Israa M. Subhi et al /J. Pharm. Sci. & Res. Vol. 10(11), 2018, 2934-2937

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Some immunological aspects of rheumatoid arthritis post treated with biological treatment (enbrel)

Israa M. Subhi, Dr.Ayaid K. Zgair*, Mohammed H. Al-Osami
Department of Biology, College of Science, University of Baghdad, Baghdad

Abstract

Rheumatoid arthritis (RA) is one of the most challenging medical challenges in the world. There are many medicines help in reducing severity of RA. The most important of these medicines are biological treatment. The studies that address the effect of the use of these drugs on the lymphocytes population are very scanty, thus present study focused on the effect of the use of endrol (biological treatment) on the number of lymphocytes and in peripheral blood. Ninety five blood samples were collected from patients suffering from chronic RA attending the rheumatology clinic of Baghdad Teaching Hospital in Iraq from September 2016 to April 2017. The patients fulfilled the 1987 American College of Rheumatology criteria for classification of RA. Different biochemical and routine lab tests were done. Flow cytometry was used to measure the percentages of CD3+, CD4+ and CD8+ cells in peripheral blood of patients with RA (given endrol), RA (not given endrol) and healthy control. It was found significant difference in the levels of ESR, ALT and ALP in patients groups as compare with control. The present study showed that endrol increased the level of ESR. While the level of ALT and ALP decrease in peripheral blood of patients treated with endrol as compared with other groups. There is no significant difference in percentage od CD3+ cells between both patients groups and control. Significant increase in percentage of CD4+ cells and decrease in CD8+ cells as copared with healthy control. It was found that there is no significant effect of endrol on the level of CD3+, CD4+ and CD8+ cells.

Keywords-Rheumatoid Arthritis, Prevalence, ESR, T lymphocytes, CD3+, CD4+, CD8+, Flow cytometry

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic disease and symptoms such as fatigue and weight loss are common. Patients with RA have persistent synovial inflammation, causing chronic pain, joint damage and progressive disability. RA is accompanied by activation of the acute phase response, with elevation of serum C-reactive protein (CRP) and the erythrocyte sedimentation rate (ESR) [1]. The prevalence of RA is approximately 0.5–1.0% and females are three times more commonly affected than males. Patients typically develop symptoms between the ages of 20–40, although RA can present at any age [2, 3]. The prevalence of RA in the US appeared to increase during the period from 2004 to 2014, affecting a conservative estimate of 1.28-1.36 million adults in 2014 [4].

Understanding of the immunological mechanisms underlying certain pathologic conditions such as RA necessitates the detailed analysis of the functional repertoire of the immune system. Since T lymphocytes play a major role in immune functions by direct interaction with targets or indirectly by the production of a variety of immunomodulatory factors, many investigations have focused on the phenotypical and functional characterization of T cells present in the inflammatory sites of RA [5].

Flow cytometry has emerged as an essential tool for investigators in the study of the complexity of the immune system and the examination of its role in health and disease. The power of this technique lies in its ability to interrogate individual cells and simultaneously measure multiple parameters on each individual cell [6].

In the last 20 years, there was a great progress in the treatment of RA which involved the use of disease-modifying anti-rheumatic drugs (DMARDs) and the improvement of immune therapies specific against molecules and cells vital in the immune-pathogenesis of RA [7]. These can significantly change the progress of RA disease. Other agents used for RA include corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs). The NSAIDs are not considered as disease modifying agents and are not to be used as monotherapy [8]. The present study aims to obtain a survey on the prevalence of rheumatoid arthritis in Iraq and Measure the number of $CD3^+$, $CD4^+$ and $CD8^+$ in peripheral blood.

MATERIALS AND METHODS

Collection of blood samples and clinical features of patients

Ninety five blood samples were collected from patients suffering from chronic RA attending the rheumatology clinic of Baghdad Teaching Hospital in Iraq from September 2016 to April 2017. The patients fulfilled the 1987 American College of Rheumatology criteria for classification of RA. They admitted their medical history, physical examination and assessment of age, gender, address, symptoms of disease, medications, other related diseases, number and types of surgeries, smoking status, and laboratory tests). The blood samples were taken immediately to lab for further experiments.

