International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304
Vol.9, No.4, pp 307-312, 2016

# Association Between Interleukin-6(IL-6) and Thrombocytosis in Rheumatoid Arthritis Patients

**Abbas Sabbar Dkhil[1]*, Farhan Hussain[1], Arafat A.Muttar[2]**

**[1]Physiology Department, Faculty of Medicine, University of Qadisiyah, Qadisiyah, Iraq**
**[2]laboratories Accreditation Department, Quality Assurance And Academic Accreditation, Supervision and Scientific Evaluation Apparatus, Ministry of Higher Education And Scientific Research, Iraq**

**Abstract:** This study was performed to determine whether the serum concentrations of interleukin (IL)-6 are elevated in patients with RA (rheumatoid arthritis) and to investigate the relationship between IL-6 levels and platelets counts in RA patients. 95 serum samples were obtained, 70 of them from patients with RA who had visited the department of Rheumatology at Al-Sadder medical city in Najaf governorate (Iraq) and 25age and sex-matched healthy controls. The authors assessed the clinical parameters of the disease, including ESR (erythrocyte sedimentation rate), CRP (C-reactive protein), and RF (rheumatoid factor). platelets counts were measured using automated hematologyanalyzer Mythic™.. Serum concentrations of interleukin-6(IL-6) were measured using an ELISA (enzyme-linked immunosorbent assay). The results of serum concentration of interleukin-6 (IL-6) were significantly elevated ($P$<0.0001) in patients with RA compared to those of healthy controls. On the other hand, platelets counts were also showed significantly increased ($P$<0.001) in patients with RA compared to those of healthy controls.. These findings suggested that interleukin-6(IL-6) directly stimulates the thromocytopoiesis and the net detectable effects in peripheral blood is thrombocytosis.

## 1. Introduction

RA (rheumatoid arthritis) is a chronic disease that causes inflammation mainly in the synovium an produces destruction and deformity of the joints. The aetiology of RA remains unclear, but it is known to be associated with genetic and environmental factors[1]. Various proinflammatory cytokines, such as IL-6, tumor necrotic factor (TNF)-α, interleukin (IL)-1β, and interferon (IFN)-γ, are increased in the synovial tissue or synovial fluid of patients with RA[2,3]. Increased levels of proinflammatory cytokines lead to the proliferation of synovial tissue, and thereby cause damage in the articular cartilage and bone destruction in the adjacent area[4,5]. In particular, IL-6 is a cytokine with various functions. When IL-6 is activated, acute inflammatory responses such as fever or anemia are induced. IL-6 promotes the proliferation of B cells and thus is involved in the production of the rheumatoid factor[6]. Recently, RA has been observed to be associated with high levels of IL-6 in the synovial membrane and serum[6,7]. This led to the speculation that IL-6 plays a pathogenic role in RA. Recently, an IL-6 receptor antagonist, tociliumab, was developed and showed clinical efficacy in the treatment of RA[8-10]. IL-6 is a pleiotropic cytokine with a wide range of biological activities on various target cells. It regulates immune responses, hematopoiesis, acute phase responses and bone metabolism[11]. Despite its important physiological roles, dysregulated overproduction of IL-6 is responsible for systemic inflammatory manifestations and abnormal laboratory findings in patients with RA. In fact, elevated IL-6 levels have been observed in both serum and synovial fluid in patients with RA, and there have been correlations between serum

IL-6 levels and clinical and laboratory indices of RA[12]. These findings have led to the concept that interference with the actions of IL-6 could represent a therapeutic approach to RA.

Rheumatoid arthritis (RA) is frequently complicated by thrombocytosis correlated with disease activity. Particular interleukins, namely interleukin (IL)-6, IL-4 and IL-1, with pro-inflammatory mediator activities have also been shown to be involved in the regulation of megakaryocytopoiesis[13,14]. The authors compared the serum concentrations of IL-6 in patients with RA and those in normal controls and then investigated the correlation between serum levels of IL-6 and the platelets counts.

## 2. Experimental

### 2.1 Subjects and clinical assessment

This study was conducted in 50 patients who had visited Al-Sadder Medical Cityat Najaf Governorate (Iraq) between February and May 2015, and who fulfilled the ACR (American College of Rheumatology) 2010 revised criteria for the diagnosis of RA[15]. Twenty age and sex-matched healthy adults without any evidence of chronic inflammatory disease served as the controls. The patients underwent thorough clinical and laboratory evaluation, including complete medical history, seropositivity test for RA (Rheumatoid Arthritis), CRP (C-reactive protein), and estimation of ESR (erythrocyte sedimentation rate). At the time of clinical assessment for disease, six milliliters of blood samples were collected intravenously from each patient, 1ml for evaluation of ESR, whereas serum samples were collected in glass tubes without anticoagulant, stored for one hour at room temperature, centrifuged (2.500 r.p.m. for 10 minutes at 4°C) and then aliquoted in plastic tubes before being stored at -20°C until analysis.

