

IMPACT OF TH17/TREGFOXP3 CELLS RATIO ON RHEUMATOID ARTHRITIS PATIENTS RECEIVING ANTI TNF- THERAPY-.

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(Received: September 25, 2021; Accepted for Publication: October 24, 2021)

ABSTRACT

In order to determine the correlation between disease activity of RA with the Th17/TregFoxp3 cells ratio in patients undertherapy with Anti-TNF-alpha which is the aim of present study, Ninety-seven patients with RA and healthy control groups were included in this case control study, eighty-four patients divided into four groups; 25 patients receiving only MTX; 18 patients receiving monotherapy (anti- TNF); 26 patients receiving combined therapy (MTX + anti-TNF); 15 patients with RA not receiving any drug (positive control). Other thirteen healthy people considered as negative control were enrolled in this study. Patients with RA were attending Basrah General Hospital, Rheumatology Unite, Biological Therapy Center for receiving anti-TNF therapy. Flowcytometry was used for measuring TregFoxp3 and Th17 markers and DAS-28 score was used for measuring RA disease activity. Anti TNF inhibitors (Infliximab and Etanercept) were measured as well. Other inflammatory and hematological parameters were also measured (ESR, total WBC, Lymphocytes, monocytes and neutrophil counts). Resulted, DAS-28 as disease activity score was significantly correlated with Th17/ TregFoxp3 ratio and with the TH17 cells count. Statistically, Th17/ TregFoxp3 ration was not correlated with BMI, morning stiffness, and duration of the disease. In a Conclusion, Th17/Treg Foxp3 ratio correlated significantly with DAS-28 as RA disease activity. The lower Tregs Foxp3 frequency, the higher the DAS score reflecting higher disease activity. In combined therapy group, disease activity was found lower than other patient groups indicating for the effect of this combination between MTX and anti-TNF. Herein, we demonstrated that the main advantage of this combined therapy in RA patients was the reversion of Th17 cell expansion.

KEY WORDS: Anti-TNF; immunotherapy; Infliximab; Etanercept; Rheumatoid Arthritis; Th17/Treg Foxp3 ratio.

INTRODUCTION

Rheumatoid arthritis (RA) a multifactorial origin autoimmune disease, associated with chronic inflammation and articular destruction in the joints. The pathogenesis determined by induction and progression of abnormal regulatory T cell (Treg) response with a shift towards a Th17 cell response (Ohkura, Kitagawa and Sakaguchi, 2013). The Treg (CD4⁺CD25⁺) cells play a role in maintain homeostasis by mediate immune tolerance and suppress autoreactive lymphocytes. This homeostasis has been found to be altered in active RA (Flores-Borja *et al.*, 2008; Chen *et al.*, 2011). Th17 cells another player in the pathogenesis of RA, they secret certain proinflammatory cytokines, like Interleukin-17A (IL-17A) (Lee, 2018). Both IL-17A and Tumor Necrosis Factor- α (TNF- α) act to induce chemokine and cytokine production from

synovial fibroblasts, resulting in destruction of cartilage (Aravena *et al.*, 2011). So, anti-TNF- α therapy has been found to improve the ability of Tregs in regulation and decreases Th17 cell populations (Ohkura, Kitagawa and Sakaguchi, 2013). This immunomodulatory therapy has faced a number of challenges. A proportion of patients (30-40 %) fail to respond to anti-TNF therapy, that make them to have adverse effects and loss of response as their disease progressively worsens (Lina *et al.*, 2011; S. *et al.*, 2012; Thomas *et al.*, 2015). The explanation papers for this irresponsiveness were scarce. Recently, neutralizing Anti-Drug Antibodies (ADA) interferes with the ability of TNF inhibitors (TNFi) to block TNF- α (Atiqi *et al.*, 2020). These blockers directed against idiotopes inside or outside the TNF-binding fragments of the anti-TNF- α (Bendtzen, 2015). Others resulted that, alteration in the Th17/Treg ratio in combination with an imbalance in the Th1/Th2

ratio are responsible for RA diseases activity and progression of RA (Chen *et al.*, 2012). Immunomodulatory drugs like Adalimumab and Etanercept have no effect on change in number or phenotype of peripheral blood Tregs in RA patients even in conjunction with methotrexate (MTX) (Blache *et al.*, 2011).

Forkhead box protein 3(FoxP3), a transcription factor responsible for the development of Tregs in the thymus, and it needed to maintain the suppressive ability of Tregs in the human peripheral blood. Decreased of FoxP3 expression might lead to conversion of Tregs from regulatory into effector cells (Th17) (Williams and Rudensky, 2007; Hori, Nomura and Sakaguchi, 2017). That might lead to Th17/Treg cells imbalance. Pro- or anti-inflammatory cytokines production is connected with this imbalance and is relevant for the development and/or progression of the disease (Aravena *et al.*, 2011). Both rely on (Transforming Growth Factor- β) TGF- β for their induction. But, when the level of Interleukin-6 (IL-6) is increased, Th17 response is favored (Zheng, Wang and Horwitz, 2008). Thus, FoxP3 expression is critical for the

identification of Tregs that carry suppressive activity. A matter which neglected by other similar study (Taha *et al.*, 2019). Th17/Treg ratio used as biomarker, predictive marker, and progression marker for many autoimmune diseases (Lee, 2018). Herein this study we hypothesized, this ratio correlated with the activity of RA disease especially among those receive anti-TNF therapy. The aim of the present study is to investigate the correlation between Th17/Treg FoxP3 cells with the RA disease activity in patients undertherapy with Anti-TNF- α .

