EVALUATION OF TL1A DIAGNOSTIC AND PROGNOSTIC VALUES IN ANKYLOSING SPONDYLITIS PATIENTS; IN SULAYMANIYAH GOVERNORATE

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Abstract

Background: Ankylosing spondylitis is a chronic inflammatory disorder primarily involving the sacroiliac joints and spine. It is associated with both articular and extra-articular clinical manifestations, including peripheral arthritis, enthesitis, and anterior uveitis.

Objectives: To compare the serum levels of tumor necrosis factor-like ligand 1a (TL1A) in ankylosing spondylitis patients and the apparently healthy persons; and to find out the association between the cytokine level and ankylosing spondylitis disease activity and spinal mobility.

Patients and Methods: Cross-sectional analytic study conducted in the division of rheumatology/Shaheed Hemin general hospital, in Sulymaniyah city from (April 2015 to September 2015), on 45 ankylosing spondylitis patients with ages ranged between 17 to 52 years. The patients interviewed, fully examined, and classified to anti-TNF treated (n=26) and non-biologic therapy treated (n=19). Then investigated for the serum levels of TL1A and the results were compared with those of 45 age and gender matched apparently healthy persons. The cytokine levels also compared with the disease activity and spinal mobility parameters.

Results: The serum levels of TL1A in ankylosing spondylitis patients were significantly higher than those of the healthy subjects, with mean and standard deviation values of (428.4±285.7 pg./ml), (217.6±64 pg. /ml) respectively, the serum levels of TL1A in non-biologics treated ankylosing spondylitis patients were significantly higher than those of the healthy subjects, with mean and standard deviation values of (576.6±337.4 pg./ml), (217.6±64 pg./ml) respectively, and for anti-TNF treated ankylosing spondylitis patients mean and standard deviation values of (320.1±181.2 pg./ml), (217.6±64 pg./ml) respectively, with (P values < 0.001).

Conclusions: Serum TL1A is up-regulated in ankylosing spondylitis, associates with disease activity; and isn't normalized by anti-TNF therapy, suggesting that TL1A may be of pathogenic and potentially of therapeutic importance in ankylosing spondylitis.

Keywords: Tumor Necrosis Factor–Like Ligand 1A (TL1A), Ankylosing Spondylitis (AS), Tumor Necrosis Factor-α (TNF-α), Bath Ankylosing Spondylitis Metrology Index (BASMI), Bath Ankylosing Spondylitis Disease Activity Index (BASDAI)

Introduction

Ankylosing spondylitis (AS) is a chronic, progressive, inflammatory rheumatic disease that primarily affects the sacroiliac joints (Sieper J et al., 2006). AS is primarily a disease of the axial skeleton, but some patients have peripheral joint involvement (Lee JH et al., 2002). AS also has some extra–articular manifestations (Vander Cruyssen B et al., 2007). The effects of environmental factors on genetically susceptible individuals are considered to be one possible mechanism for the pathogenesis of AS. It is known to be a highly genetic disease and there is a strong genetic association of MHC molecules with AS, especially human leukocyte antigen-B27 (HLA-B27) (M. F. Shamji et al., 2008).

Epidemiology

AS usually initially presents during the third decade of life, and rarely after the age of 45 years. The prevalence of AS is generally believed to be between 0.1% and 1.4% globally (Akkoc N, 2008). There is some gender disparity within AS, with reported gender ratios of around 2:1 (male: female), although this estimate has also been shown to vary considerably between studies and across time (Braun J and Sieper J, 2007) (Silman AJ and Hochberg MC, 2001).

Classification Criteria

According to modified New York criteria, a patient can be classified as having definite AS if at least one clinical criterion (Inflammatory back pain, limitation of lumbar spine or limitation of chest expansion) plus radiologic criterion (bilaterally grade 2 or unilateral grade 3-4 sacroiliitis) are fulfilled (van der Linden et. al, 1984).