### 2.2 Study Design

The patients were classified according to the duration of RA disease as follows: GI (Group I): the group of patients with disease length of (less than one year) was considered as the group with very early disease duration. GII (Group II): the group of patients with disease length of (1-5) years was considered as the group with early disease duration. GIII (Group III): the group of patients with disease length of (6-15) years was considered as the group with median disease duration. Group IV (GIV): the group of patients with disease length of (16-25) years was considered as the group with long disease duration. GV (Group V): the group of patients with disease length of (more than 25) years was considered as the group with very long disease duration.

### 2.3 Methods

#### 2.3.1 Rapid test

RF (rheumatoid factor) and C-reactive protein were performed by a rapid latex agglutination test kit for the presumptive detection of the RF and C-reactive protein in serum.

#### 2.3.2 ESR (Erythrocyte sedimentation rate)

The ICSH (International Committee on Standardization in Hematology) recommends the use of the Westergren method [16].

#### 2.3.3 Platelet count assessment

Platelets counts were performed on EDTA blood using Mythic™ 18 (RINGELSAN CO., Turk) at Virology Laboratory of AL-Sadder Medical City in Najaf Governorate

#### 2.3.4Assessment of serum IL-6

The serum concentration of IL-6 was measured using an AssayMax ELISA (enzyme-linked immunosorbent assay) kit (Assaypro, USA) at Virology Laboratory of AL-Sadder Medical City in Najaf Governorate. Fifty microliters each of serum sample and assay diluent were placed in each well of a 96 well plate coated with a monoclonal mouse IgG against IL-6. This mixture was incubated for two hours at room

temperature, and each well was aspirated and washed five times with wash buffer. Subsequently, 50µL of Biotinylated IL-6 Antibody was added to each well and incubated for two hours. Again, each well was washed five times with wash buffer. Following this, 50 µL of Streptavidin-Peroxidase Conjugate was added per well and incubated for 30 minutes and each well was aspirated and washed five times with wash buffer. Subsequently, 50 µL of substrate solution, which was prepared with equal amounts of stabilized hydrogen peroxide ($H_2O_2$) and tetramethylbenzidine, was added for a 20 minute reaction under dark conditions. The reaction was quenched by the addition of 50 µl stop solution (0.5 N of HCl). Within 30 minutes, the optical density was measured at a wavelength of 450 nm using the bioelisa reader ELx 800 (Molecular Device Co., biokit, CA, USA). The serum concentration of IL-6 was determined based on a standard concentration curve. The correlation coefficient (r) of the standard concentration curve was 0.990.

### 2.4 Statistical analysis

Data analyses were performed with SAS version 9.1 (SAS Institute Inc., Cary, NC, USA). All of the descriptive variables were expressed as the mean ±SE (standard error). The correlations between the concentrations of IL-6 and iron status were tested using Pearson's correlation test. The group analyses were performed using one-way ANOVA and Tukey's post-hoc analyses. For all tests, a p value less than 0.05 was considered statistically significant.

## 3. Results

### 3.1. Serum concentration of IL-6

Serum level of IL-6 of RA patients showed significant increase ($P< 0.0001$) in the average values comparatively to the healthy group. In the very early duration n of the disease was much higher (Fig. 1).

### 3.2. Platelets (PLT) ($\times 10^9$/L)

Platelets count values in the patients groups of RA patients exhibited highly statistically significant increase ($P<0.01$) in the average values in all groups of patients suffering from RA except GV of duration compared to the healthy control group (Figure.2).

### 3.3. Relationship of IL-6 levels to platelets counts

Serum concentration of IL-6 correlated positively and significantly with platelets counts ($r = 0.2071$, $p = 0.0854$) (Fig. 3) and (Table. 1)

### 3.3. Relationship of IL-6 levels to ESR

Serum concentration of IL-6 correlated positively and significantly with the highest coefficient correlation with ESR ($r = 0.9776$, $p<0.0001$) (Fig. 4) and (Table. 1).

