

# Association of Serum Concentrations of Proinflammatory Cytokines and Hematological parameters in Rheumatoid Arthritis Patients

Abbas Sabbar Dakhil

Faculty of Medicine, University of Al-Qadisiyah, Iraq, 58001

## Abstract

Rheumatoid joint inflammation is an immune system illness of multi factorial etiology portrayed by aggravation of the joints and presence of autoantibodies coordinated against various autoantigens. This study was achieved to define the levels of serum interleukin-6 (IL-6), interleukin -1alpha, tumor necrotic factor-alpha (TNF- $\alpha$ ) and interleukin-8 in rheumatoid joint inflammation patient and its association with hematology biomarkers in those patients.

70 serum samples from RA patients were collected besides 25 (compatible with gender and age) served as control subjects. Clinical considerations of ailment were measured, involving sero-positivity test for each rheumatoid factor, c-reactive protein and erythrocyte precipitation rate Complete blood count (CBC) was performed utilizing mechanized haematology analyzer (Mythic™). The levels of cytokines were assessed by an enzyme-labeled immunosorbent assay (ELISA).

The concentration of interleukin-6 (IL-6), interleukin -1alpha, tumor necrotic factor- alpha (TNF- $\alpha$ ) and interleukin-8 were considerably rising (P value <0.0001) in persons suffering from Rheumatoid joint inflammation paralleled to whom of intact individuals. In spite of the fact that there was no noteworthy relation between proinflammatory interleukins levels and erythrocyte count, hemoglobin, hematocrit and indices of red cell, the proinflammatory cytokines in RA patients demonstrated a huge relationship with thrombocytes, differential white blood cells, and total white cell count.

This investigation presumed that the concentrations of interleukin-6 (IL-6), interleukin -1alpha, tumor necrotic factor- alpha (TNF- $\alpha$ ) and interleukin-8 were altogether raised in RA patients and firmly corresponded with haematological modifications. So, these discoveries recommend the conceivable part for proinflammatory of cytokines in the pathogenesis of rheumatoid joint inflammation.

**Keyword:** Rheumatoid joint inflammation; interleukin-6; proinflammatory cytokines; interleukin-8; tumor necrosis factor (TNF- $\alpha$ )

## 1. INTRODUCTION

Rheumatoid joint inflammation or rheumatoid arthritis (RA) defined as ceaseless ailment which causes irritation for the most part within synovia and yields devastation and disfigurement of the joints. The causes of this autoimmune disease are mysterious; however, it is well documented to be related to hereditary and environmental factors [1].

Different pro-inflammatory interleukins, interleukin-6 (IL-6), interleukin -1alpha, interleukin-8 and tumor necrosis factor- alpha (TNF- $\alpha$ ) are raised inside fluid of synovium with RA [2, 3]. High serum availability of these interleukins prompt the expansion of fluid of synovia and accordingly elicit damage within joint cartilage and bone pulverization near the contiguous area [4, 5]. Specifically, IL-6 is a cytokine with different capacities. Once this cytokine is stimulated, a severe inflammatory reaction like pyrexia or anaemia is promoted and activates B lymphocytes propagation therefore is elaborated in the autoantibodies generation like rheumatoid factor [6]. Tumor necrotic factor- alpha (TNF- $\alpha$ ) is a multipotent cytokine with various functions and strong proinflammatory impacts, and is ensnared in numerous incendiary and immune system diereses and is an individual from a gathering of cytokines that empower the acute-phase response [7]. Proinflammatory cytokines, for example, interleukin -1alpha, interleukin-8 (chemokine) and tumor necrosis factor- alpha (TNF- $\alpha$ ) mediate the irritation and subsequent attracting the inflammatory cells to the site of inflammation particularly synovia which

excite bone and articular pulverization in patients with RA [8]. Tumor necrosis factor-alpha (TNF- $\alpha$ ), interleukin -1alpha, and interleukin-6 (IL-6) specifically, are accountable as the excitable interleukins of various acute phase proteins, for example, hepatocyte ferritin protein and c-reactive protein [9].

