

ОРИГИНАЛНИ СТАТИИ
ORIGINAL ARTICLES

TENASCIN-C AND INTERLEUKIN-17 UP-REGULATION IN AXIAL SPONDYLOARTHRITIS PATIENTS

M. S. Al-Hindawi¹, A. M. Al-Gebori¹, M. H. M. Alosami²

¹Applied Sciences Department, Applied Chemists Division, University of Technology, 10001 Baghdad – Iraq

²Department of Medicine, Rheumatology Unit, Colleges of Medicine, University of Baghdad, 10001 Baghdad – Iraq

Abstract. Background: Axial spondyloarthritis (axSpA) is an inflammatory, systemic rheumatic condition that mostly affects the axial skeleton. Tenascin-C (TN-C) is a hexameric glycoprotein of considerable size, upregulated in many inflammatory conditions, while Interleukin-17 (IL-17) is a cytokine that plays an important role in SpA symptoms. **Objective:** to examine the serum levels of TN-C and IL-17 in Iraqi axSpA patients and investigate the upregulation between these parameters and the disease characteristics. **Patients and Methods:** Seventy-four axSpA patients and 28 age- and sex-matched controls were studied. Fifty-four patients received a tumor necrosis factor inhibitor (TNFi) and 20 did not. Serum TN-C and IL-17 concentrations were determined using the ELISA technique. The Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) was assessed. For statistical tests, Chi Square, Mann Whitney, Independent Samples T, and Pearson's correlation tests were used. **Results:** Patients mean age was 35.6 ± 0.9 years, 65 males and 9 females, and disease duration was 5.18 ± 0.6 years. 47.4% was smokers, 47% had inflammatory low back pain, and HLA-B27 was present in 91%. Mean BASDAI was 6.1 ± 0.4 in non-TNFi and 2.6 ± 0.3 in those on TNFi. The serum TN-C concentration was significantly increased in patients, especially those on non-TNFi (79 ± 9.6 pg/mL) compared to those on TNFi (69.1 ± 3.2 pg/mL) and control (53 ± 3.9 pg/mL), where ($p = 0.003$) between patients and controls, while no significant differences ($p = 0.21$) were found between TNFi and non-TNFi. Serum IL-17 was significantly elevated in those receiving TNFi (877.9 ± 257 pg/mL) compared to non-TNFi (487.2 ± 234 pg/mL) and control (182.4 ± 36.6 pg/mL), where ($p = 0.033$) between patients and controls, while no significant differences ($p = 0.3$) were found between TNFi and non-TNFi. The TN-C significantly correlated with erythrocyte sedimentation rate ($r = 0.27$, $p = 0.02$) and with hemoglobin (Hb) concentration ($r = 0.26$, $p = 0.02$). TN-C and IL-17 were non-significantly related ($r = 0.14$, $p = 0.2$). **Conclusions:** AxSpA patients demonstrated high levels of serum TN-C, particularly in those not receiving TNFi, and high IL-17 levels in those receiving TNFi, suggesting that there is an association with tissue injury from the disease and stimulation of immunological and resident tissue cells. Patients who had not received TNFi showed significantly higher disease activity than others.

Key words: axial spondyloarthritis, Tenascin-C, Interleukin-17, BASDAI

INTRODUCTION

Axial spondyloarthritis (axSpA) is an inflammatory, systemic rheumatic condition with a wide range of symptoms. The axial skeleton, sacroiliac joint (SIJ) and spine, is mostly affected [1]. In addition to the peripheral joint inflammation, entheses, and low back pain (LBP), axSpA causes extra-musculoskeletal or systemic symptoms [2]. The axSpA refers to patients with structural damage seen on radiographs (radiographic axSpA) commonly known as ankylosing spondylitis (AS) and patients without structural

damage seen on radiographs (non-radiographic axSpA, nr-axSpA) [3]. Nr-axSpA represents an early stage of inflammation while AS represents the progress of the disease [4].

