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# The effect of biological anti-TNF-α therapy, originator and biosimilar, used to treat ankylosing spondylitis was studied based on clinical and blood counts and an inflammatory marker

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## ABSTRACT

Ankylosing spondylitis (AS) is a common inflammatory rheumatic disease that affects the axial skeleton. It causes inflammatory back pain, structural and functional problems, and a lower quality of life. If an AS patient does not respond to NSAIDs, the FDA has approved several anti-TNF therapy originators and biosimilars. This study aims to determine how well anti-TNF treats ankylosing spondylitis using a traditional complete blood count, clinical signs, and inflammatory markers. Blood samples were collected from 81 AS patients from two groups: 67 AS patients treated with anti-TNF therapy and 14 newly diagnosed patients who were not given treatment (naïve); in addition, 65 healthy individuals were enrolled in the study as a control group. The complete blood count showed a significant difference between the three groups in lymphocyte, neutrophil, and platelet counts (P = 0.001). Positive controls (14/14) treated with NSAIDs had higher disease activity than established patients (32/67) treated with an anti-TNF drug (P = 0.0001). There is a higher significant difference in inflammatory markers between patients with AS and positive controls than in healthy individuals (p = 0.001). In conclusion, AS patients had decreased activity with anti-TNF therapy but progressed on radiographic examination.

**Keywords:** *ankylosing spondylitis, anti-TNF therapy, clinical signe, blood counts . inflammatory marker*

## 1. INTRODUCTION

Ankylosing spondylitis (AS) is a common inflammatory rheumatic disease that affects the axial skeleton. It causes inflammatory back pain, structural and functional problems, and a lower quality of life. How AS is treated has dramatically changed since tumour necrosis factor alpha (TNF-α) blockade was developed. Before these drugs came out, the only options for treatment were non-steroidal anti-inflammatory drugs (NSAIDs). For decades, NSAIDs [1] and a good response to nonsteroidal anti-inflammatory drugs (NSAIDs) have been a sign of AS.

For decades, NSAIDs [1] and structured exercise programs [2] have been the standard way for people with ankylosing spondylitis to treat their spinal symptoms. Physical therapy and regular exercise are likely to affect the outcome of the condition, depending on how helpful they are at an early stage (for example, when the disease is very active) and how much they help. Drugs may indicate a worse prognosis. Some researchers have even said that taking NSAIDs regularly, instead of only when needed, might slow the progression of radiography over two years [3]. Since the initial approval of originator's TNF inhibitors, the FDA has approved several biosimilars, which are not generic products but closely resemble the originals. These have only slight differences in the clinically inactive ingredients' molecular structures, which are about the same in purity, safety, and efficacy [4].

The FDA has approved biosimilars to infliximab, etanercept, and adalimumab in the last five years. CT-P13 (RemsimaTM), the biosimilar to infliximab (Remicade), is approved by the European Medicines Agency (EMA) for use in all indications for which reference infliximab is approved, including rheumatoid arthritis, ankylosing spondylitis, Crohn's disease, ulcerative colitis, psoriatic arthritis, and psoriasis. The Food and Drug Administration approved Amgen's ABP 501, also known as adalimumab-atto (Amjevita), as the first biosimilar to adalimumab in 2016.

A standard blood test called a complete blood count (CBC) evaluates the composition, proportion, characteristics, types, and numbers of red blood cells, white blood cells, and platelets in a blood sample [5]. White blood cells (WBCs) are essential to immune system and will increase during disease or infection. Neutrophils, produced by the bone marrow, are among the "first responders" to any disease or infection. Neutrophils make up roughly 50% of total WBCs. Simply multiplying your total WBCs by 50% will yield the absolute neutrophil count (ANC), which does not require isolating and counting each neutrophil individually. The reference range of values for an ANC test can vary based on a person's age and other factors. Most labs consider a routine ANC between 2,500 and 6,000 cells/mcL.

Acute-phase reactants are important mediators produced in the liver during inflammation. Interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α) are acute-phase protein regulators or producers, as are interferon-gamma (IFN-γ).

Both ESR and CRP are inflammatory biomarkers in the acute phase, but CRP is a more sensitive and accurate reflection of the acute phase of inflammation than ESR. The half-life of CRP is constant. The ESR will remain elevated for several days until excess fibrinogen is removed from the serum. This study aims to find out how well anti-TNF therapy works to treat ankylosing spondylitis by using a traditional complete blood count, clinical signs, and inflammatory markers.

## 2. PATIENTS AND METHODS

### *2.1 Study Population*

A prospective case-control study was conducted at Basrah Teaching Hospital. The study reviewed 81 AS patients from two groups: 67 AS patients treated with anti-TNF therapy and 14 newly diagnosed patients who were not given treatment (naïve). All patients were assessed according to the Assessment of SpondyloArthritis International Society (ASAS) criteria for a pivotal treatment center included in the rheumatology and biological therapy clinics at Basrah Teaching Hospital. Patients received anti-TNF therapy (Infliximab, Etanercept, Remsima, and Amjevita) within more than three months from the time of sample collection. In addition, 65 healthy individuals were enrolled in the study as a control group. Disease activity was assessed using the AS Bath Disease Activity Index (BASDAI) [6], where patients' BASDAI scores were recorded during the clinical materials sampling.