Patients and Methods

A. Study population

A total of ninety-seven participants were included in this case control study, conducting from August/2019 to February/2020. Eighty-four of them were patients with RA attending Basrah General Hospital, Rheumatology Unite, Biological Therapy Center for receiving anti-TNF therapy. Negative control group includes thirteen healthy individuals. The number, age, and gender of participants according to group are shown in below Table-A.

Table (A): Characteristic feature of patients and control groups

Group No.	Character	N	Age range (years) (median)	Females No. (%)
G1	Methotrexate (MXT) only	25	(20- 79) (52)	18 (72.0)
G2	Monotherapy (anti-TNF)	18	(33- 69) (53)	11 (61.1)
G3	Combined therapy (antiTNF + MXT)	26	(25- 71) (50)	24 (92.3)
G4	Positive control (Naïve patients)	15	(20- 65) (48)	10 (66.7)
Total		84	(20 -79) (50)	71 (73.2)
G5	Negative control (Healthy)	13	(25- 67) (43)	8 (61.5)

Information about socio-demographic and clinical features were obtained prior to collect the blood sample from all participants. Information include: body weight, treatment information, clinical presentation and disease activity. The Clinical parameters was obtained according to 2010 EULAR/ ACR criteria (DAS 28). Patients receiving anti-TNF from at least six doses and their age more than 18 years were

included in the study. Patients with other autoimmune or chronic diseases, switch to other drugs, irregular therapy, overlap diseases, receiving corticosteroid drugs, or pregnant women were excluded.

B. Sampling and processing

Five ml of blood sample were collected from all participants for flowcytometry and other laboratory investigations.

Flow Cytometry work

BriCyteE6 (Mindray, China) flowcytometry was used for T cells phenotyping which equipped with dual-laser based optics. A red diode laser and a blue diode laser is used for exciting 4 to 6 colors of fluorescence lights. For Treg (CD4+CD25+Foxp3+) cells enumeration flowcytometry: After RBC lysis, ten ul of Mouse anti-human CD4 fluorescein conjugated FITC (Becton Dickinson, San Jose, CA, USA, BD) monoclonal antibodies (mAb) and Mouse anti-human CD25 (IL-2R α) phycoerythrin PE labelled (BD) mAbs were incubated for 45 min. Following surface staining, cells were fixed and permeabilized using BD Fixation-permeabilization Kit. The cells then were re-suspended in 250 ul by cytofix and incubated for 20 minutes at 4° C then washed. Ten ul of polyclonal anti-human/mouse/rat Foxp3-Allophycocyanin, APC conjugated (e Bioscience, USA), were incubated with the cells overnight in the dark. Unstained cells, as isotypic control, were used to exclude autofluorescence.

Th17 cells were quantified by recombinant human IgG1 to CD196 (CCR6) and CD194 (CCR4) ((Bio-Connect Diagnostics B.V. The Netherlands). According to product instructions, the recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry was 1:50 for up to 106 cells/100 μ L. Up to 106 was re-suspended nucleated cells per 98 μ L of buffer. Then 2 μ L was added of the antibody. Mixture was mixed well and incubated for 10 minutes in the dark in the refrigerator (2–8 °C). Attention made for avoiding the higher temperatures and/or longer incubation times to avoid non-specific cell labeling. cells were washed by adding 1–2 mL of buffer and centrifuged at 300 \times g for 10 minutes. Then the supernatant was aspirated completely. Re-suspended cell pellet in a suitable amount of buffer and analyzed by flowcytometry. Treg FoxP3/Th17 ratio was calculated according to the output resulted from flowcytometry. Enzyme immunoassay for the qualitative determination of antibodies in serum to Etanercept (Enbrel[®]) and Infliximab (Remicade[®]):

Anti TNF- α inhibitors antibodies (ADA) (Infliximab and Etanercept) were measured as well for each participants by using ELISA method by using Matriks Biotek[®] kits. According to manufactures instructions, the ADA were measured for only three groups of the

study (monotherapy, combined therapy and control positive groups).

Other blood parameters: total white blood cells; Total lymphocytes; neutrophil; monocytes (10^9 cell/L); and ESR were done for each participants.

Statistical analysis

Data was entered and analyzed by using the statistical package for social sciences (SPSS) software for windows, version 26. The data in the present study was nonparametric. Therefore, the statistical methods used in accordance with this type of data. Descriptive statistics were presented as median for quantitative data, frequencies and percentages (%) according to the types of the variables. Chi-square and Fishers Exact Test were used to compare the proportion and frequencies, Kruskal Wallis Test used to compare the median of different study groups, Level of significance (P value) of ≤ 0.05 considered significant. Spearman Test was used for correlation between variables in the study.