Tenascin-C (TN-C) is a hexameric glycoprotein found in the extracellular matrix, upregulated in many inflammatory conditions. It is the first of the four tenascins (C, W, R, and X) in the tenascin family, and its expression pattern is unique [5,6]. The structure of TN-C subunits contains four regions: N-terminal, epidermal growth factor – like repeats, fibronectin type III

-like repeats, and C-terminal globular fibrinogen (gFG) domain [7]. The elevation of TN-C has been associated with persistent inflammation in many disorders like rheumatoid arthritis (RA), malignancies, psoriatic arthritis (PsA), asthma, fibrosis, and systemic lupus erythematosus (SLE) [6, 8]. In addition, TN-C levels are elevated in children who have enthesitis-related juvenile idiopathic arthritis (JIA) and many of them develop AS later. TN-C is released when the joint is infected or traumatically injured, although it does not exist in mature healthy cartilage [9]. TN-C expression is temporary, and tissue levels revert to normal when tissue healing is completed. Through a variety of mechanisms, TN-C promotes both adaptive and innate immune responses in arthritic joints. TN-C's C-terminal gFG induces the pro-inflammatory cytokines and chemokines release in autoimmune disease patients such as RA [10], which increases the risk of osteoporosis around the afflicted joint, erosive synovitis, bone damage, and cartilage deterioration [11, 12].

Interleukin (IL)-17 is produced by T-helper 17 (Th17) cells [13], although it can also be produced by tissue-resident memory cells, natural killer T cells, mucosal-associated invariant T cells, T-cytotoxic 17 cells, mast cells, and neutrophils. Multiple cell types are affected by the IL-17 signal, which also triggers the production of pro-inflammatory mediators such as tumor necrosis factor (TNF), colony-stimulating factors, IL-1, and IL-6 [14, 15]. The significant role of IL-17 in SpA symptoms is in increasing the expression of genes associated with inflammation in target cells, including fibroblasts and keratinocytes, which causes an increase in the synthesis of cytokines, chemokines, and other mediators linked to the joints, skin, and entheses by enhancing TNF. There is evidence of decreased disease activity seen with IL-17 inhibitors in AS, psoriasis, and PsA [16].

In this research, we aimed to find serum levels of TN-C and IL-17 and then investigate the association and effects of these parameters in patients with axSpA treated or not with TNF inhibitors (TNFi).

MATERIALS AND METHODS

Study design

The study was a case-control study, in which seventy-four patients with axSpA treated at Baghdad Teaching Hospital were included. Fifty-four had previously been treated with Etanercept, which is a TNFi treatment, and 20 had never been treated with a TNFi. Twenty-eight age- and sex-matched healthy control subjects were also considered. Blood samples were taken, separated into gel and EDTA tubes, centrifuged for 20 min, and subsequently frozen at -20°C .

The samples were obtained under the approval of the University of Technology (UOT) Human Research Ethics committee and the study was done in accordance with the requirements of the World Medical Association Declaration of Helsinki Ethical Principles (2013).

Inclusion criteria

The inclusion criteria were a diagnosis of axSpA in accordance with the Assessment of Spondylarthritis International Society (ASAS) [17] at Baghdad Teaching Hospital, divided according to treatment, and all patients were older than 18 years. Informed consent was obtained from all patients involved in the study.

Exclusion criteria

According to the exclusion criteria, all individuals who have a history of malignancies, thyroid diseases, cardiovascular problems, and one or more additional autoimmune diseases such as RA, SLE, PsA, diabetes mellitus, etc., were excluded. Furthermore, pregnant or lactating women were also excluded.

Study tests

Laboratory investigations included complete blood count, liver function tests (serum alanine transaminase and aspartate transaminase), renal function tests (blood urea nitrogen and serum creatinine), erythrocyte sedimentation rate (ESR), and human leukocyte antigen B27 (HLA-B27) genotyping. The Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) was assessed [18].

The sandwich ELISA technique was employed to measure the human serum TN-C (Cat. No.: MBS2703991; MyBioSource, USA) and IL-17 (Cat. No.: MBS764076; MyBioSource, USA), which utilizes a purified particular antibody to capture antigen from serum raising its specificity and sensitivity.

Statistical analysis

For data analysis, the Statistical Package for Social Sciences (SPSS) version 26 was utilized. Results were presented as number, percentage, median interquartile range (IQR), and mean \pm standard error of the mean (SEM). Mann Whitney, Chi Square, and Independent Samples T tests were utilized to compare data. The Pearson's correlation test was considered. Significance was set at $p < 0.05$.

RESULTS

Main results and characteristics

The mean age of 74 cases was 35.6 ± 0.9 years and there were 65 males and 9 females with 5.18 ± 0.58 years disease duration, 47.4% of patients were smokers, 47% had inflammatory LBP, and HLA-B27

was present in 91%. The demographic distribution of the patients is presented in table 1.