Cytokines in AS

Activation of inflammatory cells leads to production of pro-inflammatory cytokines including TNF-α along with anti-inflammatory cytokines. However, an imbalance in favor of pro-inflammatory signals leads

to inflammation and subsequently to bone destruction and erosion (van der Heijde D et. al, 2008[1]). In contrast to rheumatoid arthritis, repair in AS is characterized by new bone formation in the form of syndesmophytes (in vertebrae) and enthesophytes (at entheses) (van der Heijde D et al., 2008[2]).

The Tumor Necrosis Factor Superfamily

The tumor necrosis factor superfamily (TNFSF) member proteins is a group of structurally related cytokines with a wide range of functions in the modulation of immunity, inflammation, differentiation, proliferation, and programmed death of many different types of cells (Baud V and Karin M, 2001) (Wajant H et al., 2003). TNFSF, composed of both ligands and receptors (TNFRSF), is one of the most successfully and frequently targeted protein families with clinical relevance to diverse diseases as cancer, autoimmunity, infections, graft rejection, inflammation and osteoporosis (Aggarwal BB et al., 2012) (Croft M et al., 2013). TNFSF and TNFRSF are critically involved in the pathogenesis of AS (Braun J et al., 1995).

While expressions of TNFSF ligands are induced largely on professional antigen-presenting cells (APCs; dendritic cells, B cells, macrophages), their expression is also reported on T cells, NK cells, mast cells, eosinophils, basophils, endothelial cells, thymic epithelial cells, and smooth muscle cells (Croft M et al., 2012).

Tumor Necrosis Factor–Like Ligand 1A (TL1A)

Tumor necrosis factor–like ligand 1A (TL1A), also called TNFSF15, or vascular endothelial growth factor inhibitor (VEGI) is a member of the TNFSF. It was identified during homology searches in an endothelial cell cDNA library. It is produced by the endothelial cells (T.-S. Migone et al., 2002), monocytes, dendritic cells (DC), T cells, NKT cells, synovial fibroblasts and chondrocytes, either upon stimulation or in situ in inflammatory sites (Bamias, G. et al., 2006) (Takedatsu, H. et al., 2008) (Zhang, J. et al., 2009) (Prehn, J. L. et al., 2004). Human TL1A consists of 251 amino acids: 35 in the cytoplasmic domain, 24 in the transmembrane region and 192 in the extracellular domain. There are two potential N-linked glycosylation sites in the TL1A amino acid sequence, specifically Asparagine residues at amino acids 133 and 229 (T.-S. Migone et al., 2002).

In contrast to the TNF-α receptors, which are expressed on essentially all cells, the receptor for TL1A, death receptor 3 (DR3), is primarily expressed on T cells, natural killer (NK) cells, and NK T (NKT) cells, thereby limiting the effects of TL1A (T.-S. Migone et al., 2002) (Meylan, F. et al., 2008). In recent years, the DR-3/TL1A axis has emerged as a key regulator of inflammation and autoimmunity in its own right, with in vivo studies of transgenic mice deficient for DR-3 or TL1A and those

overexpressing TL1A or dominant-negative forms of DR-3 providing compelling evidence for an essential role of the DR-3/ TL1A axis in many models of inflammatory and autoimmune disease (Bamias, G. et al., 2006) (Calder CJ and Wang EC, 2012).

Like other members of the TNFSF, TL1A enhances T cell proliferation and cytokine production of T cells activated in vitro. Proliferation is particularly enhanced in cells sub-optimally activated in the absence of CD28, and this effect is more pronounced in memory vs. naïve T cells, perhaps because of greater expression of the full-length isoform of DR3 in memory T cells (Bamias, G. et al., 2006) (Meylan, F. et al., 2008).