Results

Table-1 shows sociodemographic features (Age, Sex, residency, and smoking tobacco) with no significant differences between patients and control groups.

The clinical features including BMI, age onset of the diseases, duration of the disease, morning stiffness (minutes), family history, history of primary and secondary failure, and DAS-28 among patients and controls groups. Resulted that only DAS-28 and family history were significantly difference between groups with prominence to patients receiving only MTX. Table-3 shows the flowcytometry outcome for all five groups. The markers Th17, Treg, Treg Foxp3, and Th17/Treg Foxp3 ratio were significantly difference ($P < 0.01$), while CD4 marker was insignificant between five groups. Laboratory investigations including WBC count, ESR, total lymphocyte count were significant difference between all five groups. Other investigation neutrophil and monocytes were insignificant difference (Table-3).

Correlations results between Th17/TregFoxP3 ratio with some clinical features were insignificant in patient's groups (Table-4). Table-5 shows the correlations between disease activity and flowcytometry outcomes including Th17/TregFoxP3 ratio. Significantly (at the level of 0.05) positive correlation between Th17 /Treg Foxp3+ T cells ratio and disease activity score in patient's groups by Spearman's correlation test ($r =$

0.231, $P= 0.034$) (Fig-1), also with Th17 ($r = 0.226$, $P= 0.039$) (Fig-2). TregFoxP3 was insignificantly correlated with disease activity in patient's groups.

Anti-TNFi (ADA) were found among those who received Infliximab more than Etanercept (table-6) with significant difference ($P<0.05$) between monotherapy, combined therapy and positive control groups.

Discussion

The ratio or balance between Th17 and Treg is critically important for pathogenesis, prognosis, and therapy of many autoimmune diseases including RA (Taha *et al.*, 2019). In animal models, re-establishing the T effector/Treg ratio can control the autoimmune responses. The published information about the correlation of this ratio as marker for immunotherapy and its correlation with disease activity were sparse. It is unclear whether the ratio of Th17/Treg in the peripheral blood of RA patients receiving anti-TNF is altered. In present study two CD markers (CD 194 and CD 196) were used for identification of Th17 by flowcytometry, revealed increased of Th17 significantly in RA patients with only methotrexate treatment than the other groups, while, Tregs (CD4⁺ 25⁺ Foxp3) were significantly increased in healthy group. In humans, Tregs constitute about 5-10% of the CD4⁺ T cell population in the peripheral blood of healthy individuals. These cells express high levels of CD25 (IL2Ra). They are also characterized by expression of the lineage-specific transcription factor -Foxp3, which is pivotal for Treg function and homeostasis (Hori, Nomura and Sakaguchi, 2017; Romano *et al.*, 2019). Foxp3 was neglected by other researchers (Blache *et al.*, 2011), so that might lead to controversy results in Treg function. In the present study, the TregFoxp3 counts approximately 50% of the total count of Treg in all patients and control groups. Therefore, we recommend to investigate Treg Foxp3 for more precision in describing their homeostasis and regulatory activity. That is come in agreement with Komatsu N, et al, 2014 (Komatsu *et al.*, 2014), resulted that exTreg cells were a subset of Treg which converted to Th17 in inflammatory environment in autoimmune arthritis. Therefore, the strengthen on future resolution and therapy of autoimmune diseases depending on stabilization of Treg Foxp3 function at same time dampen proinflammatory cytokines like IL-

17. So, it reasonable, in the present study, Th17/Treg Foxp3 ratio used as biomarker.

Important result in present study was Th17/Treg Foxp3 ratio correlated significantly with DAS-28 a matter which represent the lower Tregs Foxp3 frequency, the higher the DAS score, reflecting higher disease activity and vice versa. In combined therapy group, disease activity was found lower than other patient groups indicating for the effect of this combination between MTX and anti-TNF. Herein, we demonstrated that the main advantage of this combined therapy in RA patients was the reversion of Th17 cell expansion. Many authors resulted unchanged outcome (Shen *et al.*, 2010; Lina *et al.*, 2011) or a decreased Th17 frequency following treatment with MTX and/or TNFi (Chen *et al.*, 2012), and the latter findings are in accordance with the current study. Anti-TNF therapy with MTX was able to effectively limit the Th17 population only in the patients with early disease (S. *et al.*, 2012). However, many papers strengthen that the effectiveness of anti-TNF therapy concerning Th17 expansion should be evaluated not earlier than 12 weeks after biologics usage irrespective of the MTX administration (Chen *et al.*, 2011; Daekh, Mohammed and Ali, 2020).

Molecularly, the imbalance between Th17 and Treg cells is supported (Zhou *et al.*, 2008), resulted both Th17 and Treg cells require the same cytokine Transforming Growth Factor- β 1 (TGF-beta1) during the early stage of differentiation. Foxp3 downregulated when other transcription factor, essential for Th17 development, like ROR γ t overexpressed especially in the presence of IL-1b and IL-6. This last research might strengthen our results. In the other side, IL-2, which is required for the expansion and maintenance of FoxP3 expressing Treg cells, has been found to inhibit the development of Th17 cells (Zheng, Wang and Horwitz, 2008). Therefore, IL-2 and IL-6 have counteractive effects of on the development and differentiation of Th17 and Treg in the periphery which may hamper immunoregulatory responses and facilitate the persistence of autoimmune inflammation.