Biochemical tests

Alkaline phosphatase (ALP), the colorimetrical method of Fishman [9] was used to measure the level of ALP in sera of patients and control groups. Aminotransferase (ALT) is an enzyme released from destroyed hepatic cells, which was detected in patients and control sera, and the standard method of Henry et al. [10] was followed to estimate the activity of ALT in patients and control sera. The standard methods were used to measure the concentration of serum creatinine and blood urea in peripheral blood of patients and healthy control [11, 12].

Measurement of lymphocytes percentages

Flow cytometer was used to estimate the number of $CD3^+$, $CD4^+$ and $CD8^+$ cells of peripheral blood of each group were collected from 25 patients. **First group** (n, 10): Patients with RA did not taken biological treatment. **Second Group (n, 10)**: Patients with RA but received biological treatment. **Third Group** (n, 10): Healthy controls. The blood samples were collected by aseptic techniques in EDTA tubes and mixed thoroughly.

Fluorescein isothiocyanate (FITC) mouse anti human-CD3 monoclonal antibody, FITC mouse anti-human CD4 monoclonal antibody, and Phycoerythrin (PE) mouse anti human-CD8 monoclonal antibody provided by (BDPharmingen/USA) were used to determine the expression of T cell markers CD3, CD4 and CD8. The mononuclear cells were gated on a forward scattered versus-side scattered cytogram. Flow cytometric analysis was carried out on isolated mononuclear cells using four –color Cyflow® Cube 6 flow cytometry device (PartecCyflow®, Germany) and data was processed using commercial software. Laser excitation was at 450nm and 560nm wavelengths [5].

Sample processing for Partec Cube6 flow cytometer was performed according to manufacturing company and the data was collected for at least 10-100 cells in the live gate per sample taken was processed using commercial software. Laser excitation was at 450nm and 560nm wavelengths.

Statistical analysis

The values were used to give a mean value and the standard deviation (SD) calculated. The differences were evaluated by using Student's t-test employing original version 8.0 software. A value of $P<0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Clinical features of RA patients

Results showed that the percentages of RA in females from 95 individuals were 85 (89%) more than in males 10 (11%). This agrees with study of Shah *et al*., 2017 in India who had a percentage of 180 (89%) for females and 23 (11%) males out of total 203 patients. It has been a common perception that RA is more severe in females than males. However, RA more frequently starts early in females, which means that the females in average are exposed to the inflammation longer than males. Thus, adjustment for disease duration is required when comparing measures of disease activity across sexes [13]. In previous study in Saudi Arabia has revealed that one hundred and thirty nine patients were studied, (mean age 46 ± 13 years), ranging from 19 - 97 years of which 118 (84%) were females; mean duration of the RA was 7.2 ± 6.4 years. Those that presented with disease duration > 1 year were 121 (75.6%) of RA patients [14]. A study in Brazil also correlates with these findings, females predominated (90%) and the mean age was 47.6 years (25-81) [15].

The age groups most affected in this study were 40-50 years with mean age of 44 ± 2.78, followed by group of more than 60 years old with mean age of 64 ± 7.21 while the mean age for 50-60 years was 51 ± 2.28 as in Table 1.

This finding agrees with previous study [16] whose mean patient age was 43.44 ± 17.96, and in another study, the mean age of the patients was 47 ± 10.92 years (range 23-69 years) [17]. In Iraq, among the 203 patients with RA, 162 (79.8 %) were women and 41 (20.2 %) were men, with a mean (SD) age of 46.9 ± 11.5 years [18]. Many studies described the demographic criteria of RA patients. The mean age of patients was in the fifth decade; this is in agreement with other study which showed that RA affects usually people above 40 years old. This is in fact caused by many reasons that depress immunity as stress, thymic depression, and longer duration of exposure to environmental antigens that cause stimulation of auto-reactive immune cells.

Table 1. Age groups of patients suffering from RA.