In the course of the most recent decade, the ideal utilization of disease modified antirheumatic drugs (DMARDs), specifically the grapple DMARD methotrexate (MTX) and the accessibility of new biologic operators have drastically upgraded the accomplishment of RA administration, for instance, tumor necrotic factor alpha (TNF $\alpha$ ) blockers (Etanercept and Golimumab), Interleukin-1 alpha (IL-1) blockers—anakinra, and Interleukin 6 (IL-6) Blockers (Tocilizumab) [10]. This research article achieved to determine the levels of serum interleukin-6 (IL-6), interleukin-1alpha, interleukin-8 and tumor necrosis factor-alpha (TNF- $\alpha$ ) and in rheumatoid joint inflammation patient and its association with hematology biomarkers in those patients.

## 2. MATERIALS AND METHODS

The study protocol was approved by the ethical committee of the Faculty of Medicine -University of Al-Qadisiyah. The current investigation was directed in seventy subjects whose had gone to the Rheumatology section of at Al-Diwaniyah teaching hospital in Al-Diwaniyah city amongst March and September 2012. All subjects were satisfied the American College of Rheumatology (ACR) 2010

reconsidered principals for the determination of rheumatoid arthritis [11]. Twenty-five (compatible with gender and age) served as control subjects with no proof of incessant inflammatory ailment. The principal of twenty eight-joint disease activity score (DAS28) was applied in the current research article and we consider utilizing the joints quantity with delicacy or enlargement. Clinical considerations of ailment were measured, involving sero-positivity test for each rheumatoid factor, c-reactive protein, and erythrocyte precipitation rate. Six milliliters of intravenous blood were gathered from every subjects and whole blood count (CBC) was assessed utilizing mechanized haematology analyzer (Mythic™). Serum samples were obtained after centrifugation (3000 rpm for ten minutes) and after that kept with deep freeze in Eppendorf tube until investigation.

**2.1 Subject Design**

In light of DAS28, the subjects were subdivided into five gatherings as takes after

1. Group I (GI): reduction (DAS28 ≤ 2.6)
2. Group II (GII): mellow (2.6 < DAS28 ≤ 3.2)
3. Group III (GIII): direct (3.2 < DAS28 ≤ 5.1)
4. Group IV (GIV): extreme (5.1 < DAS28)
5. Group V (GV): the gathering of Subjects with whose DAS28 surpassed 5.1

**2.2 Methods**

**2.2.1 C-reactive protein (CRP) and serological test rheumatoid factor (RF)**

Rheumatoid factor latex serological test and C - reactive protein were measured by agglutination test.

**2.2.2 Erythrocyte Sedimentation Rate (ESR)**

ESR was measured according to the Westergren method procedure which recommended by International Committee for Standardization in Haematology (ICSH) [12].

**2.2.3 Assessment of Complete Blood Count (CBC)**

Complete blood count was conducted on anticoagulant blood utilizing Mythic™ eighteen (Ringelsn co., Turk) in Haematology Laboratory of AL-Diwaniyah hospital.

**2.2.4. Assessment of IL-8, IL-6, TNF-alpha, and IL-1alpha**

The serum level of interleukin-6 (IL-6), interleukin -1alpha, tumor necrotic factor- alpha (TNF-α) and interleukin-8 were assessed by an AssayMax enzyme-labeled immunosorbent assay (ELISA) kit (Assaypro, USA).

**2.2.5 Data analysis**

Parametric statistical analyses were accomplished by ANOVA followed posthoc Tukey's test using GraphPad

Prism software version 6. Pearson correlation analysis was also performed to evaluate the correlation between studied parameters. The limit of significance was set at 5% (Motulsky, 2003).