TL1A-associated pathways represent targets of potential therapeutic importance, as they are implicated in the pathogenesis of several inflammatory diseases, including inflammatory bowel disease (Bamias, G. et al., 2006), atherosclerosis (Kang YJ et al., 2005) and rheumatoid arthritis (Borysenko CW et al., 2005). Also, identification of a link between the DR3/TL1A pathway and the spondyloarthritides was first demonstrated when single-nucleotide polymorphisms (SNPs) located in the direct vicinity of the TNFSF15 gene were shown to be strongly associated with predisposition to spondylo-arthropathies including AS (E. Zinovieva et al., 2009).

Current Therapies and Future Novel Therapies

Non-steroidal anti-inflammatory drugs (NSAIDs), including selective cyclo-oxygenase 2 inhibitors, are the recommended first-line pharmacological therapy for AS, but many patients achieve insufficient symptom control with NSAIDs alone. Conventional disease-modifying antirheumatic drugs (DMARDs) are of limited use. Sulfasalazine and methotrexate are not effective for the treatment of patients with axial disease. (Braun J et al., 2010). TNF antagonists (etanercept, infliximab, golimumab and adalimumab) have demonstrated clinical efficacy in short-term and long-term clinical trials (Désirée van der Heijde et al., 2015). But, for some patients, the initial response to anti-TNF-α agents diminishes over time and they are switched to another anti-TNF agent (Haberhauer G et al., 2010). However, if TNF-blockade fails to control AS disease activity, no other treatment options are currently available (Rajalingham S and Das S., 2012).

Bath Ankylosing Spondylitis Metrology Index (BASMI)

The BASMI is included in the Assessment of SpondyloArthritis international Society (ASAS) core sets as the preferred measure of spinal mobility. It has been used in clinical trials of anti–tumor necrosis factor agents in AS patients (Brandt J et al., 2003) (Brandt J et al., 2000), and more recently was the outcome measure used to show that spinal mobility is

determined by both spinal inflammation and by structural damage (Machado P et al., 2010).

Bath Ankylosing Spondylitis Disease Activity Index (BASDAI)

BASDAI is a score used to measure patient-reported disease activity in patients with ankylosing spondylitis. The instrument was first published in 1994 using visual analog scales (Garrett S et al., 1994). The BASDAI has been endorsed by the Assessment of SpondyloArthritis international Society (ASAS) for the measurement of disease activity (Zochling J et al., 2008). The BASDAI has been the most frequently used measure of disease activity in clinical trials (Brandt J et al., 2000), and is recommended to assess response to anti–tumor necrosis factor therapies in AS patients (Braun J et al., 2003).

Patients and Methods

Forty five AS patients enrolled in this cross-sectional analytic study, which had been diagnosed according to the Modified New York criteria, which include the followings:

1. Radiological criterion:

- Sacroiliitis at least grade 2 bilaterally or grade 3 or 4 unilaterally.

2. Clinical criteria:

- Low back pain and stiffness for more than 3 months that improves with exercise but is not relieved by rest.
- Limitation of motion of the lumbar spine in both the sagittal and frontal planes.
- Limitation of chest expansion relative to normal values correlated for age and gender.

A definite diagnosis of ankylosing spondylitis requires the radiological criterion and at least one clinical criterion.

The AS patients were recruited from the department of rheumatology in Shaheed Dr. Hemin general teaching hospital and the center of rheumatology and rehabilitation in Sulaymaniyah city. Forty five apparently healthy control subjects were selected among paramedical personnel and apparently healthy blood donors (age and gender matched with the AS patients) at Sulaymaniyah central blood bank, after obtaining their informed consent.

Both AS patients and normal controls were recruited in the period from April 2015 to September 2015. The normal controls were interviewed; general information recorded, past medical and drug history taken, physical examination and laboratory investigations done (including complete blood count and viral screen).

A protocol was designed for all AS cases to record age, gender,

address, occupation, duration of illness, drug history, BASDAI, BASMI. Verbal informed consent was taken from all cases. A proper history and physical examination were undertaken and all clinical and laboratory parameters involved in BASMI and BASDAI were measured.