Age groups	Percentage	Mean ± SD of ages
1-10 years	1.05%	7 ± 0
11-20years	1.05%	15 ± 0
21-30 years	8.42%	26.12 ± 2.41
31-40 years	6.31%	34.6 ± 2.06
41-50 years	36.84%	44.93 ± 2.78
51-60 years	18.94%	51.5 ± 2.28
<60 years	27.36%	64.5 ± 7.21

The incidence of RA increases with age, and seems to reach a plateau from the age of 60 years. Moreover, RA patients with mean age more than 40 years and more than 50 years had significantly higher RA duration than the under-40 and under-50 year age groups [17].The medical history revealed that 37% of patients have also suffered from hypertension, 22% diabetes, 17% other cases including tuberculosis, urinary tract infection, psoriasis and others. History of tuberculosis (TB) or previous exposure to contacts or family histories are documented, screening for latent TB are obtained and if positive, it is asked whether prophylactic treatment with isoniazide (INH) was given [20].

RA associates with excessive morbidity and mortality from cardiovascular disease (CVD) which may be due to multiple causes. Several risk factors, such as hypertension (HT), smoking, dyslipidaemia, as defined by National Cholesterol Education Program and insulin resistance are thought to be more prevalent in RA and may be important contributors. HT is one of the most important modifiable risk factors for the development of CVD in the general population. The reported prevalence of HT among patients with RA varies from 3.8% to 73%. The very wide range of reported prevalence of HT in RA is explained by the different populations assessed, the varied sample sizes and significant differences in the definition of HT used [21]

Smoking has also been associated with RA by 5% of cases of this study. Smoking has been shown to be a risk factor for RA. Gender interacts with smoking by an unknown mechanism, which leads to differential risk of RA [22].

The disease duration for this study ranged from 1 to more than 10 years with mean of 2 ± 0.88 years as in Table 2.

Shah, et al. (2017) showed that duration of disease ranged from 1-10 years with a mean of 2.55 ± 2.09 years, approximately 23% of cases have acquired the disease from their relatives [17].

Table 2: showing the duration of RA disease

	Duration of Disease			
	less than 1 year	1-5years	5-10years	<10 years
	13%	42%	19%	19%
Mean ± SD	4.33 ± 3.67	2.05 ± 0.88	4.62 ± 1.57	14.9 ± 4.2

Clinical lab tests for blood samples collected from patients with RA

Table 3 shows several clinical tests that were done to patients and healthy control groups. The study showed that there is no significant difference was observed between first and second groups and control as well, in all tests except ESR, ALT and ALP. Previous randomized controlled trials have documented that Methotrexate (MTX) or leflunomide (LEF) monotherapy are both associated with a significantly increased incidence of ALT and/or ALP elevations [23]. The white blood cell count (WBC) is typically normal, but may be slightly elevated in non-treated group. ESR is typically elevated because the elevation of globuline protein and most inflammation serum factors [24]. In another study indicated that elevated transaminase levels in RA patients on MTX are rarely related to MTX-specific liver lesions (5%), but frequently to autoimmune hepatitis (AIH) histological lesions (41%) [25].

Table 3. Clinical tests for patients suffering with RA that treated with biological therapy, non treated with biological therapy and healthy control. ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; S, significant difference; NS, non-significant different; a, significant to RA- Biological treated group; b significant difference to control group; P value of 0.05 considered as significant difference. All data presented in mean ± SD (standard deviation).

Test	Group 1, RA- Non biological treated	Group 2, RA- Biological treated	Group 3, Control	T test
ESR mm/h	$34.5 \pm 19.9^{a,b}$	41.7 ± 24^{b}	8.75 ± 3.4	S
Hb g/dl	12.56 ± 1.2	12.9 ± 1.6	13.6 ± 4.1	NS
PCV %	37.5 ± 3.7	40.4 ± 5.06	42.5 ± 2.9	NS
WBCs cell/ml	$7.04 \pm 1.9 * 10^{6}$	$7.74 \pm 2.3 * 10^{6}$	$7.53 \pm 1.8 * 10^{6}$	NS
Urea mg/dl	29.1 ± 2.8	24.81 ± 11.7	30.1 ± 3.7	NS
Creatinine mg/dl	0.83 ± 0.29	0.76 ± 0.1	0.9 ± 0.1	NS
ALT U/l	$30.2 \pm 10.8^{a,b}$	18.5 ± 6.3^{b}	22.1 ± 3.2	S
ALP U/l	$43.8 \pm 17^{a,b}$	21.6 ± 7.5^{b}	17.5 ± 4.7	S

Mononuclear cells

Flow cytometry analysis was done to measure the percentage of $CD3^+$, $CD4^+$ and $CD8^+$ cells in peripheral blood of each group were collected from patients and healthy control (Figure 1).