### *2.2 Sampling*

Blood samples were collected and placed in three different tubes. Three millilitres of blood were drawn into an EDTA tube to perform a CBC assay. The blood was mixed gently using an electric roller mixer and then processed. The

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results were collected by printing them from the fully automated 5-part hematological analyzer machine. To determine the CRP titer, another 3 ml of blood was placed into a gel tube and left at room temperature for 10 to 15 minutes to clot. Then it was centrifuged at 4,000 xg for 5 min. Serum was collected from the tubes and transferred to a COBAS Integra 4000 Plus machine, and the results were then documented. To conduct an ESR test, 2 ml of blood was drawn into a unique sodium citrate tube. The sample was mixed gently, and the line was placed in an ESR analyzer (the ESRA-20 Bioevopack machine). The results were then identified and documented.

### *2.3 Statistical analysis*

All analyses were performed using SPSS software v21.0 (IBM, Chicago, USA). It illustrates the basic features of invoking SPSS Statistics from an external Java application. The Shapiro–Wilk test was used to determine the normality of the variables. The differences between parameters that are not normally distributed were analyzed with the KruskalWallis test alternative to the one-way ANOVA test and Friedman's test alternative to the two-way NOVA test and were defined as mean rank. A categorical variable was compared using chi-square and Fisher exact test analysis.

## 3. RESULTS

The ages of all participants ranged from 20 to 63, and there were no obvious differences between AS patients, positive and, healthy control groups. In the three groups, the male- to- female ratio was nearly similar, AS patients, positive control, and healthy control (60/7, 12/2, and 58/8, respectively). Although there were more males than females, there was no distinction between the three groups (P=0.807). There was no clear difference between the groups regardless of whether the place of residence was central or peripheral (P= 0.126).

There was no discernible difference in the duration of disease onset of established and biologically naïve patients with AS (P= 0.95). Positive controls (14/14) treated with NSAID had higher disease activity than established patients (32/67) treated with an anti-TNF drug (P=0001). Radiographic changes in the sacroiliac joint showed more progression and destruction in patients with established AS, with 22/67 (32.8%) having grade 4 joint damage compared to 2/12 (14.4%) in naive patients, a significant difference (P= 0.001). There were no significant differences in hip involvement between the two groups (P= 0.744). Uveitis was more common in naïve patients, but osteoporosis and cardiopulmonary disease were more common in establishing patients (P= 0.001). In comparison to NSAIDs in biologically naïve AS patients, all established patients who received antiTNF therapy were most taken etanercept drug (P=0.001).Table 1

**TABLE 1:** Comparison of clinical features and treatment effects between patients with AS and positive controls (biologically naïve AS patients)

| Characteristic | Established AS Patients<br>No. (%)<br>Mean ±SD | Naïve patients<br>No. (%)<br>Mean ±SD | P value |
|---|---|---|---|
| Duration of disease onset (years) | 10.57±6.74 | 10.43±10.6 | 0.95 |
| BASDAI | 4.485±0.269 | 5.986±0.374 | |
| Active | 32(47.8) | 14(100) | 0.001 |
| Inactive | 35(52.2) | | |
| Sacroiliac joint grading | | | |
| Grade1 | 8(11.9) | 2(14.3) | 0.001 |
| Grade2 | 17(25.4) | 4(28.6) | |
| Grade3 | 20(29.9) | 6(42.9) | |
| Grade4 | 22(32.8) | 2(14.3) | |
| Hip arthritis | 12(17.9) | 2(14.3) | 0.744 |
| Extra-spinal manifestation | | | |

| | | | |
|---|---|---|---|
| Ocular | 5(7.5) | 2(14.3) | 0.001 |
| Cardiopulmonary | 1(1.5) | 0(0) | |
| Osteoporosis | 6(9 | 0(0) | |
| Type of treatment | | | |
| NSAID | 0(0) | 14(100) | 0.001 |
| Infliximab | 13(19.4) | | |
| Remsima | 4(6) | | |
| Etanercept | 44(65.7) | | |
| Amjevita | 6(9) | | |

There is a higher significant difference in inflammatory markers between patients with AS and positive controls than in healthy individuals (p= 0.001). The complete blood count showed a significant difference between the three groups in lymphocyte, neutrophil, and platelet counts (P= 0.001).Table 2

**TABLE 2:** Inflammatory markers and complete blood count comparisons between groups of AS patients, positive controls, and healthy controls.