Table 1. Patients demographic distribution

Characteristics	Patients n = 74
	Median (IQR)
Age	36.5 (28.75-41)
BMI	26.675 (24.385-29.328)
ESR (mm in 1st hour)	14 (8-32.5)
BASDAI	2.85 (1.2-5.8)
Disease duration (years)	5.18 ± 0.6
Gender	n (%)
Male	65 (88)
Female	9 (12)
Clinical parameters	n (%)
Inflammatory back pain	35 (47)
HLA-B27 genotype	67 (91)
Treatment	n (%)
bDMARD (TNFi)	54 (73)
No treatment (non- TNFi)	20 (27)
Smoking	n (%)
Smoker	36 (48)
Non-smoker	38 (52)

HLA-B27: human leukocyte antigen B27, BASDAI: bath ankylosing spondylitis disease activity index, TNFi: tumor necrosis factor inhibitor, BMI: body mass index, ESR: erythrocyte sedimentation rate, bDMARD: biological disease modifying anti-rheumatic drug, IQR: interquartile range.

The characteristics of the patients receiving TNFi or not and the control are presented in table 2, and results showed a significant changes between axSpA patients and healthy control in TN-C with $p = 0.003$, IL-17 with $p = 0.033$, ESR with $p = 0.017$, and hemoglobin (Hb) with

$p = 0.004$ levels in the other hand between TNFi and non-TNFi there are no significant differences in TN-C, IL-17, and Hb levels but the significant differences was in ESR with $p < 0.001$ and disease activity as BASDAI with $p < 0.001$. Other parameters such as age, BMI, AST, ALT, BUN, and creatinine have shown no significant differences between groups.

Gender and smoking differences

Gender differences between patients in TN-C, IL-17, ESR, BASDAI and disease duration are presented in table 2. There were no significant differences either in smokers and non-smoking patients nor between males and females, but an increase in TN-C and IL-17 towards female's samples.