The mean percentage of $CD3^+$ non treated group with biological drug was 71.3 ± 4.2, while the mean percentage in biological treated group was 69.25± 3.2 and the mean percentage in healthy control group was 68.1± 2.2 %. No significant difference in percentage of CD3+ cells was observed in non-treated and treated groups as compared with healthy control group (P>0.05). There was no significant difference between non biological and biological treated groups in case of CD3+cells (P>0.05). Feuchtenberger *et al.* (2008) showed that the percentage of CD3+ before biological therapy was 66±10% while after treatment with biological therapy anti-TNF-α agent. Furthermore, they found that the percentage of CD3+ cells in peripheral blood of patients with RA and treated with rituximab for 12 months was 73 ± 8 % [26].

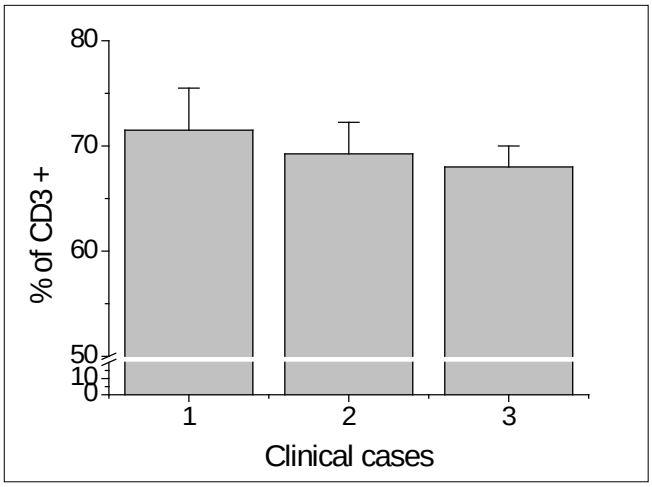

Figure 1. Percentages of CD3+ cells in peripheral blood of patients suffering from RA. The percentage of CD4+ was calculated from total mononuclear cells. 1, No biological treated group; 2: Biological treated group; 3: Healthy control group.

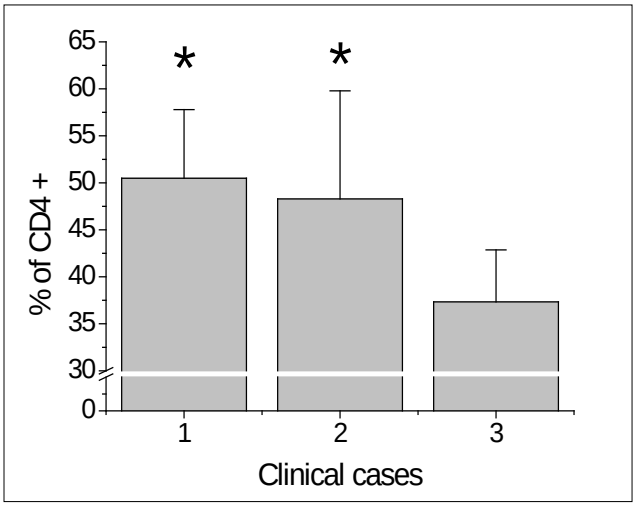

Figure 2. Percentages of CD4+ cells in peripheral blood of patients suffering from RA. The percentage of CD4+ was calculated from total mononuclear cells. 1, Non biological treated group; 2: Biological treated group; 3: Healthy control group. Asterisks indicate a significant difference from control group; P < 0.05 considered to be significant difference.

Percentage of CD4+ cells in this study was calculated from total percentage of CD3+ cells (Figure 2).