Concerning immunogenicity to TNF antagonists, in present study Etanercept has lower immunogenicity than Infliximab. That result may be due to the well-known infliximab is a purified, recombinant DNA-derived chimeric human-mouse IgG monoclonal antibody and contains murine heavy (H) and

light (L) chain variable regions, ligated to genomic human heavy and light chain constant regions, while Etanercept is a fusion-protein between a human IgG1 Fc-tail and the TNF-receptor type 2 (Levin, Wildenberg and van den Brink, 2016; Atiqi *et al.*, 2020; Bodio *et al.*, 2020). A fact which make infliximab more immunogenicity than Etanercept. Studies on rheumatology patients identified different genetic factors associated with reduced response to infliximab (Talotta *et al.*, 2015). However, patient-specific predictors of infliximab immunogenicity have not been identified so far. That gap has precluded a rational tailoring of individualized therapy, namely, prescribing combination MTX-infliximab therapy (combined therapy group) to those patients with high risk of infliximab immunogenicity.

In a conclusion, Th17/Treg Foxp3 ratio correlated significantly with DAS-28 as RA disease activity. The lower Tregs Foxp3 frequency, the higher the DAS score reflecting higher disease activity. In combined therapy group, disease activity was found lower than other patient groups indicating for the effect of this combination between MTX and anti-TNF. Herein, we demonstrated that the main advantage of this combined therapy in RA patients was the reversion of Th17 cell expansion.

prescribing combination MTX-infliximab therapy to those patients with high risk of infliximab immunogenicity, sparing them from developing antibodies to infliximab and its risks and side effects.

Acknowledgements

The authors would like to thank Dr Muhammad AlMussawi for his keen assistant in flowcytometry work, without which, this study would not have been possible.

Disclosure

The authors declared no conflicts of interest. No funding was received for this study.

REFERENCES

- Aravena, O. *et al.* (2011) 'Anti-TNF therapy in patients with rheumatoid arthritis decreases Th1 and Th17 cell populations and expands IFN- γ -producing NK cell and regulatory T cell subsets', *Immunobiology*, 216(12), pp. 1256–1263. doi: 10.1016/j.imbio.2011.07.006.
- Atiqi, S. *et al.* (2020) 'Immunogenicity of TNF-Inhibitors', *Frontiers in Immunology*, 11(February), pp. 1–13. doi:

10.3389/fimmu.2020.00312.

- Bendtsen, K. (2015) 'Immunogenicity of anti-TNF- α biotherapies: II. Clinical relevance of methods used for anti-drug antibody detection', *Frontiers in Immunology*, 6(APR), pp. 1–5. doi: 10.3389/fimmu.2015.00109.
- Blache, C. *et al.* (2011) 'Number and phenotype of rheumatoid arthritis patients' CD4+CD25hi regulatory T cells are not affected by adalimumab or etanercept', *Rheumatology*, 50(10), pp. 1814–1822. doi: 10.1093/rheumatology/ker183.
- Bodio, C. *et al.* (2020) 'Personalized medicine in rheumatoid arthritis: How immunogenicity impacts use of TNF inhibitors', *Autoimmunity Reviews*, 19(5), p. 102509. doi: 10.1016/j.autrev.2020.102509.
- Chen, D. Y. *et al.* (2011) 'Increasing levels of circulating Th17 cells and interleukin-17 in rheumatoid arthritis patients with an inadequate response to anti-TNF- α therapy', *Arthritis Research and Therapy*, 13(4), p. R126. doi: 10.1186/ar3431.
- Chen, J. *et al.* (2012) 'Comprehensive evaluation of different t-helper cell subsets differentiation and function in rheumatoid arthritis', *Journal of Biomedicine and Biotechnology*, 2012. doi: 10.1155/2012/535361.
- Daekh, N. A. K., Mohammed, K. A. and Ali, N. H. (2020) 'Polymorphism of HLA-B27 among Ankylosing Spondylitis Patients in Basrah, Iraq', *Scientific Journal of Medical Research*, 04(13), pp. 12–16. doi: 10.37623/sjmr.2020.41303.
- Flores-Borja, F. *et al.* (2008) 'Defects in CTLA-4 are associated with abnormal regulatory T cell function in rheumatoid arthritis', *Proceedings of the National Academy of Sciences of the United States of America*, 105(49), pp. 19396–19401. doi: 10.1073/pnas.0806855105.
- Hori, S., Nomura, T. and Sakaguchi, S. (2017) 'Control of regulatory T cell development by the transcription factor Foxp3', *Journal of Immunology*, 198(3), pp. 981–985. doi: 10.1126/science.1079490.
- Komatsu, N. *et al.* (2014) 'Pathogenic conversion of Foxp3 + T cells into TH17 cells in autoimmune arthritis', *Nature Medicine*, 20(1), pp. 62–68. doi: 10.1038/nm.3432.
- Lee, G. R. (2018) 'The balance of th17 versus