**3. RESULTS**

**3.1 Clinical qualities of the subjects**

Table 1 revealed the clinical and statistical information of the RA patients. The median age of patients was 48.3 ± 2.42 years (extent: 26-74 years), involved thirteen males and fifty-seven females. Patients were subdivided into five gatherings as per disease activity score (DAS28) as takes after 10 in the reduction (DAS 28 less than or equal to 2.6), 19 gentle (DAS28 greater than 2.6 and less than or equal to 3.2), 20 direct (DAS28 greater than 3.2 and less than or equal to 5.1), 11 extreme (DAS28 greater than 5.1), and 10 the gathering of patients with whose DAS28 surpassed 5.1. While the mean age of the controls was 43±3.33 years (ranged between 23-76 years), and they involved six men and 19 nineteen women.

**3.2 Complete blood count**

Data examination demonstrated a huge reduction (P value <0.0001) in erythrocytes, hemoglobin, hematocrit and red cell indices amongst the RA patients contrasted with the control as shown in (Table 2) though for total white blood cell, differential white blood cell and thrombocytes of the investigation gatherings, measurably huge (P value less than 0.0001) high esteems were resolved at RA subjects contrasted with the intact subjects.

**3.3 Serum cytokines levels**

Data examination uncovered the huge increment (P value <0.0001) in serum interleukin-6 (IL-6), interleukin -1alpha, tumor necrotic factor- alpha (TNF-α) and interleukin-8 in rheumatoid joint inflammation patient contrasted with the control subjects as shown in Table 3.

**3.4 Correlations between measured cytokines levels and haematological biomarkers**

Serum concentration of serum interleukin-6 (IL-6), interleukin -1alpha, tumor necrotic factor- alpha (TNF-α) and interleukin-8 are correlated positively with TLC and platelets. Serum level of cytokines demonstrated a negative relationship with LYM%. Then again there were converse connections between cytokines, RBC, Hb, MCH and MCHC (Fig. 1-Fig. 4).

**Table1: Clinical with Demographic subjects Characteristics**

| Character                        | Healthy subjects | patients with RA (n=70) |                      |                      |                |                     |
|----------------------------------|------------------|-------------------------|----------------------|----------------------|----------------|---------------------|
|                                  |                  | (DAS 28 ≤ 2.6)          | (2.6 < DAS 28 ≤ 3.2) | (3.2 < DAS 28 ≤ 5.1) | (5.1 < DAS 28) | DAS 28 exceeded 5.1 |
| No. of subjects                  | 25               | 9                       | 20                   | 19                   | 13             | 9                   |
| Age                              | 41±1.33          | 42±4.12                 | 45±4.11              | 48±1.12              | 46±4.9         | 56±5.13             |
| Female/male ratio                | 20/5             | 6/4                     | 16/3                 | 18/2                 | 9/2            | 8/2                 |
| Disease duration (years) (range) |                  | 0.44±0.05               | 2.17±0.33            | 7.44±2.54            | 18.4±0.66      | 25.5±0.8            |
| %RF positive patients            |                  | 92.5%                   | 96.8%                | 91.2%                | 91%            | 94%                 |
| ESR (mm/hour)                    | 20.9±0.4         | 56.1±4.2                | 55.6±2.6             | 58.4±5.16            | 68.19±6.6      | 49.3±3              |
| C-RP% positive patients          |                  | 91%                     | 96%                  | 93%                  | 92.9%          | 89%                 |