The blood samples were taken from both AS patients and healthy controls for the estimation of the serum levels of TL1A. Then, the levels of the cytokine were compared to BASDAI and BASMI scores of each patient.

Disease activity of AS patients was assessed through Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), and spinal mobility was measured through Bath Ankylosing Spondylitis Metrology Index (BASMI).

The Human TL1A ELISA kit was a quantitative assay kit for detection of human TL1A in serum, plasma and biologic fluids using a (Sandwich Immunoassay Technique). The TL1A ELISA kit was from (MyBioSource). The detection range of the cytokine in the serum is (78-2500 pg/ml), and it's used for research purposes only (not for diagnostic purposes).

Statistical Analysis

All patients' data entered using computerized statistical software; Statistical Package for Social Sciences (SPSS) version 17 was used. Descriptive statistics presented as (mean ± standard deviation) and frequencies as percentages. **Kolmogorov Smirnov analysis verified the normality of the data set.** Multiple contingency tables conducted and appropriate statistical tests performed, t-test was used to compare between two means. One way ANOVA analysis was used to compare between more than two means. Pearson Correlation test was used to assess relationship between continuous variables. In all statistical analysis, level of significance (p value) set at ≤ 0.05 and the result presented as tables and/or graphs.

Results

Demographic Data

A total of 45 ankylosing spondylitis (AS) patients were included in this study with mean age of 33.6±3.8 years. Male AS patients were more than females with male to female ratio as 2.7:1.

Mean AS duration of studied patients was 7.6±7.3 years, 44.4% of them had AS duration more than 5 years.

Mean age of controls was 33.6±8.8 years, Males were more than females.

No significant differences were observed between AS patients and controls regarding gender (p=0.3), and age (p=0.07).

Table 1: Demographic Characteristics of AS Patients and Controls

Variable	AS		Control		χ^2	P
	No.	%	No.	%		
Age					8.4*	0.07
< 20 years	3	6.7	1	2.2		
20-29 years	13	28.9	11	24.4		
30-39 years	17	37.8	29	64.4		
40-49 years	10	22.2	3	6.7		
≥ 50 years	2	4.4	1	2.2		
Gender					0.8	0.3
Male	33	73.3	29	64.4		
Female	12	26.7	16	35.6		

Clinical Data

Enthesitis was present among 57.8% of AS patients and peripheral arthritis was present among 42.2% of them.

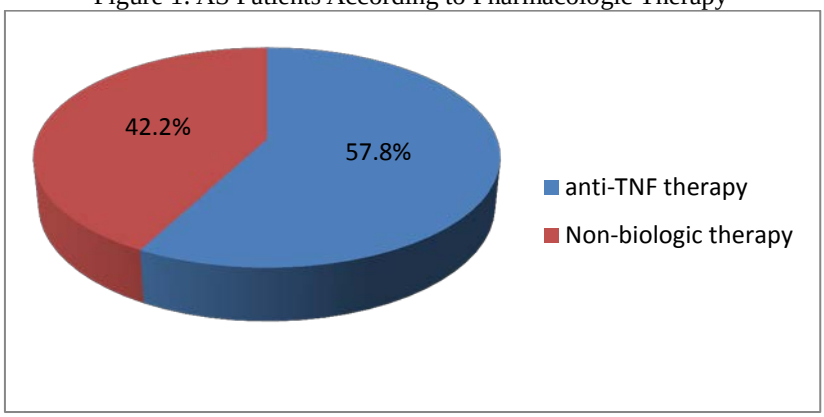
Table 2: Clinical Findings of AS Patients

Variable	No.	%
Enthesitis		
Present	26	57.8
Absent	19	42.2
Total	45	100.0
Peripheral arthritis		
Present	19	42.2
Absent	26	57.8
Total	45	100.0

AS Patients According to Treatment Methods

More than half (57.8%) of AS patients were on anti-TNF therapy and 42.2% of them were on non-biologic therapy.

