is 32% higher in the cases of this study.

Nevertheless, no significant linear correlation between *TGF-*BI** gene expression and the clinical progression of AS disease was found. This is consistent with the study done in 2011 by Ali Taylan et al. on serum levels of T helper 17-related cytokines. They found significantly higher levels of TGF- $\beta$  and IL-6 in AS patients compared to healthy individuals. However, no significant correlation between disease activity (measured by BASDAI) and the serum levels of TGF- $\beta$  was found in their study as well [13]. Nonetheless, the absence of a significant linear correlation does not reject the possibility of a correlation between TGF- $\beta$  in patients and their clinical progression. The pattern of progression in AS is not exactly predictable [14]. The inflammation is not constant in the course of the disease [15]. It might increase as the disease progresses, and it might also decline after spine restriction because of ankylosis. Therefore, the nonlinear correlation can possibly be present between TGF- $\beta$  and the clinical progression of AS disease, and further research is needed on the inflammation in the course of AS disease.

Regarding the dual role of TGF- $\beta$ , the debate about the mechanism by which this cytokine affects AS disease can possibly be discussed through the recent usage of biologic drugs which have inhibitory effects on the inflammatory pathway of TGF- $\beta$  (activation of Th17, therefore causing further function of its cytokines), which has achieved favorable results [16]. Secukinumab, a human monoclonal antibody against

interleukin 17A, was first approved for the treatment of psoriasis and psoriatic arthritis in 2014. It is also being investigated for the treatment of AS, but so far, the studies have not provided sufficient data to prove Secukinumab efficacy on AS [17, 18]. However, in a study by Baeten et al., Secukinumab caused a rapid reduction in the clinical and biological signs of active AS [16]. The effectiveness of Secukinumab in AS was also stated by Blair et al. in their 2016 study [19].

Controversially, Medhat Shehata et al. found a significant increase of TGF- $\beta$  in AS patients who had responded to a special therapeutic method, and their diseases turned inactive compared to the group who had not responded to the therapy. This suggests that TGF- $\beta$  plays an anti-inflammatory role in AS disease [20]. This result is consistent with the Brown et al. review of the role of non-major-histocompatibility-complex genes in AS. According to this study, TGF- $\beta$  plays a small role in the pathogenesis of ankylosing spondylitis, and some other explanation exists for the linkage between TGF- $\beta$  and AS disease [21].

In the present study, a matched control group was used to reduce confounding bias. However, the small sample size can possibly lead to less external validity. Convenient sampling was also a limitation in the present study.

## Conclusion

This study purposed to investigate the expression of *TGF-*BI** gene in ankylosing spondylitis. It can be concluded that the increase of this cytokine in AS patients with active disease can possibly demonstrate its activity in the disease. This activity can be a regulatory role as a response to inflammation in the body or the augmentation of inflammation which exacerbates the disease. Therefore, further research needs to be done in this field to resolve this dilemma.

## Conflicts of interest

The author declares no conflicts of interest.

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## Osteomalacia with Looser zones caused by celiac disease

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Celiac disease is considered a malabsorption syndrome and is characterized by chronic small intestinal disease caused by hypersensitivity to the gliadin fraction of gluten. Celiac disease comes with diarrhea, occasional steatorrhea, weight loss, and other complications which might be caused by anemia. Reports of osteomalacia as the only symptom of celiac disease are very rare; however, osteomalacia can be a detected sign of celiac disease. Herein is described a case of osteomalacia with a Looser zone in a 39-year-old woman who had low bone mineral density caused by severe osteomalacia associated with chronic celiac disease. In patients with pain in the spine and proximal muscle, the risk of osteomalacia should be considered in any kind of diagnosis.

**Keywords:** bone loss, celiac disease, Looser zone, osteomalacia.

### Introduction

Celiac disease is an autoimmune inflammatory disease of the small intestine caused by the ingestion of gluten, a part of wheat protein, in genetically susceptible people [1]. Typically, the disease demonstrates diarrhea, occasional steatorrhea, weight loss, and anemia [2]. There are a handful of reports of osteomalacia with Looser zones as the first presenting symptom of celiac disease. Celiac disease is known to be associated with an increased risk of osteoporosis. Osteomalacia, as a symptom of celiac disease, is believed to be the result of decreased absorption of Vitamin D that occurs, in turn, because of improper functionality of the small intestine. It has been reported that the prevalence of celiac disease in Iran, even in low-risk subjects, is higher than that of western countries, being one out of every 166 healthy blood donors [3]. The present article discusses a patient who was referred to the medical center with low bone mineral density due to longstanding osteomalacia with a Looser zone and celiac disease.

### Patient and Observation

A 39-year-old woman was referred to the Rheumatology Clinic in Ghaem Medical Center, Mashhad University of Medical Sciences, Mashhad, Iran, in June 2016. She complained of experiencing pain in her spine and proximal muscle for the past three

years. Because of her body pain which lasted a long time, fibromyalgia was first considered, but a bone chemistry analysis revealed low vitamin D, calcium, and phosphate levels, elevated bone alkaline phosphatase, and high parathyroid hormone (PTH) levels which are typical in celiac disease. Plain films showed a Looser zone in the left femur. Clinical examination indicated bilateral proximal muscle atrophy, and the patient complained of weakness in the upper and lower extremities. Her hip range of motion was limited and accompanied by pain. The patient had a waddling gait. Laboratory tests (Table 1) were significant for microcytic anemia, hypocalcemia, hypophosphatemia, and high serum alkaline phosphatase. Her PTH level was high and Vitamin D (25-OH Vitamin D) level was low. Results of anti-gliadin and anti-endomysial antibody tests were positive.