- treg cells in autoimmunity', *International Journal of Molecular Sciences*, 19(3), pp. 1–14. doi: 10.3390/ijms19030730.
- Levin, A. D., Wildenberg, M. E. and van den Brink, G. R. (2016) 'Mechanism of action of anti-TNF therapy in inflammatory bowel disease', *Journal of Crohn's and Colitis*, 10(8), pp. 989–997. doi: 10.1093/ecco-jcc/jjw053.
- Lina, C. *et al.* (2011) 'Combined treatment of etanercept and MTX reverses Th1/Th2, Th17/Treg imbalance in patients with rheumatoid arthritis', *Journal of Clinical Immunology*, 31(4), pp. 596–605. doi: 10.1007/s10875-011-9542-6.
- Ohkura, N., Kitagawa, Y. and Sakaguchi, S. (2013) 'Development and Maintenance of Regulatory T cells', *Immunity*, 38(3), pp. 414–423. doi: 10.1016/j.immuni.2013.03.002.
- Romano, M. *et al.* (2019) 'Past, present, and future of regulatory T cell therapy in transplantation and autoimmunity', *Frontiers in Immunology*, 10(JAN). doi: 10.3389/fimmu.2019.00043.
- S., A. *et al.* (2012) 'Incomplete response of inflammatory arthritis to TNF α blockade is associated with the Th17 pathway.', *Annals of the rheumatic diseases*, 71(10), pp. 1741–1748. Available at: [http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L366349584%0Ahttp://sfx.library.uu.nl/utrecht?sid=EMBASE&issn=14682060&id=doi:&atitle=Incomplete+response+of+inflammatory+arthritis+to+TNF \$\alpha\$ +blockade+is+associated+with+the+Th17+pa](http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L366349584%0Ahttp://sfx.library.uu.nl/utrecht?sid=EMBASE&issn=14682060&id=doi:&atitle=Incomplete+response+of+inflammatory+arthritis+to+TNF%alpha+blockade+is+associated+with+the+Th17+pa).
- Shen, H. *et al.* (2010) 'Infliximab educes the Frequency of Interleukin 17-Producing Cells and the Amounts of Interleukin 17 in Patients with heumatoid Arthritis', *Journal of Investigative Medicine*, 58(7), pp. 905–908. doi: 10.2310/JIM.0b013e3181eb9895.
- Taha, H. A. *et al.* (2019) 'Investigating the Balance between Th17/Treg Cells in Rheumatoid Arthritis and its Association with Disease Activity', *Journal of Child Science*, 9(1), pp. E75–E83. doi: 10.1055/s-0039-1693158.
- Talotta, R. *et al.* (2015) 'Paradoxical Expansion of Th1 and Th17 Lymphocytes in Rheumatoid Arthritis Following Infliximab Treatment: a Possible Explanation for a Lack of Clinical Response', *Journal of Clinical Immunology*, 35(6), pp. 550–557. doi: 10.1007/s10875-015-0182-0.
- Thomas, S. S. *et al.* (2015) 'Comparative Immunogenicity of TNF Inhibitors: Impact on Clinical Efficacy and Tolerability in the Management of Autoimmune Diseases. A Systematic Review and Meta-Analysis', *BioDrugs*, 29(4), pp. 241–258. doi: 10.1007/s40259-015-0134-5.
- Williams, L. M. and Rudensky, A. Y. (2007) 'Maintenance of the Foxp3-dependent developmental program in mature regulatory T cells requires continued expression of Foxp3', *Nature Immunology*, 8(3), pp. 277–284. doi: 10.1038/ni1437.
- Zheng, S. G., Wang, J. and Horwitz, D. A. (2008) 'Cutting Edge: Foxp3 + CD4 + CD25 + Regulatory T Cells Induced by IL-2 and TGF- β Are Resistant to Th17 Conversion by IL-6 ', *The Journal of Immunology*, 180(11), pp. 7112–7116. doi: 10.4049/jimmunol.180.11.7112.
- Zhou, L. *et al.* (2008) 'Provided Mice for Fate-Mapping Experiments', 453(7192), pp. 236–240.

Table (1): Sociodemographic features of Patients and controls.

Sociodemographic features		MTX n (25)	Monotherapy n (18)	Combined therapy n (26)	Positive control n (15)	Negative control n (13)	P. value
Age (years)	Median	52.00	53.50	50.00	48.00	53.00	0.360
	(min-max)	(20-79)	(33-69)	(25-71)	(20-65)	(25-67)	
Sex	Male n (%)	7 (28%)	7 (38.9%)	2 (7.3%)	5 (26.8%)	5 (38.5%)	0.073
	Female n (%)	18 (72%)	11 (61.1%)	24 (92.7%)	10 (73.2%)	8 (61.5%)	
Residency	Urban n (%)	4 (16%)	5 (27.8%)	8 (30.8%)	3 (20%)	4 (30.8%)	0.724
	Rural n (%)	21 (84%)	13 (66.7%)	18 (69.2%)	12 (80%)	9 (69.2%)	
Smoking	smoking n (%)	3 (12.0%)	3 (16.7%)	5 (19.2%)	3 (20.0%)	0 (0.0%)	0.502
	Nonsmoking n (%)	22 (88.0%)	15 (83.3%)	21 (80.8%)	12 (80.0%)	13 (100%)	