The percentage of CD4+ cells was 50.49 ± 7% in non-biological treated group, while the mean percentage of this type of cell in peripheral blood of biological treated group was 48.29 ± 11.4, and this percentage was 37.3 ± 7.3 in healthy control group. Significant increase was seen between the percentage of CD4+ in treated and non-treated group as compared with healthy control group (P<0.05). While, there is no significant difference was observed in percentage of CD4+cells in peripheral blood of biological and non-biological treated group.

Lamour *et al*. (1992) showed that there is no difference in percentage of CD4+ cells in RA patients did not get biological treatment as compared with healthy controls (42 + 11% versus 39 + 6%) [27]. Feuchtenberger *et al.,* 2008 showed that the percentage of CD4+ before therapy was 47 ± 11 % while after treatment with anti-TNF-α agent was 52 ± 9 % after 12 months of the course of treatment [24].

Percentage of CD8+ in this study was calculated from total percentage of CD3+ (Figure 3). The results of current study showed that the mean percentage of CD8+ cells in peripheral blood of patients with RA who did not get biological treatment was 18.47± 5.1 % while the mean percentage with the biologically treated group was 19.28±8.01%, and the mean percentage in healthy controls was 27.3±4.5 %. There was a significant decrease in the percentage of CD8+ cells in peripheral blood of patients with RA who non treated with biological treatment and who treated with biological treatment (P<0.05) as compared to the percentage of this type of cell in peripheral blood of healthy control group, while there was no significant difference in the percentages of CD8+ cells in peripheral blood of patients with RA who treated with biological treatment and who not treated with biological treatment.

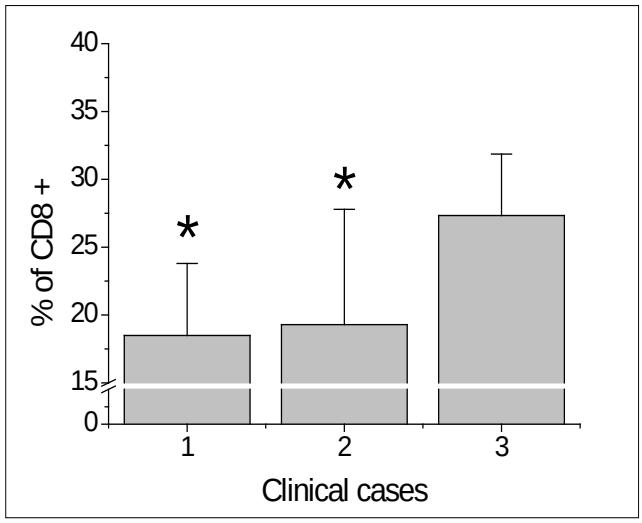

Figure 3. Percentages of CD8+ cells in peripheral blood of patients suffering from RA. The percentage of CD8+ was calculated from total percentage of isolated mononuclear cells. 1, Non biological treated group; 2: Biological treated group; 3: Healthy control group. Asterisks indicate a significant difference from control group; P < 0.05 considered to be significant difference.

During the last few years it was found that RA is characterized by profound changes in the immune system, both local (synovitis) and systemic (changed phenotypes and functions of peripheral blood CD4+ and CD8+ T lymphocytes). The direction of changes in the immune system as reported in the literature seems to be somewhat confusing, as both more activation of immune system and immune senescence of $CD4^+$ T cells have been reported in RA patients. However, immune senescence in healthy people is also associated with increased systemic inflammation and the

appearance of more activated T cells in the blood [16]. Lamour *et al*., 1992 showed in their study that decreased levels of CD8+ in RA patients without biological treatment 21 +7% versus 32+±6% in healthy controls. In human autoimmune arthritis, the phenotype of CD8+ T-cell subsets in peripheral blood is skewed towards an activated or inflammatory phenotype. Indeed, the frequency of CD8+ cells is increased in the peripheral blood of patients with RA compared with age-matched healthy donors. Like CD4+ T cells, CD8+ T-cell functions can be controlled by negative co-stimulatory signals and regulatory cells [27]. The alteration of these regulatory mechanisms might sustain inflammation in autoimmune arthritis [26].