Figure 1: AS Patients According to Pharmacologic Therapy



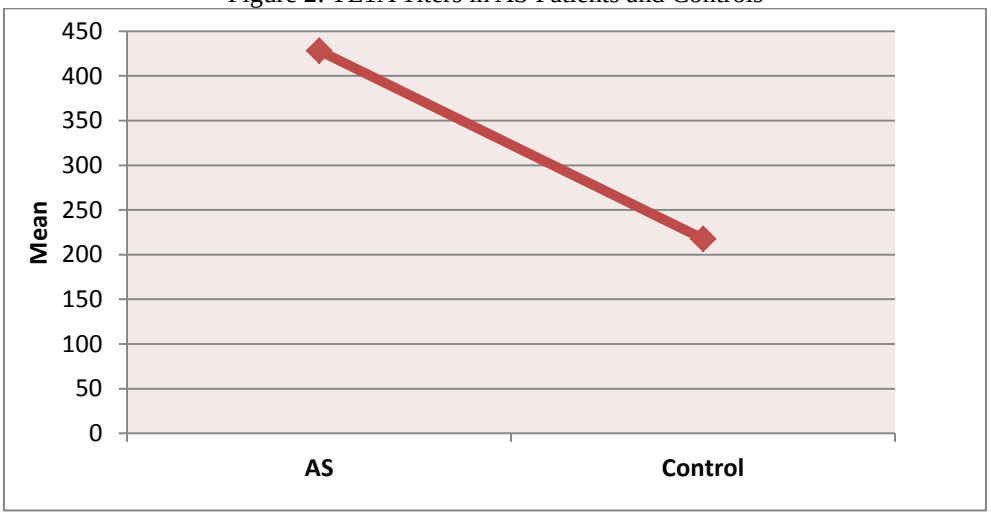
Laboratory Findings

A significant association was observed between TL1A titer and AS ($p<0.001$).

Table 3: TL1A Titers in AS Patients and Controls

Variable	AS Mean±SD	Control Mean±SD	t-test	P
TL1A titer	428.4±285.7	217.6±64	4.8	**<0.001**

Figure 2: TL1A Titers in AS Patients and Controls



There were highly significant differences between serum TL1A levels of non-biologic treated AS cases and controls ($p< 0.001$).

There were highly significant differences between serum TL1A levels of anti-TNF treated AS cases and controls ($p< 0.001$).

There were highly significant differences between serum TL1A levels of anti-TNF treated AS cases and AS cases on non-biologic therapy ($p< 0.002$).

Table 4: TL1A Titers in the Three Groups

Study Groups	TL1A Mean±SD
AS cases on anti-TNF therapy	320.1±181.2
AS cases on non-biologic therapy	576.6±337.4
Controls	217.6±64
ANOVA (P value)	**<0.001**