Table (2): clinical features of patients and controls groups

Features		MTX	Monotherapy	Combined therapy	Positive control	P. value
BMI	n	25	18	26	15	0.228
	Median (min-max)	25.7100 (16.30 - 40.00)	30.4800 (24.34-34.67)	27.6250 (2.50-44.92)	27.4100 (3.00-37.57)	
Age onset of the disease (years)	n	22	18	26	13	0.163
	Median (min-max)	44.00 (15-78)	37.00 (17-59)	30.00 (18-59)	44.00 (14-55)	
Duration of the disease diagnosis (years)	n	22	18	26	13	0.257
	Median (min-max)	1.00 (0.5-30)	0.50 (0.75-13)	2.00 (0.75-30)	2.50 (1-16)	
Morning stiffness (munities)	n	25	18	26	15	0.330
	Median (min-max)	30.00 (0-120)	15.00 (0-120)	22.50 (0-120)	20.00 (0-60)	
DAS-28	n	25	18	26	15	*0.044
	Median (min-max)	4.86 (1-6)	3.61 (2-6)	3.93 (2-7)	3.49 (2-6)	
Family history	Yes n (%)	4 (16%)	4 (22.2%)	11 (42.3%)	1 (6.7%)	*0.041
	No n (%)	21 (84%)	14 (77.8%)	15 (57.7%)	14 (93.3%)	
History of primary and secondary failure	Yes n (%)	0 (0%)	2 (11.1%)	1 (3.8%)	-	0.241
	No n (%)	25 (100%)	16 (88.9%)	25 (96.2%)	15 (100%)	

Table (3): Flowcytometry outcomes for patients and controls groups.

Marker		MTX N (25)	Monotherapy N (18)	Combined therapy N (26)	Positive control N (15)	Negative control N (13)	Sig. at level of
CD4	Median (min-max)	32.200 (15.4-66.7)	31.500 (6.3-60.7)	32.450 (7.4-62.2)	36.300 (18.0-61.8)	29.070 (22.0-41.0)	0.613
Th17	Median (min-max)	19.200 (4.3-32.1)	3.100 (0.7-6.7)	4.200 (1.7-14.0)	17.300 (7.1-28.6)	0.900 (0.3-4.5)	**0.01
Treg	Median (min-max)	1.5000 (0.54-4.20)	3.7000 (0.26-7.50)	2.3700 (0.69-3.90)	1.8000 (0.90-5.70)	11.6500 (2.10-19.23)	**0.01
Foxp3	Median (min-max)	0.7000 (0.03-2.70)	3.6000 (0.20-11.00)	1.8000 (0.06-9.30)	0.7000 (0.03-2.70)	9.2000 (1.92-15.27)	**0.01
Th17/ Foxp3	Median (min-max)	20.4300 (2.95-690.00)	0.7050 (0.18-13.50)	2.9100 (0.18-155.00)	28.5700 (7.93-830.00)	0.1100 (0.03-0.61)	**0.01

Table(4): Laboratory investigations for patients and controls groups.

Features		MTX N (25)	Monotherapy N (18)	Combined therapy N (26)	Positive control N (15)	Negative control N (13)	Sig. at level of
ESR (mm/hr)	Median (min-max)	50.00 (5-105)	40.00 (8-95)	31.00 (6-110)	35.00 (5-96)	14.00 (7-20)	0.01
Total WBC (10 ⁹ cell/L)	Median (min-max)	7.4300 (1.00-18.00)	8.1000 (4.00-12.00)	7.2500 (4.20-15.00)	7.1500 (3.50-15.07)	8.4000 (4.80-9.90)	0.767
Total Lymphocyte (10 ⁹ cell/L)	Median (min-max)	2.5900 (1.11-7.00)	2.3500 (0.90-5.20)	2.6000 (1.52-4.70)	2.0000 (1.40-3.37)	1.3000 (0.32-1.80)	0.01
Neutrophil (10 ⁹ cell/L)	Median (min-max)	4.0000 (1.00-11.40)	5.0000 (3.00-8.40)	3.9500 (0.40-11.00)	4.5300 (1.20-12.20)	3.8500 (0.36-6.20)	0.200
Monocytes (10 ⁹ cell/L)	Median (min-max)	0.3500 (0.10-1.01)	2.8000 (0.47-9.90)	1.3500 (0.19-5.90)	0.4700 (0.12-0.93)	0.1800 (0.10-0.80)	0.01

Table(5): Spearmann's correlations values between Th17/TregFoxp3 ratio and clinical features of patients.