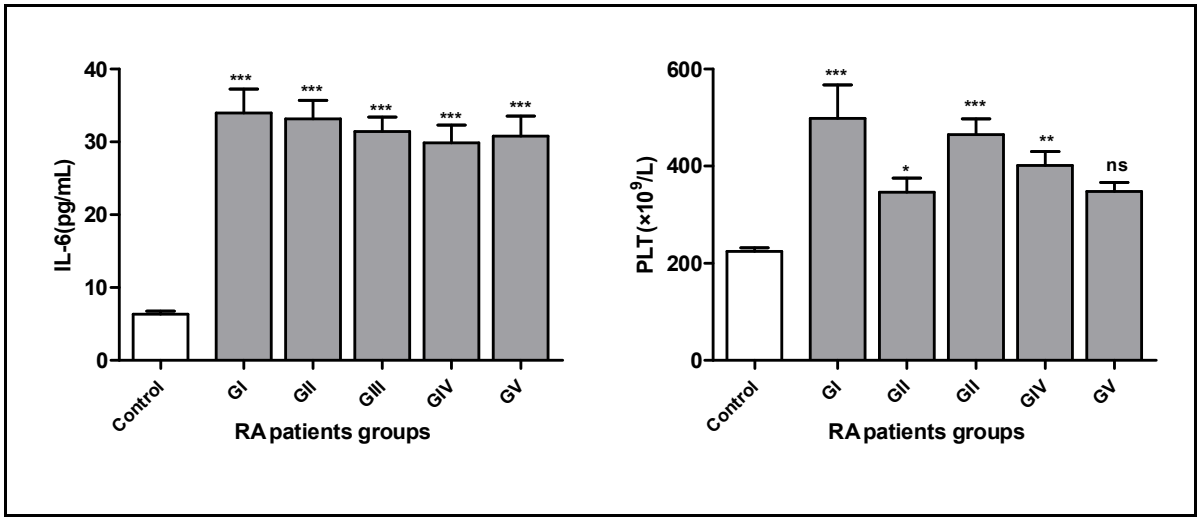


**Fig 1serum levels of IL-6 in healthy group and in the five groups of patients suffering from rheumatoid arthritis.**
Data are expressed as means ± standard error (SE). *** indicate significant difference based on Tukey's multiple comparison tests. $P< 0.0001$

**Fig 2PLT in healthy group and in the five groups of patients suffering from rheumatoid arthritis.**
Data are expressed as means ± standard error (SE). *** indicate significant difference based on Tukey's multiple comparison tests. $P< 0.0001$
**: indicate very significant (P value 0.001to 0.01).
*indicate significant (P value 0.01to 0.05). ns indicate not significant (P value >0.005).

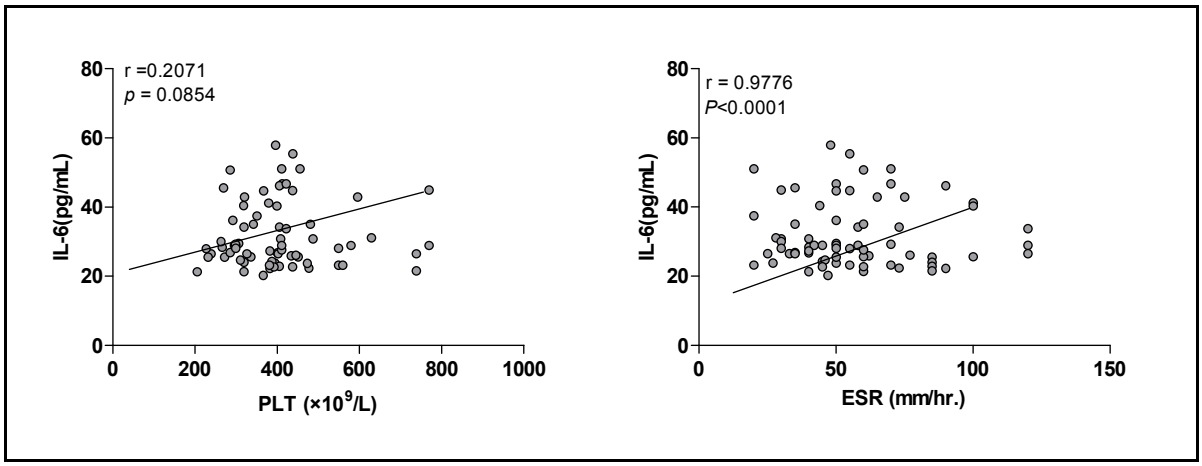


**Fig 3 The Correlation between Serum Interleukin-6 (IL-6) Concentration (pg/mL) and platelet count ($\times 10^9$/L)in Rheumatoid Arthritis Patients**
***Pearson's correlation analysis was performed**

**Fig 4The Correlation between Serum Interleukin-6 (IL-6) Concentration (pg/mL) and ESR (mm/hr.)in Rheumatoid Arthritis Patients**
***Pearson's correlation analysis was performed**