Interestingly, clinical response to treatment with other biologic agents, such as abatacept or rituximab, has been associated with a decrease in IFNγ-producing and IL-17-producing CD8+ T cells, or a decrease in total CD8+ T-cell counts, respectively. Although most of the beneficial effects of biologic drugs in autoimmune arthritis have been attributed to their ability to antagonize the function of CD4+ T cells, they also have a clear effect on the CD8+ T-cell compartment [28].

Another study of absolute number of CD3+ lymphocytes showed no significant changes during the time of B cell depletion and during the regeneration phase up to 1 year after B cell depletion compared to before therapy. The absolute numbers for CD4+ and CD8+ cells showed no significant changes either. Nevertheless they observed some changes in the composition of the peripheral lymphocyte pool. The population of CD3+ cells was significantly increased within 1-12 months with rituximab ($p < 0.05$). This was related to a statistically significant increase of CD4+ T cells. CD8+ T cells did neither show significant changes in the absolute numbers nor in the relative frequency. Feuchtenberger *et al.*, 2008 showed that the percentage of CD8+ before therapy was 26 ± 7 % while after treatment by the biological anti-TNF-α agent rituximab was 26 ± 9 % after 12 months of the course. Despite the fact that a significant increase in the relative frequency of CD3+ cells and in the CD4+ subset within 1-12 months after rituximab ($p < 0.05$), the absolute numbers of CD3+ and CD4+ T cells not changed. This can be due to lack or reduced levels of B-cells at the time of study. Due to extensive pretreatment before rituximab investigated patients had low lymphocyte counts. Therefore, absolute numbers of T cells were generally low [26].