Figure 3: TL1A Titers in the Three Groups

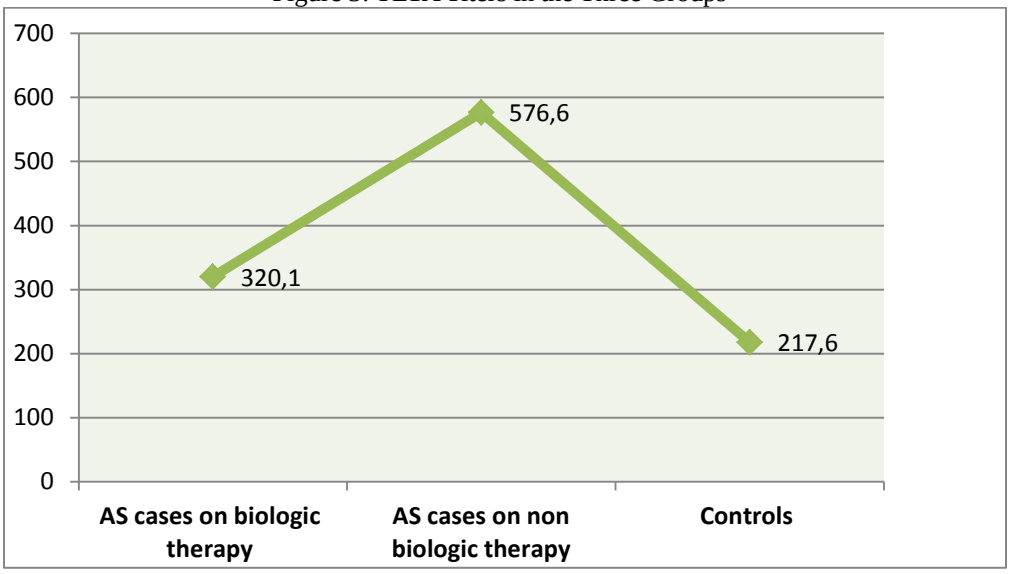
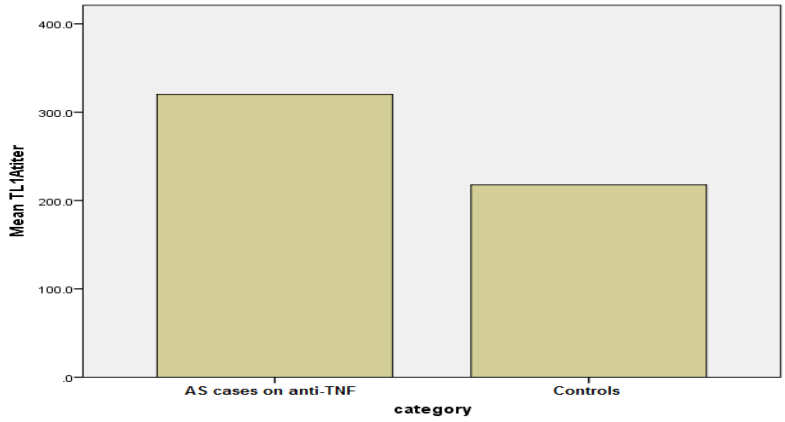


Figure 4: TL1A titers of AS Patients on anti-TNF and Controls



BASDAI and BASMI Scores of the Patients

Mean BASDAI scores of AS patients were 3.8±1.3, 44.4% of AS patients were active according to BASDAI scores. Mean BASMI scores of AS patients were 3.7±1.9.

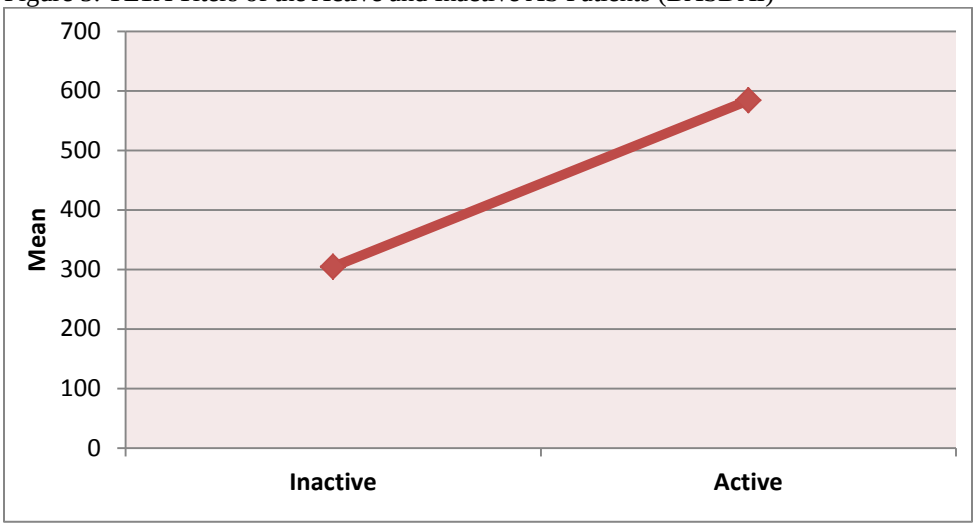
A significant association was observed between active AS patients (BASDAI score≥4) and TL1A titer (p=0.001).