Parameters	TH17/TregFoxP3	
	P. value	r. value
BMI	0.227	0.133
Age of onset	0.363	0.104
Duration of the disease	0.465	-0.085
Morning stiffness	0.121	-0.171

Table (6): level of Anti TNFi in patients and groups

Anti-TNFi		Monotherapy N=18	Combined Therapy N=26	Positive control N=15	p. value
Anti-infliximab	+ (%)	3 (16)	4 (15)	2 (13)	0.050
	Median	7.73	34.35	10.70	
	(Mix-Min)	(19.700-1.160)	(42.900-17.800)	(50.040-2.100)	
Anti-Etanercept	+ (%)	1(5)	2(7.2)	2(13)	0.134
	Median	0.52	0.48	0.61	
	(Mix-Min)	(0.650-0.360)	(2.010-0.370)	(1.050-0.390)	

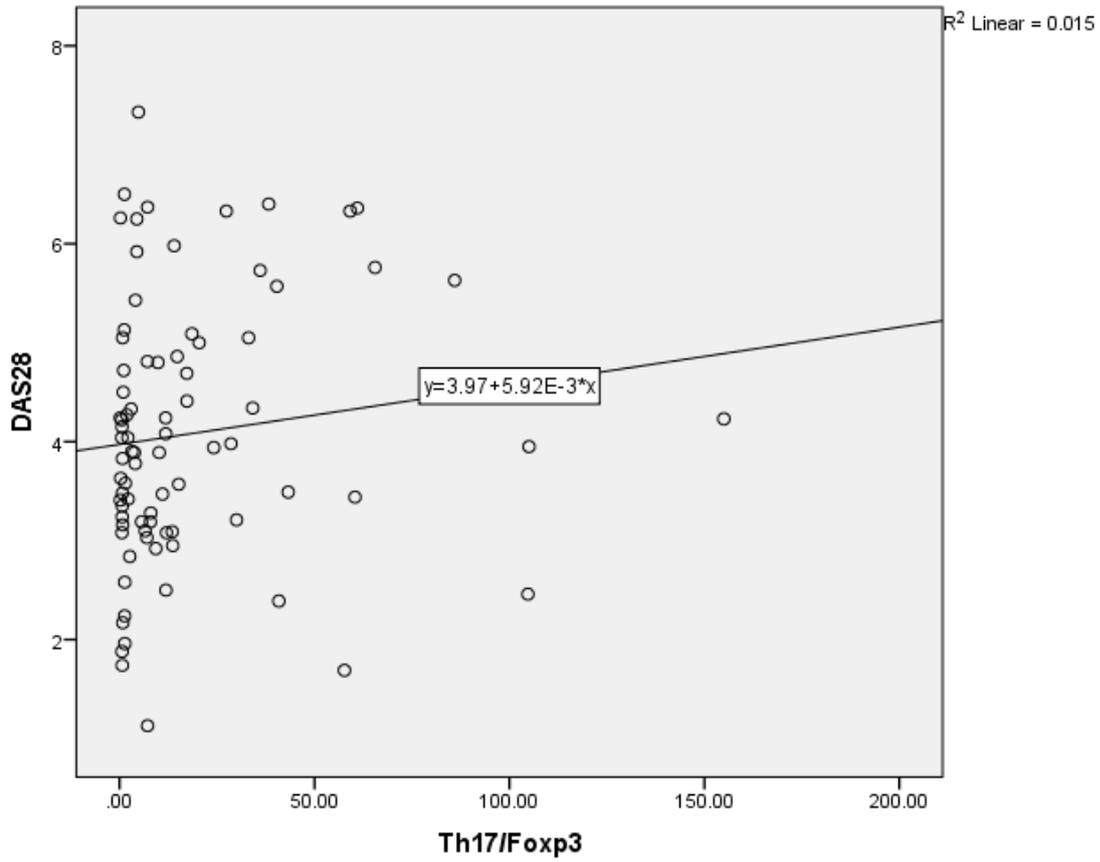


Fig. (1): Chart showing a positive significant (at the level of 0.05). Correlation between Th17 /Treg Foxp3+ T cells ratio and disease activity (DAS-28). score in patient's groups by Spearmann's correlation test ($r = 0.231$, $P = 0.034$)