**Table2: Complete blood count in healthy control and in rheumatoid arthritis patients.**

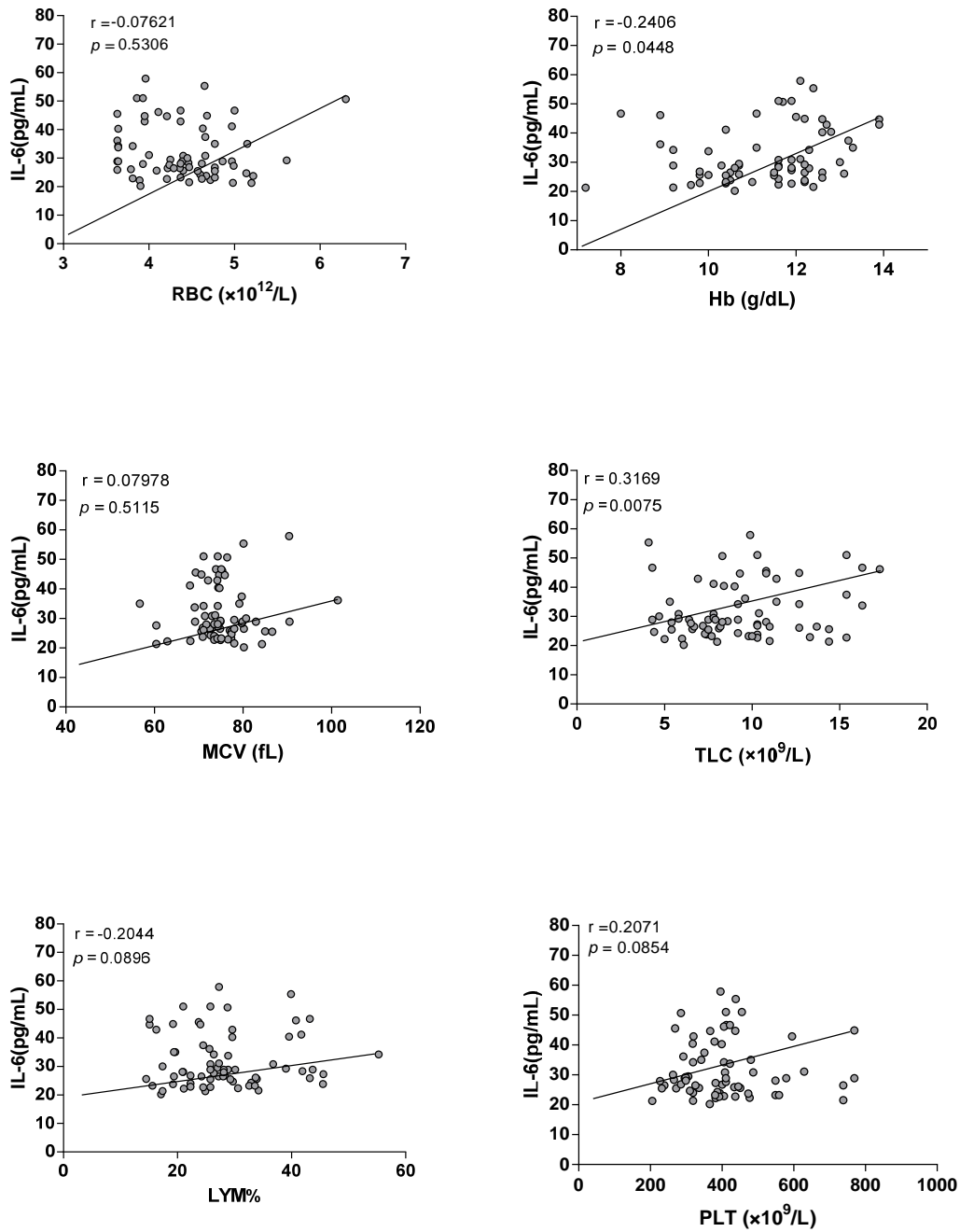
| Parameters                                   | Healthy control (n=25) | RA patients (n=70)                      |  |  |  |  |                                  |
|--|------------------------|---|--|--|--|--|----------------------------------|
|  |                        | Disease Activity Score (DAS28)          |  |  |  |  | P                                |
|  |                        | (DA S28 ≤ 2.6)                          | (2.6 < DAS 28 ≤ 3.2)                   | (3.2 < DAS28 ≤ 5.1)                    | (5.1 < DAS 28)                         | DAS28 exceeded 5.1                     |                                  |
| Erythrocytes (×10 <sup>12</sup> /L)          | 6.07±0.02              | 4.20±0.130 <sub>a</sub> <sup>***</sup>  | 4.33±0.12 <sub>a</sub> <sup>*</sup>    | 4.22±1.12 <sub>a</sub> <sup>**</sup>   | 3.17±0.38 <sub>a</sub> <sup>*</sup>    | 3.65±0.64 <sub>a</sub> <sup>**</sup>   | Less than 0.0001 <sup>***</sup>  |
| Hemoglobin (g/dL)                            | 14.44±0.21             | 11.22±0.29 <sub>a</sub> <sup>***</sup>  | 11.33±0.19 <sub>a</sub> <sup>***</sup> | 10.12±0.22 <sub>a</sub> <sup>***</sup> | 11.06±0.31 <sub>a</sub> <sup>***</sup> | 11.43±0.12 <sub>a</sub> <sup>***</sup> | Less than 0.0001 <sup>***</sup>  |
| Hematocrit %                                 | 46.55±0.23             | 33.12±0.23 <sub>a</sub> <sup>***</sup>  | 33.12±0.56 <sub>a</sub> <sup>***</sup> | 32.59±0.12 <sub>a</sub> <sup>*</sup>   | 33.32±0.54 <sub>a</sub> <sup>***</sup> | 33.22±0.25 <sub>a</sub> <sup>*</sup>   | Less than <0.0001 <sup>***</sup> |
| Mean cell volume (fL)                        | 91.87±0.12             | 76.22±1.67 <sub>a</sub> <sup>*</sup>    | 74.22±0.33 <sub>a</sub> <sup>***</sup> | 75.65±0.12 <sub>a</sub> <sup>*</sup>   | 76.65±0.21 <sub>a</sub> <sup>***</sup> | 71.87±0.95 <sub>a</sub> <sup>***</sup> | Less than <0.0001 <sup>***</sup> |