Results of further laboratory investigations, including C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), creatinine, and thyroid function tests, were within normal ranges. Bone mineral density was measured using dual energy X-ray absorptiometry (DEXA) Lunar DPX-L (Lunar Corporation, Madison, WI), and results were low: 0.813 g/cm<sup>2</sup> (2.33 SD below the mean) for the femoral neck and 0.806 g/cm<sup>2</sup> (2.36 SD below the mean) for the lumbar spine. Plain radiology films showed a Looser

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zone in the left proximal femur bone (Fig. 1). The diagnosis of osteomalacia was then confirmed. The diagnosis of celiac disease of this patient was confirmed by positive IgA and IgG anti-gliadin, anti-tissue transglutaminase antibody tests, endoscopic detection of inflammation, and atrophy of duodenal mucosa. The patient's clinical and laboratory responses to a gluten-free diet, iron, and calcium-vitamin D were good, which is indicative of celiac disease.

**Table 1.** Results of laboratory evaluation

Laboratory evaluation	Patient	Reference values
Calcium (mg/dl)	7.5	8–10.6
Phosphate (mg/dl)	2.1	2.5–4.5
Albumin (g/L)	36	35–52
Alkaline phosphatase (U/L)	605	50–305
25-hydroxy vitamin D (nmol/L)	<9	25–150
Parathyroid hormone (pmol/L)	83	14–72
Antigliadin Ab. IgG (u/ml)	46	Up to 5
Anti-tissue transglutaminase Ab. (u/ml)	>153	up to 20

### Discussion

Samuel Gee first described celiac disease in 1888. He reported patients with malabsorption associated with a reversible atrophy of intestinal mucosa of the small bowel [4]. It is suggested that celiac disease is common in Caucasian family members, and it is strongly associated with HLA phenotypes B8, BR3, and DQw2 [5]. CD is an autoimmune inflammation disease of the small intestine caused by the ingestion of gluten, a factor of wheat protein, in persons who are genetically

at risk [6]. Osteomalacia was first noted in coexistence with celiac disease more than 64 years ago. In 1953, the relationship between celiac disease and osteomalacia was first reported. The osteomalacia that is associated with celiac disease is believed to result from the decreased absorption of Vitamin D caused by improper functionality of the small intestine in absorbing this vitamin. Therefore, the recommendation is that patients with celiac disease be assessed for osteoporosis. In a similar case, Frikha et al. expressed that osteomalacia with Looser zones is a late complication of celiac disease which, in turn, occurs because of poor compliance to a gluten-free diet [7]. On the other hand, osteomalacia may be the presenting feature of celiac disease, and vitamin D supplementation could be an effective mode of therapy.

Dual Energy X-ray Absorptiometry (DEXA) bone densitometry is the best method available today for diagnosing low bone mineral density [8]. This is advocated as a showing for an antibodies test for celiac disease in young patients who have osteoporosis [9]. Clinical examination shows the indication of bilateral, proximal muscle atrophy and simultaneous weakness in the upper and lower extremities [10]. In the current case, the patient's hip range of motion was limited and painful. A recent article stressed the fact that celiac disease often presents with fatigue and anemia rather than the consequences of malabsorption. Clinical or biochemical evidence of osteomalacia in patients with celiac disease is rare now, and recently, in two large series of celiac patients, there were no reports of osteomalacia evidence [11].



**Fig.1.** A looser zone in the left thigh bone

Improvement in bone density in the current patient was notable after six months of treatment. Hence, a regimen of a gluten-free diet together with vitamin D supplementation was proven to be very effective. Osteomalacia is a major feature of celiac disease in young women. The current patient had both clinical and radiological signs of osteomalacia (Looser zone) that preceded the diagnosis by a significant period of time. Moreover, she had features of malabsorption as well. Celiac disease that is not treated is linked to malabsorption. Most fat-soluble vitamins are absorbed in the distal small bowel; nonetheless, extensive villous atrophy from long-standing gluten enteropathy can lead to weak absorption of vitamin D and other vitamins [12].

In osteomalacia patients, the diagnosis of celiac disease should be considered, although the biochemical features of osteomalacia appear late in the process of vitamin D depletion, and preliminary features may include some increase in parathyroid hormone and reduction in 25-hydroxy cholecalciferol levels [13]. It is also recommended that parathyroid hormone blood

levels be measured. This test helps the doctor determine whether the celiac disease is being controlled, whether excessive bone loss is going on, and whether sufficient calcium is being received by the patient. Unquestionably, a gluten-free diet is a must-have component of therapy for celiac disease [14]. After the diagnosis of osteomalacia was made in the current case, treatment with a gluten-free diet and supplementary calcium and vitamin D with bisphosphonates caused progressive improvement in symptoms as expected [15].

### Conclusion

It is recommended that a celiac disease diagnosis be performed in any patient with osteomalacia. Moreover, it is essential that a gluten-free diet be imposed for the purpose of celiac disease therapy [16]. Due to the risk of osteomalacia, the treatment of celiac disease should be started to prevent bone loss complications.

### Conflict of interest

The authors declare no conflicts of interest.

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