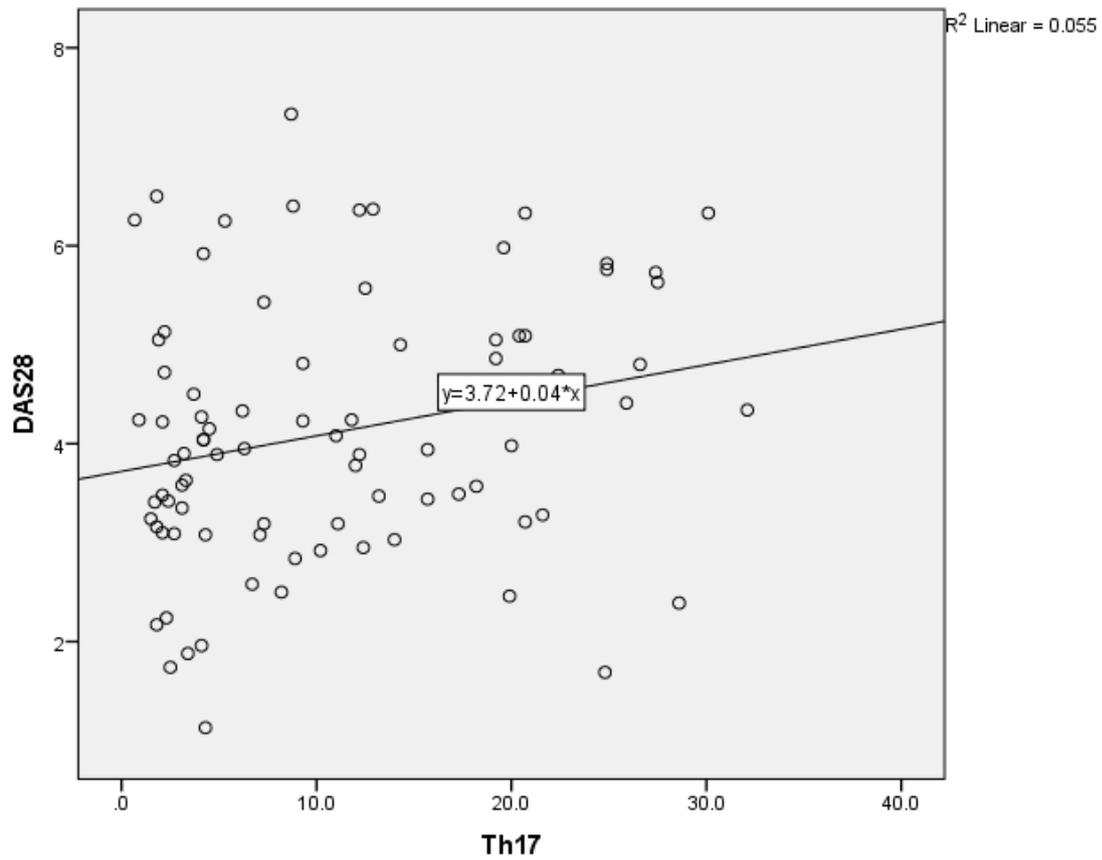


Fig. (2): Chart showing a positive significant (at the level of 0.05) correlation between Th17 T cells and disease activity score in patient's groups by Spearman's correlation test ($r = 0.226$, $P = 0.039$).

تأثير نسبة خلايا Th17 / TregFoxp3 في مرضى التهاب المفاصل الرثوي الذين يتلقون علاجًا مضادًا لعامل TNF- α

الخلاصة

من أجل تحديد العلاقة بين نشاط مرض التهاب المفاصل الروماتويدي مع نسبة خلايا Th17 / TregFoxp3 في المرضى تحت المعالجة بمضاد TNF-alpha وهو الهدف من الدراسة الحالية ، تم تضمين سبعة وتسعين مريضًا مصابًا بالتهاب المفاصل الروماتويدي ومجموعات تحكم صحية في هذه الحالة دراسة مراقبة ، أربعة وثمانين مريضًا مقسمة إلى أربع مجموعات ؛ 25 مريضًا يتلقون MTX فقط ؛ 18 مريضًا يتلقون علاجًا أحاديًا مضاد لـ TNF ؛ 26 مريضًا يتلقون علاجًا مشتركًا MTX + anti-TNF ؛ 15 مريضًا مصابًا بالتهاب المفاصل الروماتويدي لا يتلقون أي دواء (مجموعة حاكمة ايجابية). تم تسجيل ثلاثة عشر شخصًا يتمتعون بصحة جيدة يعتبرون حاكمة سلبًا في هذه الدراسة. كان المرضى الذين يعانون من التهاب المفاصل الرثياني يحضرون إلى مستشفى البصرة العام ، وحدة الروماتيزم ، مركز العلاج البيولوجي لتلقي العلاج المضاد لعامل نخر الورم. تم استخدام قياس التدفق الخلوي لقياس علامات TregFoxp3 و Th17 واستخدمت درجة DAS-28 لقياس نشاط مرض التهاب المفاصل الروماتويدي. تم قياس مثبطات TNF Infiximab و Etanercept أيضًا. كما تم قياس المعلمات الالتهابية والدموية الأخرى ESR ، إجمالي WBC ، الخلايا الليمفاوية ، وحيدات العدلات وعدد الخلايا. الناتج ، كان DAS-28 كدرجة نشاط المرض مرتبطة بشكل كبير مع نسبة Th17 / TregFoxp3 ومع عدد خلايا Th17. TH17 احصائيا ، لم تكن نسبة Th17 / TregFoxp3 مرتبطة بمؤشر كتلة الجسم وتيبس ومدة المرض. في الختام ، ارتبطت نسبة Th17 / Treg Foxp3 بشكل كبير مع DAS-28 كنشاط مرض التهاب المفاصل الروماتويدي. كلما انخفض تردد Tregs Foxp3 ، زادت درجة DAS التي تعكس نشاطًا أعلى للمرض. في مجموعة العلاج المشترك ، وجد أن نشاط المرض أقل من مجموعات المرضى الأخرى مما يشير إلى تأثير هذا المزيج بين MTX ومضاد TNF. هنا ، أوضحنا أن الميزة الرئيسية لهذا العلاج المركب في مرضى التهاب المفاصل الروماتويدي كانت

ارتداد تمدد خلايا Th17.

الكلمات المفتاحية: Anti-TNF ؛ العلاج المناعي. إنفليكسيماب. إتانرسبت. التهاب المفصل الروماتويدي؛ نسبة

Th17 / Treg Foxp