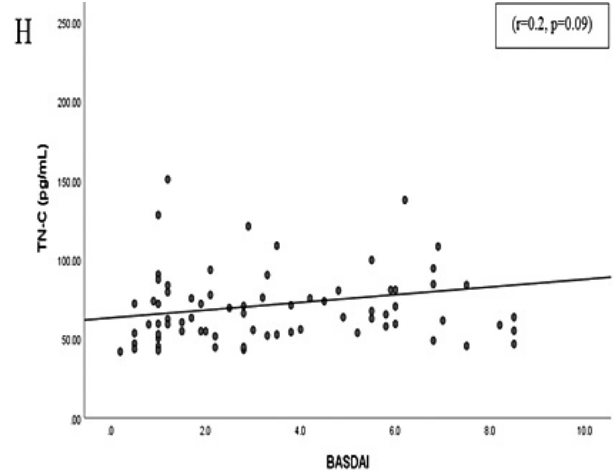
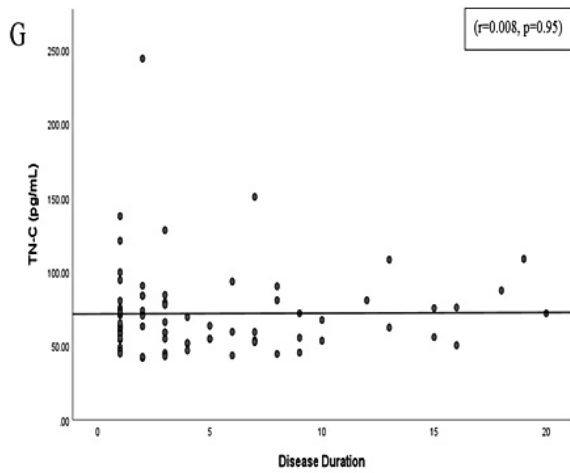
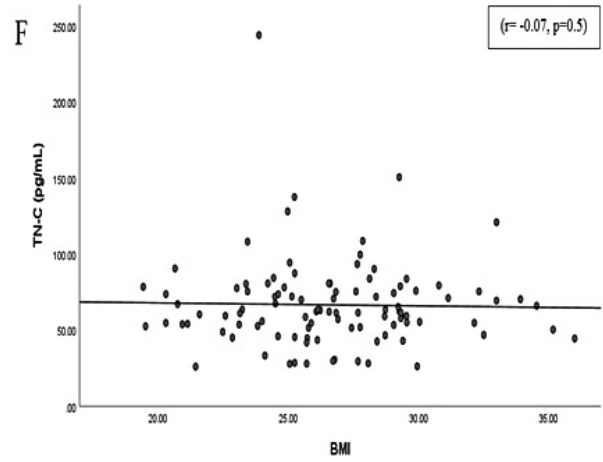
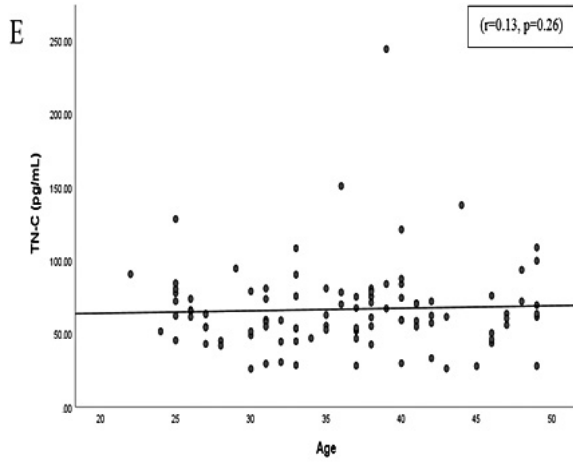
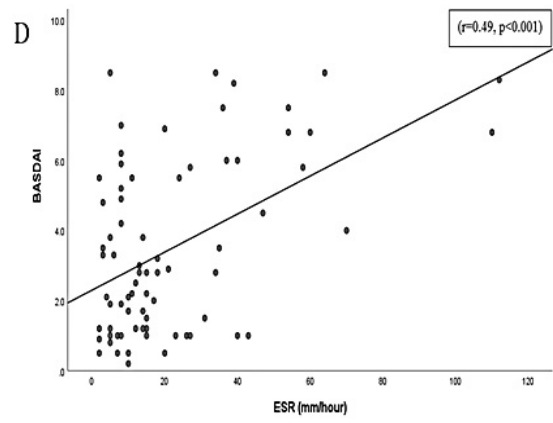
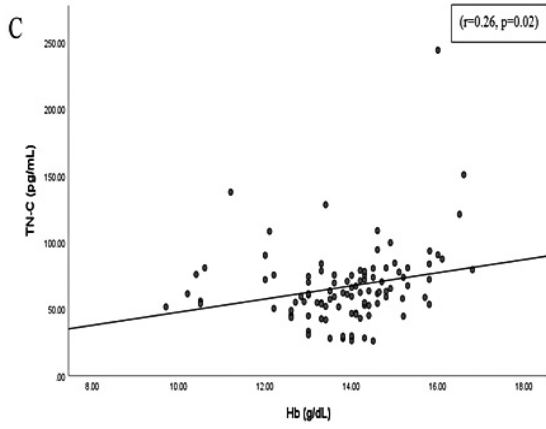
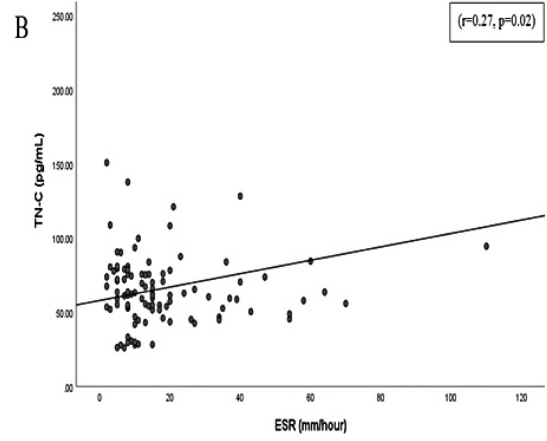
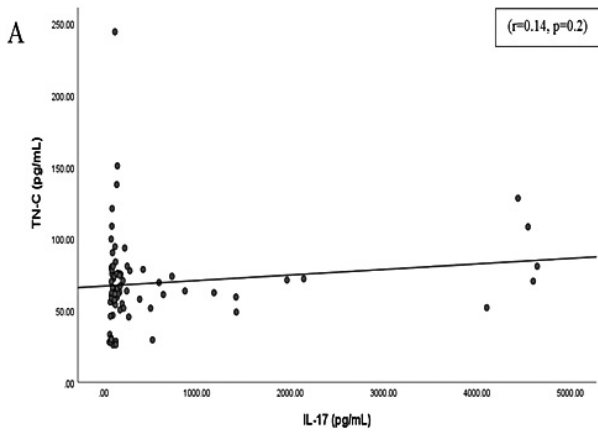
Correlation test results