| Mean cell hemoglobin (pg)                    | 33.21±0.44             | 24.41±0.35 <sub>a</sub> <sup>***</sup>  | 23.32±2.47 <sub>a</sub> <sup>***</sup> | 25.42±0.49 <sub>a</sub> <sup>***</sup> | 23.42±5.02 <sub>a</sub> <sup>***</sup> | 23.33±0.12 <sub>a</sub> <sup>***</sup> | Less than <0.0001 <sup>***</sup> |
| Mean cell hemoglobin concentration (g/dL)    | 34.12±0.65             | 33.81±0.21 <sub>a</sub> <sup>ns</sup>   | 34.32±0.14 <sub>a</sub> <sup>ns</sup>  | 33.87±0.43 <sub>a</sub> <sup>**</sup>  | 33.32±0.40 <sub>a</sub> <sup>*</sup>   | 33.2±0.12 <sub>a</sub> <sup>ns</sup>   | 0.031 <sup>**</sup>              |
| Total leukocytes count (×10 <sup>9</sup> /L) | 6.89±0.43              | 9.32±0.01 <sub>a</sub> <sup>ns</sup>    | 7.39±0.12 <sub>a</sub> <sup>n</sup>    | 8.14±0.22 <sub>a</sub> <sup>ns</sup>   | 8.03±0.12 <sub>a</sub> <sup>n</sup>    | 8.09±1.98 <sub>a</sub> <sup>ns</sup>   | 0.2280 <sup>ns</sup>             |
| Lymphocytes %                                | 32.03±0.11             | 26.22±0.22 <sub>a</sub> <sup>ns</sup>   | 30.33±0.77 <sub>a</sub> <sup>ns</sup>  | 28.22±0.22 <sub>a</sub> <sup>ns</sup>  | 28.76±0.20 <sub>a</sub> <sup>ns</sup>  | 20.43±0.23 <sub>b</sub> <sup>**</sup>  | 0.0084 <sup>**</sup>             |
| Monocytes %                                  | 3.22±0.32              | 12.34±2.43 <sub>a</sub> <sup>**</sup>   | 11.56±0.67 <sub>a</sub> <sup>**</sup>  | 9.87±0.56 <sub>a</sub> <sup>ns</sup>   | 16.29±0.13 <sub>a</sub> <sup>***</sup> | 12.98±0.18 <sub>a</sub> <sup>*</sup>   | Less than 0.0001 <sup>***</sup>  |
| Granulocytes %                               | 59.22±0.14             | 64.54±0.44 <sub>a</sub> <sup>ns</sup>   | 66.34±0.43 <sub>a</sub> <sup>ns</sup>  | 63.55±0.76 <sub>a</sub> <sup>ns</sup>  | 65.32±0.56 <sub>a</sub> <sup>ns</sup>  | 74.22±0.31 <sub>b</sub> <sup>***</sup> | 0.0042 <sup>**</sup>             |
| Thrombocytes (×10 <sup>9</sup> /L)           | 227.3±6.44             | 496.4±44.03 <sub>a</sub> <sup>***</sup> | 345.5±34.1 <sub>a</sub> <sup>*</sup>   | 464.2±22.6 <sub>a</sub> <sup>***</sup> | 403.3±54 <sub>a</sub> <sup>**</sup>    | 346.7±19.33 <sub>a</sub> <sup>ns</sup> | Less than 0.0001 <sup>***</sup>  |