REFERENCES

1. Sudoł-Szopińska I, Jans L, & Teh J. (2017). Rheumatoid arthritis: what do MRI and ultrasound show? *J. ultrasonography.*17 (68):5.
2. Gabriel S E, & Michaud K. (2009). Epidemiological studies in incidence, prevalence, mortality, and comorbidity of the rheumatic diseases. Arthr.res. ther. 11(3): 229.
3. Al-Dalaan A, Al S B, Bahabri S, Biyari T, Al MS, & Mousa M. (1998). The prevalence of rheumatoid arthritis in the Qassim region of Saudi Arabia. Ann.Saudi med. 18(5): 396-397.
4. Hunter TM, Boytsov N N, Zhang X, Schroeder K, Michaud K, & Araujo A. B. (2017). Prevalence of rheumatoid arthritis in the United States adult population in healthcare claims databases, 2004–2014. Rheum. Inter. 37(9):1551-1557.
5. Walter G J, Fleskens V, Frederiksen K S, Rajasekhar M, Menon B, Gerwien J G, & Taams L S. (2016). Phenotypic, Functional, and Gene Expression Profiling of Peripheral CD45RA+ and CD45RO+ CD4+ CD25+ CD127low Treg Cells in Patients With Chronic Rheumatoid Arthritis. Arth. Rheum. 68(1):103-116.
6. Soloski M J, & Chrest F J. (2013). Disease Mechanisms in Rheumatology—Tools and Pathways: Multiparameter Flow Cytometry for Discovery of Disease Mechanisms in Rheumatic Diseases. Arth. Rheum. 65(5):1148-1156.
7. Moreland LW. General principles of management of rheumatoid arthritis in adults.
8. Singh J A, Furst D E, Bharat A, Curtis J R, Kavanaugh A F, Kremer J M, & Bridges S L (2012). 2012 Update of the 2008 American College of Rheumatology recommendations for the use of disease-modifying antirheumatic drugs and biologic agents in the treatment of rheumatoid arthritis. Arth. care res.64(5), 625-639.
9. Fishman WH (1990) Alkaline phosphatase isozymes: recent progress. Clin Biochem 23:99–104
10. Henry RJ, Chiamori N, Golub OJ, Berkman S (1960) Revised spectrophotometric methods for the determination of glutamic oxalacetic transaminase, glutamic pyruvic transaminase and lactic acid dehydrogenase. Am J Clin Pathol 34:381–398.
11. Wyss, M., and Kaddurah-Daouk, R., 2000, Creatine and creatinine metabolism. Physiol. Rev. 80:1107–1213.
12. Kurzer F et tal. Urea in the history of organic chemistry: Isolation from natural sources. J Chem Educ 1956; 33, 9: 452-59.
13. Van Vollenhoven R F. (2009). Sex differences in rheumatoid arthritis: more than meets the eye. BMC med. 7(1): 12.
14. Abdul-Aziz S and El-Deek B. (2014). A Single Center Experience in Biological Therapy for the Treatment of Rheumatoid Arthritis in Saudi Arabia.O.J.RA.4:199-206.
15. Azevedo A F B, Petribú K C L D, Lima M D N, Silva A S D, Rocha Filho J D A, Mariano M H Q D A, and Rushansky E. (2015). Quality of life of patients with rheumatoid arthritis under biological therapy. Rev.Assoc.Med.Bras. 61(2): 126-131
16. Pawłowska J, Smoleńska Ż, Daca A, Witkowski J M, and Bryl E. (2011). Older age of rheumatoid arthritis onset is associated with higher activation status of peripheral blood CD4+ T cells and disease activity. Clin. Experim. Immun. 163(2): 157-164.
17. Shah S, Baba M, Khaliq A, Jeelani I, Shah S,and Nadeem S. (2017). Lipid Profile in Rheumatoid Arthritis and its Relation with Inflammatory Markers. Insights Biomed. 2(1):1-6.
18. Abdul-Qahar ZH, Al-Osami MH and Al-Asay FZ. (2013).Prevalence of Metabolic Syndrome in Iraqi Patients with Rheumatoid Arthritis.J.Dent.Med.Sci. 11(1):69-72.
19. Marder W, Vinet É, and Somers E C. (2015). Rheumatic autoimmune diseases in women and midlife health. Women's midlife heal. 1(1): 11.
20. Panoulas V F, Metsios G S, Pace A V, John H, Treharne G J, Banks M J, and Kitas G D (2008). Hypertension in rheumatoid arthritis. Rheum. 47(9): 1286-1298.
21. Mehta P K, Wei J and Wenger N K. (2015). Ischemic heart disease in women: a focus on risk factors. Trends in CVD. Med. 25(2): 140-151.
22. Tobón G J, Youinou P, and Saraux A. (2010). The environment, geo-epidemiology, and autoimmune disease: rheumatoid arthritis. J. autoimmune. 35(1): 10-14.
23. Curtis J R, Beukelman T, Onofrei A, Cassell S, Greenberg J D, Kavanaugh A and Kremer JM. (2010). Elevated liver enzyme tests among patients with rheumatoid arthritis or psoriatic arthritis treated with methotrexate and/or leflunomide. Anna. Rheum. Dis. 69(01): 43-47.
24. Gossec L, Smolen J S, Gaujoux-Viala C, Ash Z, Marzo-Ortega H, Van Der Heijde D and Braun J. (2012). European League against Rheumatism recommendations for the management of psoriatic arthritis with pharmacological therapies.Ann. Rheum. Dis. 71(1): 4-12
25. Toulemonde G, Scoazec J Y and Miossec P. (2012). Treatment with etanercept of autoimmune hepatitis associated with rheumatoid arthritis: an open label proof of concept study. Ann.Rheum. Dis. 71(8):1423-1424.
26. Feuchtenberger M, Müller S, Roll P, Waschbisch A, Schäfer A, Kneitz C and Tony H P (2008). Frequency of regulatory T cells is not affected by transient B cell depletion using anti-CD20 antibodies in rheumatoid arthritis. O. Rheum. J. 2: 81-88.
27. Lamour A, Jouen-beades F, Lees O, Gilbert D, Loet X and Tron F. (1992). Analysis of T cell receptors in rheumatoid arthritis: the increased expression of HLA-DR antigen on circulating γδ+ T cells is correlated with disease activity. Clin.Exper.Immun. 89(2): 217-222.
28. Petrelli A and Van Wijk F. (2016). CD8+ T cells in human autoimmune arthritis: the unusual suspects. Nat. Rev. Rheum. 12(7): 421-428.