Correlation results between TN-C and IL-17 concentration with the characteristics of patients are illustrated in figure 1. TN-C and IL-17 were non-significantly related ($r = 0.14$, $p = 0.2$). TN-C has a significant correlation with ESR and Hb ($r = 0.27$, $p = 0.02$) ($r = 0.26$, $p = 0.02$) respectively. BASDAI and ESR showed a strongly significant correlation ($r = 0.49$, $p < 0.001$). TN-C has a non-significant relation with age ($r = 0.13$, $p = 0.26$), BMI ($r = -0.07$, $p = 0.5$), disease duration ($r = 0.008$, $p = 0.95$), and BASDAI ($r = 0.2$, $p = 0.09$). IL-17 has a non-significant relation to age ($r = -0.04$, $p = 0.77$), BMI ($r = 0.007$, $p = 0.96$), disease duration ($r = 0.016$, $p = 0.25$), BASDAI ($r = 0.03$, $p = 0.86$), ESR ($r = -0.05$, $p = 0.73$), and Hb ($r = -0.11$, $p = 0.4$).

Table 2. The characteristics of axial spondyloarthritis (axSpA) patients receiving or not receiving anti-tumor necrosis factor inhibitors and healthy control

Factor mean ± SEM or n(%)	AxSpA (n = 74)	Control (n = 28)	p	TNFi (n = 54)	non-TNFi (n = 20)	p
Male:female	65:9 (7.2:1)	24:4 (6:1)	0.77	46:8 (5.8:1)	19:1(19:1)	0.25
Age (years)	35.6 ± 0.9	37.3 ± 1.3	0.304	35.4 ± 1.05	36.1 ± 1.5	0.74
Disease duration (years)	5.18 ± 0.6	–		6.44 ± 0.7	1.75 ± 0.45	< 0.001
BMI	26.98 ± 0.43	25.7 ± 0.5	0.088	26.98 ± 0.5	26.98 ± 0.8	0.99
Smoking	36 (48.6)	10 (35.7)	0.24	27 (50)	9 (45)	0.7
BASDAI	3.5 ± 0.3	–		2.6 ± 0.3	6.1 ± 0.4	< 0.001
Hb (g/dL)	14.1 ± 0.16	13.2 ± 0.25	0.004	14 ± 0.2	14.5 ± 0.2	0.17
ESR (mm/1sth)	21.96 ± 2.6	11.5 ± 0.94	0.017	16.1 ± 2	38.6 ± 7.2	< 0.001
AST (U/L)	23.6 ± 1.2	21.4 ± 1.2	0.32	22.2 ± 1.3	27.2 ± 2.9	0.07
ALT (U/L)	27.4 ± 2.0	24.6 ± 1.0	0.4	26.9 ± 2	29 ± 5.2	0.64
BUN (mg/dL)	26.5 ± 0.8	25.9 ± 1.3	0.7	26.2 ± 0.9	27.1 ± 1.5	0.63
Creatinine (mg/dL)	1.04 ± 0.24	0.75 ± 0.03	0.5	1.1 ± 0.3	0.8 ± 0.03	0.51
TN-C (pg/mL)	71.8 ± 3.5	53.04 ± 3.8	0.003	69.1 ± 3.2	79 ± 9.6	0.21
IL-17 (pg/mL)	727.6 ± 182.4	182.4 ± 36.6	0.033	877.9 ± 257	487.2 ± 234	0.3
HLA-B27	67 (91)	–	–	54 (100)	13 (65)	< 0.001

TN-C: tenascin-C, IL-17: interleukin-17, axSpA: axial spondyloarthritis, BMI: body mass index, TNFi: tumor necrosis factor inhibitor, BASDAI: Bath ankylosing spondylitis disease activity index, Hb: hemoglobin, ESR: erythrocyte sedimentation rate, ALT: alanine transaminase, AST: aspartate transaminase, BUN: blood urea nitrogen. Significant values at $p \leq 0.05$ are in bold.