| Inflammatory markers and CBC test | Patient with AS | Positive controls | Healthy controls | P. value |
|---|---|---|---|---|
| CRP (mg/L) | 10±3.4 | 19±4.6 | 1.7±09 | 0.001 |
| ESR (mm/hr) | 30±4.3 | 38±.21 | 3.56±2.5 | 0.001 |
| Leukocyte (x10/mm3 ) | 7.24±1.32 | 8.322±1.60 | 7.83±1.08 | 0.737 |
| Lymphocyte (x10/mm3 ) | 2.92±1.02 | 3.51±09 | 2.31±0.65 | 0.001 |
| Neutrophil (x10/mm3) | 6.72±2.32 | 6.53±2.32 | 3.81±1.52 | 0.001 |
| Monocyte(x10/mm3) | 0.62±0.1 | 0.56±0.2 | 0.336±0.1 | 0.129 |
| Platelet (x10/mm3) | 376.68±95.1 | 397.78±105 | 254.8±45.9 | 0.001 |

The significant level was $P \leq 0.05$

## 4. DISCUSSION

Clinical features and treatment options for patients with established and biologically naïve AS In the present study, there was no significant difference in disease onset duration between established AS patients and biologically naïve AS patients (P = 0.95); the disease onset duration means of established AS were 10.57±6.74 with a 95% confidence interval of 12.47-8.96, while the disease onset duration means of biologically naïve AS were 10.43±10.6 with a 95% confidence interval of 20.63-0.23. This could be because the same disease was treated with different medicines at different times. This study showed a significant decrease in disease activity in established AS patients treated with anti-TNF therapy compared to biologically naïve AS patients treated with NSAIDs. The percentage of disease activity in established AS patients (32/67; 47.8%) was lower than in biologically naïve AS patients (14/14; 100%; P = 0.001). These results are inconsistent with a study of 59 patients with AS. The study reviewed 11 patients who were refractory to conventional therapy and treated with etanercept and showed the mean BASDAI decreased from 7.1±1.6 before treatment to 4.2± 1.8 at three months after the etanercept treatment (P = 0.001) [7]. This could be due to the effects of anti-TNF therapy on TNF-α cytokine inhibition, which reduces BASDAI domine, fatigue, axial skeleton symptoms, peripheral arthritis symptoms, enthesitis symptoms, and morning stiffness. In the current study, even though anti-TNF therapy was given to patients with established AS, conventional radiograms showed progression to grade 4 sacroiliitis in 22/67 (32.8%) of them. These findings agree with a study on AS patients treated with anti-TNF therapy. There was a significant improvement in clinical disease activity scores and inflammatory markers 12 weeks after anti-TNF initiation, but there was an increase in the frequency of

circulating IL-17A-producing cells. This was also observed 12 weeks after anti-TNF initiation in AS patients by D. N. Hull et al. [8]. This might be a ubiquitous characteristic of inflammatory suppression by TNF inhibition instead of being a disease-specific phenomenon. One reason for disease progression in AS patients treated with anti-TNF therapy is this drug effect.

Inflammatory markers and complete blood count in the AS, biologically naïve, and healthy control groups In our study, more significant differences in inflammatory markers were found in both ESR and CRP in established and biologically naïve AS patients, more toward biologically naïve AS patients with a CRP mean of 19±4.6. An ESR mean of 38±2.1 than for established AS patients with a CRP mean of 10±3.4 and an ESR mean of 30±4.3. There were also significant differences in blood cell counts between established and biologically naïve AS patients and healthy individuals, with lymphocyte, neutrophil, and platelet counts increasing (P = 0.001) but no difference in leukocyte and monocyte counts (P = 0.737 and P = 0.129, respectively). This increase in cell count may cause more disease activity in biologically naïve AS than in established AS, according to a study of 30 patients with AS and 30 healthy donors. Neutrophils showed an increase in TNF-α, IL-6, ERAP1, and IL-10, whereas lymphocytes showed a rise in TNF-α and IL-2eIKK. The monocyte (MON) analysis demonstrated a reduction in the MON1 percentage and increased MON2 and MON3 rates. IL1 and IL6 levels went up in MON1, while IL-10, IL-23, MCP1, MIP1a, ERAP1, and TFeIKK levels went up in MON3 [9].

## CONCLUSION

The observation of significantly decreased inflammatory markers and lymphocyte, neutrophil, and platelet counts in AS patients treated with ant-TNF as a result of a decrease in the activity of the disease but patients with radiographically progressive disease due to other pathways.

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### *Author contributions*

Husham A. Aldaoseri, Naael Hussein Ali, and Firas Al-Mubarak collected the data. Naael Hussein Ali and Firas Al-Mubarak reagents and quality control were provided. Aldaoseri performed the experiments and analyzed the data. All of the authors contributed to the article and approved the final version. All authors read and approved the final manuscript.

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### *Availability of data and materials*

All the data generated or analyzed during the current study are available from the corresponding author. If a reasonable request is made, the corresponding author will respond.

### *Declarations*

### *Ethical agreement*

The Ministry of Higher Education and Scientific Research; the University of Basrah College of Medicine, 030403/034/2022; and the Basrah Health Department at the Ministry of Health looked over and ethically approved the study protocol. Before the data were collected, each participant signed a permission form. This was done after the study's purpose was explained, and they were ensured that the data would be safe. Relevant guidelines and regulations have been followed for all methods.

### *Consent for publication*

Not applicable

### *Competing interests*

All authors have no relevant financial or nonfinancial interests to disclose.

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