**Table 1: The Correlation between Serum Interleukin-6 (IL-6) Concentrations (pg/mL),platelet count ($\times 10^9$/L)and ESR (mm/hr.)in Rheumatoid Arthritis Patients.**

| Cytokine (pg/mL) | PLT($\times 10^9$/L) | | ESR (mm/hr.) | |
|---|---|---|---|---|
| | r | *p* | r | *p* |
| IL-6 | 0.2071 | 0.0854 | 0.9776 | <0.0001*** |

***Pearson's correlation analysis was performed**

## 4. Discussion

The current study showed that serum concentrations of IL-6, were significantly elevated in patients with RA compared to those in healthy controls (Fig. 1). As seen in previous reports, this finding supports the hypothesis that IL-6 is involved in the pathogenesis of RA [16-20]. In addition to these previous observations, the significant increase in the IL-6 cytokine observed in the current study indicates that this cytokines might play a role in inducing inflammatory responses or mediating anti-inflammatory responses in the pathogenesis of RA[21]. The present study also revealed significant increased in platelets counts(Fig. 2). The results of this study indicate thrombocytosis associated with all groups of patients who suffering from rheumatoid arthritis. Gasparyan and his co-workers have shown in their RA study an increased number of platelets and platelet-derived proteins (growth factors) within the synovium and synovial fluid[22]. Chung *et al*., (2007) are also showed that circulating platelets are an abundant source of prothrombotic agents closely related to inflammatory markers, and play a crucial role in the initiation and propagation of vascular disease[23].

Megakaryocytes are sensitive to IL-6, which is a mediator of thrombocytosis, although this effect seems to be indirect, mediated through the induction of thrombopoietin, and is common to other inflammation related cytokines[24].

Reactive megakaryocytopoiesis increases circulating platelets count and triggers hyperactivity. Hyperactive platelets target synovial membranes with subsequent local rheumatoid inflammation. The exact pathogenetic mechanism(s) that causes increased platelet counts in RA are still unknown. Recent investigations indicated that proinflammatory pleiotropic cytokines of RA also have megakaryocytopoietic/thrombopoietic properties[25].

Other authors also showed that the activated platelets, alone or together with other inflammatory cells and mediators, may play a significant role in thrombus formation, synovial microcirculation, and destruction of cartilage[26,27].

In summary, the authors found that the serum concentrations of IL-6 were significantly increased in patients with RA compared with those of normal controls. The cytokine levels were significantly correlated inflammatory state represented by ESR. Taking together with handling of platelets count, interleukin-6 IL-6 plays a major role in the pathogenesis of RA including thrombocytosis.

## References

1. HochbergMC, Silman AJ,. Smolen,JS, Weinblatt,ME, Weisman MH. Rheumatology. In: A. J. MacGregor, A. J. Silman, editors, Classification and epidemiology, 4th ed. Mosby, Spain,2008 pp. 755-62.
2. McInnesIB, SchettG. Cytokines in the pathogenesis of rheumatoid arthritis, Nat Rev Immuno.,2007, l7;429-42.
3. BrennanF, BeechJ. Update on cytokines in rheumatoid arthritis, CurrOpinRheumato.,2007, l19; 296-301.
4. HuberLC,DistlerO, TarnerI, GayRE, GayS, PapT. Synovial fibroblasts: Key players in rheumatoidarthritis. Rheumatology.,2007,45; 669-75.
5. SzekaneczZ, KochAE. Macrophages and their products in rheumatoid arthritis, CurrOpin Rheumato.,2007, l19; 289-95.
6. MadhokR, CrillyA, WatsonJ, CapellHA.Serum interleukin 6 levels in rheumatoid arthritis: Correlations with clinical and laboratory indices of disease activity. Ann Rheum Dis., 1993, 52; 232-4.
7. KnudsenLS, ChristensenIJ, LottenburgerT, SvendsenMN, NielsenHJ, NielsenL, et al,Pre-analytical and biological variability in circulating interleukin 6 in healthy subjects and patients with rheumatoid arthritis. Biomarkers.,2008, 13; 59-78.
8. SmolenJS, BeaulieuA, Rubbert-RothA, Ramos-RemusC, RovenskyJ, AlecockE., et al, Effect of interleukin-6 receptor inhibition with tocilizumab in patients with rheumatoid arthritis (OPTION study): A double-blind, placebo controlled Randomised Trial. Lancet.,2008, 371; 987-97.
9. GenoveseMC, McKay JD, Nasonov EL,MyslerEF,. da SilvaNA, Alecock E, et al, Interleukin-6 receptor inhibition with tocilizumab reduces disease activity in rheumatoid arthritis with inadequate