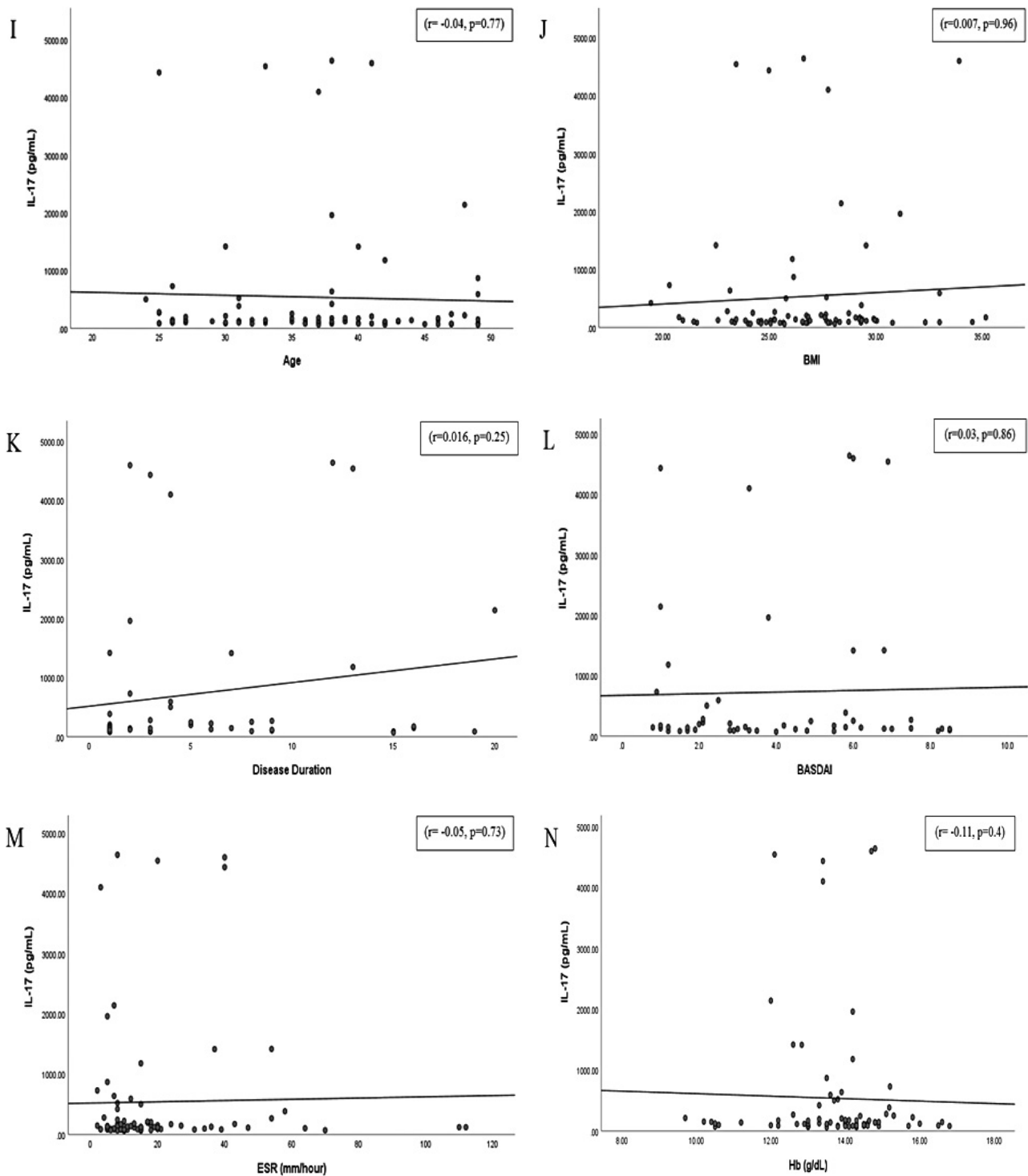


Figure 1. Correlation results between TN-C and IL-17 concentration with the characteristics of patients. (A) Correlation between TN-C and IL-17 ($r = 0.14$, $p = 0.2$). (B) between TN-C and ESR ($r = 0.27$, $p = 0.02$). (C) between TN-C and Hb ($r = 0.26$, $p = 0.02$). (D) between BASDAI and ESR ($r = 0.49$, $p < 0.001$). (E) between TN-C and age ($r = 0.13$, $p = 0.26$). (F) between TN-C and BMI ($r = -0.07$, $p = 0.5$). (G) between TN-C and disease duration ($r = 0.008$, $p = 0.95$). (H) between TN-C and BASDAI ($r = 0.2$, $p = 0.09$). (I) between IL-17 and age ($r = -0.04$, $p = 0.77$). (J) between IL-17 and BMI ($r = 0.007$, $p = 0.96$). (K) between IL-17 and disease duration ($r = 0.016$, $p = 0.25$). (L) between IL-17 and BASDAI ($r = 0.03$, $p = 0.86$). (M) between IL-17 and ESR ($r = -0.05$, $p = 0.73$). (N) between IL-17 and Hb ($r = -0.11$, $p = 0.4$).