There was no significant association between the BASMI scores of the AS patients and their TL1A titers (p=0.1).

Table 5: TL1A Titers of the Active and Inactive AS Patients (BASDAI)

Variable	Inactive AS Mean±SD	Active AS Mean±SD	t-test	P
TL1A titer	304.2±182.5	583.7±318.3	3.7	**0.001**

Figure 5: TL1A Titers of the Active and Inactive AS Patients (BASDAI)



Discussion

Ankylosing spondylitis (AS), a prototype of seronegative spondyloarthropathies, is a chronic inflammatory disease mainly affecting the axial skeleton. In addition to an HLA-B27 association (Marcilla M and López de Castro JA, 2008) (López de Castro JA, 2007), the pathogenesis of AS may involve a preceding infection (Kim TH, 2005) and an abnormal cytokine expression (Cañete JD, 2000). A number of studies that examined cytokine levels in AS have shown varied results. There seemed to have a general trend toward raised serum pro-inflammatory cytokine levels. However, serum anti-inflammatory cytokines such as IL-10 were also demonstrated to be increased in AS patients. Besides, both CD4+ and CD8+ T cells are considered to participate in the inflammatory processes of AS (Cañete JD, 2000) (Schirmer M, 2002). Among pro-inflammatory cytokines in AS, TNFSF and TNFRSF members are critically involved (Braun J et al., 1995).

Thirty three (73.3%) of our patients were males while twelve (26.7%) were females, with a female to male ratio of (2.7:1), which was close to the ratio of (Lee W et al., 2007) and (Boonen A et al., 2003); who reported male to female ratio of (2-3 : 1) among AS patients.

The mean of the ages of AS patients was (33.6±3.8) years, which was close to that of (Feldtkeller E et al., 2003), who found in a survey of 1080 AS patients that the age at diagnosis of HLA-B27 positive and negative patients was 39.1 and 33.2 years, respectively.

Peripheral arthritis was present in 42.2% of AS patients. This result was close to what was mentioned by (Tae-Jong Kim et al., 2014); who found that peripheral arthritis occurs in 30-40% of AS patients across the course of

their disease, but lower than what (Ji-Hyun Lee et al., 2002) concluded, who found that among 257 cases of adult AS, 61% of them had peripheral arthritis.

Among our cases, 26 of them (57.8 %) had enthesitis. This result was near to what was obtained by (Spadaro A et al., 2011); who found enthesitis through clinical examination in 63.3% of 36 AS cases, although he found a higher number of enthesitis when he examined the cases by ultrasonography.

A significant association was observed between TL1A titer and AS ($p<0.001$). This association remained significant even in patients treated with biological anti-TNF therapy.

There was a significant association between AS cases on non-biologic therapy and TL1A titer ($p<0.001$). This result was close to that of (Maria Konsta et al., 2013); who referred also to highly significant difference ($P = 0.042$).

Although the serum level of TL1A was affected by anti-TNF therapy (576.6±337.4 in non-biologic treated vs. 320.1±181.2 in anti-TNF treated) there was a significant difference between serum TL1A levels of anti-TNF treated AS cases and controls ($p< 0.001$).

The level of serum TL1A remained significantly different between AS cases treated with non-biologic therapy and those treated with biologic anti-TNF therapy (p value 0.002).

Convincing evidence from several short term randomized controlled trials indicates that treatment of AS with anti-TNF agents is safe and efficacious (Braun J et al., 2002) (Gorman JD et al., 2002) (Van Den Bosch F et al., 2002). However, as (Baeten D et al., 2001) and (Zou JX et al., 2003) noted, a subset of patients fails to respond or does not sustain initial responses. As for our study, 11 of our anti-TNF treated cases (42.3%) have active disease according to BASDAI scores.