response to disease-modifying antirheumatic drugs: The tocilizumabin combination with traditional disease-modifying antirheumatic drug therapy study.Arthritis Rheum.,2008, 58; 2968-80.
10. EmeryP, KeystoneE,TonyHP, CantagrelA, van VollenhovenR, SanchezA, et al, IL-6 receptor inhibition with tocilizumab improves treatment outcomes in patients with rheumatoid arthritis refractory to anti-tumour necrosis factor biologicals: Results from a 24-week multicentrerandomised placebo-controlled trial. Ann Rheum Dis.,2008, 67; 1516-23.
11. AkiraS, TagaT, KishimotoT,Interleukin-6 in biology and medicine.AdvImmunol.,1993, 54; 1-78.
12. SackU, KinneR, MarxT, et al, Interleukin-6 in synovial fluid is closely associated with chronic synovitis in rheumatoid arthritis.RheumatolInt.,1993, 13; 45–51.
13. ErtenliI, KirazS, OztürkMA, HaznedaroğluI,CelikI and CalgüneriM. Pathologic thrombopoiesis of rheumatoid arthritis. RheumatolInt.,2003,23(2); 49-60.
14. ErtenliI, HaznedaroğluI, CelikI,CalgüneriM, and KirazhiS. Cytokines affecting megakaryocytopoiesis in rheumatoid arthritis with thrombocytosis. Rheumatol Int.,1996,16(1);5-8.
15. AletahaD, NeogiT, SilmanA J.Rheumatoid arthritis classification criteria: An American college of rheumatology/European League against rheumatism collaborative initiative. Ann Rheum Dis.,2010,62(9); 2569-2581.
16. International Council for Standardization in Hematology (Expert Panel on Blood Rheology), ICSH recommendations for measurement of erythrocyte sedimentation rate, J ClinPathol.,1993, 46; 198-208.
17. OkamotoH, YamamuraM, MoritaY, HaradaS, MakinoH, OtaZ. The synovial expressionand serum levels of interleukin-6, interleukin-11, leukemia inhibitory factor, and oncostatin M in rheumatoid arthritis and SLE. Arthritis Rheum., 1997, 40; 1096-105.
18. RobkT, GladalskaA, StepieńH, RobakE. Serum levels of interleukin-6 type cytokines and soluble interleukin-6 receptor in patients with RA. Mediators Inflamm.,19987; 347-53.
19. Waring PM, CarrollGJ, KandiahDA, BuirskiG, MetcalfD. Increased levels of leukemia inhibitory factor in synovial fluid from patients with rheumatoid arthritis and SLE. Arthritis Rheum.,1993, 36; 911-5.
20. NishimotoN, KishimotoT. Interleukin-6: from bench to bedside. Nat ClinPractRheumato.,2006, l2; 619-26.
21. Soo-Jin Chung, KwonJY, ParkMC, ParkYB, LeeSK. The Correlation between increased serum concentrations of interleukin-6 family cytokines and disease activity in rheumatoid arthritis patients.Yonsei Med J.,2011, 52 (1); 113-120.
22. GasparyanAY, Stavropoulos-KalinoglouA,MikhailidisDP,TomsTE, DouglasMJ, and KitasGD. Platelet Function in Rheumatoid Arthritis: Arthritic and Cardiovascular Implications. Rheumatology International., 2010, 31(2);153-164.
23. ChungI, ChoudhuryA, andLipGY. Platelet activation in acute decompensated congestive heart failure. Thromb Res., 2007, 120; 709-13.
24. PablosÁlvarezJL. Interleukin 6 in the Physiopathology of Rheumatoid Arthritis. RheumatolClin.,2009, 5(1); 34-39.
25. GasparyanAY, Stavropoulos-KalinoglouA, SandooA, and KitasGD. Mean Platelet Volume in Patients with Rheumatoid Arthritis: the Effect of anti-TNF-alpha Therapy. Rheumatology International.,2010, 30(8);1125-1129.
26. Gonzalez-GayMA,Gonzalez-JuanateyC, Miranda-FilloyJA, Garcia-PorruaC, LlorcaJ, and MartinJ. Cardiovascular disease in rheumatoid arthritis. Biomed Pharmacother., 2006, 60; 673-7.
27. CaporaliR, PallaviciniFB, FilippiniM,GorlaR, MarchesoniA, FavalliEG, ,Sarzi-PuttiniP, AtzeniF, and MontecuccoC. Treatment of rheumatoid arthritis with anti-TNF-alpha agents: a reappraisal. AutoimmunRev.,2009, 8;274-80.

*****