Table 3. Gender and smoking differences between axial spondyloarthritis patients

Parameter	Gender differences		P	Smoking differences		p
	Male (n = 65)	Female (n = 9)		Smoke (n = 36)	No smoke (n = 38)	
TN-C (pg/mL)	69.27 ± 3.68	90.08 ± 9.8	0.052	71.39 ± 6.1	72.18 ± 3.7	0.9
IL-17 (pg/mL)	653.16 ± 177.7	1083.47 ± 643.8	0.38	835.72 ± 335.1	654.43 ± 209.1	0.6
ESR (mm/1st h)	21.66 ± 2.85	24.11 ± 7.08	0.76	22.97 ± 4.6	21.03 ± 2.8	0.7
BASDAI	3.36 ± 0.3	4.7 ± 0.9	0.13	3.503 ± 0.42	3.55 ± 0.4	0.9
Hb	14.39 ± 0.14	11.97 ± 0.37	< 0.001	14.72 ± 0.15	13.504 ± 0.23	< 0.001

TN-C: tenascin-C, IL-17: interleukin-17, ESR: erythrocyte sedimentation, BASDAI: Bath ankylosing spondylitis disease activity index. Significant values at $p \leq 0.05$ are in bold.

DISCUSSION

The serum TN-C and IL-17 concentrations were higher in axSpA patients, including both TNFi and non-TNFi groups, compared to the control. Additional laboratory testing outcomes, such as liver and renal function tests, showed a non-significant change between the patient groups compared to the control.

The expression of TN-C is linked to tissue damage and is activated by a variety of stressors, involving physical pressure, pro-inflammatory mediators, human growth hormone, hypoxia, and reactive oxygen species [19]. TN-C elevation in the serum is considered unspecific and associated with tissue injury as well as the stimulation of immunological and resident tissue cells [20]. In addition, the ability of TN-C to stimulate a pro-inflammatory cytokines production linked to axSpA pathogenesis, such as TNF and IL-17, is supposed to be related to the gFG domain in TN-C that binds to toll-like receptor 4 on monocytes, macrophages, and dendritic cells [21]. In an arthritic animal model, TN-C instillation intra-articularly was shown to induce synovial inflammation, in contrast to mice lacking TN-C, which showed rapid joint inflammation resolution and disease severity were reduced; this supports the findings that TN-C elevation can stimulate pro-inflammatory pathways [22]. Also, as a part of its mechanism, TN-C may bind to several cell surface receptors, such as heparan sulfate proteoglycans and integrins, to modify cytoskeletal organization and cell spreading in arthritic joint diseases [23]. Since TN-C is a key driver of new bone formation and our study's findings showed increased levels in non-TNFi patients, targeting TN-C may provide an alternative strategy for patients with high disease activity due to delayed diagnosis or whose bone remodeling process has already begun, making it no longer adequate to just stop the inflammation [24].

The current study results of IL-17 levels support findings presented in previous studies [25, 26] reporting an increased serum IL-17 in patients with axSpA.

The elevation of IL-17 in this study might be due to the presence of the HLA-B27 genotype that most patients seem to have, especially in the TNFi group, which is related to Th17 activation, the primary IL-17 source [27]. Furthermore, higher numbers of IL-17-producing Th17 cells have previously been observed in SpA animal models in patients with long disease duration [28], and there is evidence that many patients who appeared to have reduced disease activity after using anti-TNF medications still exhibited high Th17 cell percentages [29]. This may explain the elevation of IL-17 levels in the TNFi group with a longer duration in comparison to the non-TNFi group, even if there is non-significant difference. It is probable that IL-17 has emerged as an immunological actor in the lack of bone regeneration in axSpA patients, which has the effect of suppressing bone regeneration by being involved in the processes that cause bone loss in those patients [30]. Given this propensity and the fact that the pathway of IL-17 plays a substantial role in axSpA development, it suggests that IL-17 may be a potential target to reduce inflammation more than TNF [31, 32] or can utilize anti-IL-17 if TNFi fails [33]. IL-17, TNF, and IL-22 inflammatory cytokines can stimulate fibroblasts to create high levels of TN-C. The limited pharmacological activity of antibodies that neutralize a particular cytokine on the radiographic progression of individuals with AS can be explained by the fact that several inflammatory cytokines can cause TN-C formation [8].