Inefficacy of anti-TNF therapy in some cases was observed by many researchers; like (Suzanne Arends et al., 2011), which followed up 220 AS cases on anti-TNF therapy and assessed their response to the therapy. At three and six months, 68% and 63% of patients were Assessments in Ankylosing Spondylitis (ASAS)20 responders, 49% and 46% ASAS40 responders, and 49% and 50% Bath Ankylosing Spondylitis Disease Activity Index (BASDAI)50 responders, respectively.

In one study; (Braun J and Sieper J, 2007) found that 30–40% of patients experience substantial disease activity despite anti-tumour necrosis factor therapy.

Other researchers; like (Kavanaugh A et al., 2006) concluded that although anti-TNF-α have a dramatic effect on the treatment of spondyloarthritis, almost 30% of the patients will have to discontinue their treatment prematurely due to loss of efficacy or side effects. These

observations drew the attention that there may be other inflammatory pathways different from TNF-α pathway, included in the pathogenesis of AS, and this is evident from cases having active disease despite anti-TNF therapy.

In another study; (S Jin et al., 2013) investigated the mechanism of TL1A induced inflammation in human CD4+ T cells and compared the effects to the TNF-α induced pathway. They concluded that both soluble and membrane TL1A directly induces pro-inflammatory cytokines from CD4+ CD161+ T cells, while these cells are resistant to TNF-α. More importantly, they discovered that TL1A induces TNF-α expression from CD161+ T cells, suggesting that TL1A is upstream of TNF-α. Soluble and membrane TL1A-induced cytokine expression was inhibited by a novel neutralizing anti-TL1A antibody, but not by an anti-TNF-α antibody, indicating that TL1A directly affects CD161+ T cells. Notably (Tae-Hwan Kim et al., 2005) and (Paul Bowness et al., 2011) concluded that, CD4+ T cells are involved in the pathogenesis of AS, and as (S Jin et al., 2013) concluded; CD161 (also known as KLRB1) is expressed on approximately 25% of their blood-resident CD4+ T cells. Others like (T.-S. Migone et al., 2002) also found that while IL-2, IFN-γ, and CD25 were all predicted as responsive to TL1A, they were surprised to see that TL1A induced TNF-α from CD4+ T cells.

Finding a biomarker for diagnosis of AS is eagerly needed, as in the context of all the inflammatory rheumatic diseases, there is an unacceptably long delay between the onset of symptoms and the time of diagnosis for AS —an average interval of 8–11 years has been reported (Feldtkeller E et al., 2003). Early diagnosis and treatment of AS is important because it can reduce the disease disability (McLeod C et al., 2007).

In this study; there was a significant association between active AS patients (BASDAI score≥4) and TL1A titer (p=0.001), which is comparable to the results of (Maria Konsta et al., 2013); who observed a positive correlation between individual levels of TL1A and BASDAI (P = 0.008).

Having a biomarker to show AS disease activity is crucial, because, in contrast to rheumatoid arthritis, where ESR and CRP correlate well with disease activity, laboratory biomarkers that correlate with disease activity in AS has proved to be elusive. Studies assessing the role of ESR and CRP in AS, suggested that they correlated poorly with disease activity (Pathan, Ejaz Mohammed Ishaq, 2013).

No significant association were observed between AS patients' BASMI scores and TL1A titers (p=0.1)**,** this was close to the results concluded by (Maria Konsta et al., 2013) who found that there were no significant association between TL1A level and AS Metrology parameters namely (Schober test, Occiput to wall distance, Cervical rotation, Cervical flexion and Lateral spinal flexion) with P values (0.87, 0.57, 0.7, 0.9 and 0.9)

respectively.

Conclusions

Serum TL1A is up-regulated in ankylosing spondylitis, associates with disease activity which could be used as a useful tool in the follow up of AS patients, and isn't normalized by anti-TNF therapy; suggesting that TL1A may be of pathogenic and potentially of therapeutic importance in ankylosing spondylitis in the future (new novel therapies), apart from TNF-blockade.

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