High levels of circulating TN-C may indicate significant joint tissue degradation and damage through a variety of pathways, suggesting that elevated serum TN-C may be both a cause and a result of active erosion in joints not only in axSpA but even in other rheumatic diseases such as RA [10]. By activating receptors on the bone marrow dendritic cells' surface and inducing signal transduction pathways that culminate in de novo cytokine synthesis and secretion, TN-C may trigger the manufacture of components specific for Th17 lymphocyte polarization

during inflammation. TN-C has a pro-inflammatory effect and is expressed frequently in inflammatory tissues and lymphoid organ zones that perform T cell-dependent activities and promote T cell differentiation and activation not just in lymphoid tissues but also in inflammatory regions [23, 34]. That tendency leads to increased IL-17 production [35] and a subset of IL-17-producing Th17 cells, a key player in the induction of autoimmune tissue damage [36].

In Iraqi patients, disease activity represented by BASDAI was shown to be higher in axSpA patients who had not received TNFi treatment. This may indicate the therapeutic significance of TNFi treatment on axSpA patients in the long-term use, especially on the SIJ and spine [37]. Additionally, there is a highly significant rise in disease duration between patient groups, which is related to the fact that the majority of patients with non-TNFi were recently diagnosed (newly diagnosed), and was confirmed by the existence of a highly significant increase in ESR towards the non-TNFi group. Since ESR is one of the most helpful indicators for monitoring the progression of inflammatory disease activity and it is associated with clinical responses to TNFi treatment, this may explain the significant correlation between ESR and BASDAI as mentioned in the results [38,39]. However, patients with axSpA had significantly higher Hb results than healthy controls, and since anemia often occurs with the onset of inflammatory disorders [40], that result was unexpected. The elevated Hb levels may be related to smoking differences, as smoking patients appear to have high Hb levels, which is consistent with previous research that linked smoking and elevated Hb levels [41]. Additionally, it was observed that the biological therapy etanercept that the TNFi group was treated with had an effect on rising Hb levels over time [42].

In the current study, results of the gender differences revealed that the means of TN-C, IL-17, ESR, and BASDAI were non-significant differences between males and females, in spite of the evidence in a previous study that male patients with axSpA have more radiological damage than females [43]. However, there was an increase in female IL-17 levels and that was unexpected since previous studies [43, 44] observed that serum IL-17 concentrations were lower in females compared to males. On the other hand, there was a slight increase in females' TN-C levels mean with a p-value that was close to significant, but to demonstrate whereas this might be a woman's sex indicator in axSpA, a larger research with more data is required. Smoking difference results also showed a non-significant difference be-

tween smoking and non-smoking patients, although the clinical response to TNFi treatment is expected to be reduced in current smokers and axSpA patients [45].

According to the findings, TN-C and ESR significantly correlate, which agrees with previous studies [9, 46]. TN-C also showed a significant correlation with Hb that agreed with others [47].

There are several limitations in this study that may be discovered, including the small number of patients with negative HLA-B27, so we could not make a comparison on this parameter. In addition, radiological information for patients was not collected. Further studies with a larger number of participants are recommended to indicate gender differences in TN-C levels in axSpA patients.

CONCLUSIONS

Patients with axSpA demonstrated high levels of serum TN-C concentrations compared to the control group, particularly in those not receiving TNFi, although there are no significant differences between TNFi and non-TNFi groups, suggesting that there is an association with tissue injury from the disease as well as the stimulation of immunological and resident tissue cells. There were also elevated patient's serum IL-17 levels compared to the control group, although there were no significant differences between TNFi and non-TNFi groups, suggesting that it's because of the presence of the HLA-B27 genotype that most patients seem to have, which is related to Th17 activation. Disease activity was significantly higher in axSpA patients who had not received TNFi treatment.

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Conflict of Interest: *The authors declare no conflict of interest.*

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**Correspondence address:**

Mohammed Salah Al-Hindawi
Baghdad, Iraq.

phone number: +9647516313825

e-mail: Mohammed.S.AlHindawi@uotechnology.edu.iq

ORCID ID: 0000-0003-1487-5943