- Values are stated by means of means ± standard error (SE).
- Stars refer significant changes according to Tukey multiple comparable analysis.
- P amount less than 0.0001
- Similar small letters refer insignificant changes amongst groups according to Tukey multiple comparable analysis.
- \*\*\* indicate extremely significant (P value <0.001).
- \*\* indicate very significant (P value 0.001 to 0.05).
- No: no significant

**Table3: Concentrations of Cytokines (pg/mL) in control subjects and in patients with rheumatoid joint inflammation.**

| Cytokine (pg/mL) | Healthy control (n=25) | RA patients (n=70)                     |  |  |  |  | P value                         |
|------------------|------------------------|--|--|--|--|--|---------------------------------|
|                  |                        | Disease Activity Score (DAS28)         |  |  |  |  |                                 |
|                  |                        | (DAS 28 ≤ 2.6)                         | (2.6 < DAS 28 ≤ 3.2)                   | (3.2 < DAS 28 ≤ 5.1)                   | (5.1 < DAS 28)                         | DA S28 exceeded 5.1                    |                                 |
| IL -6            | 5.23±0.23              | 33.23±1.65 <sub>a</sub> <sup>***</sup> | 33.14±0.54 <sub>a</sub> <sup>***</sup> | 31.33±-.87 <sub>a</sub> <sup>***</sup> | 29.43±0.23 <sub>a</sub> <sup>***</sup> | 30.43±0.87 <sub>a</sub> <sup>***</sup> | Less than 0.0001 <sup>***</sup> |
| TNF-alpha        | 5.43±0.54              | 33.34±0.32 <sub>a</sub> <sup>***</sup> | 3324±2.63 <sub>a</sub> <sup>***</sup>  | 33.9±2.32 <sub>a</sub> <sup>***</sup>  | 41.14±5.98 <sub>a</sub> <sup>***</sup> | 26.43±0.45 <sub>b</sub> <sup>***</sup> | Less than 0.0001 <sup>***</sup> |
| IL-1alpha        | 2.67±0.49              | 29.32±2.19 <sup>***</sup>              | 26.87±0.74 <sup>***</sup>              | 28.22±0.76 <sup>***</sup>              | 27.87±0.98 <sup>***</sup>              | 25.23±0.56 <sup>***</sup>              | Less than 0.0001 <sup>***</sup> |
| IL-8             | 11.61±0.13             | 41.51±3.23 <sup>***</sup>              | 40.56±3.98 <sup>***</sup>              | 49.68±3.93 <sup>***</sup>              | 41.45±3.97 <sup>***</sup>              | 35.87±6.24 <sup>***</sup>              | Less than 0.0001 <sup>***</sup> |

- Values are stated by means of means ± standard error (SE).
- Stars refer significant changes according to Tukey multiple comparable analysis.
- Similar small letters refer insignificant changes amongst groups according to Tukey multiple comparable analysis.
- P amount less than 0.0001
- \*\*\* indicate extremely significant (P value <0.001).



**Fig.1: Relationship between interleukin-6 and hematological biomarkers in rheumatoid arthritis patients**

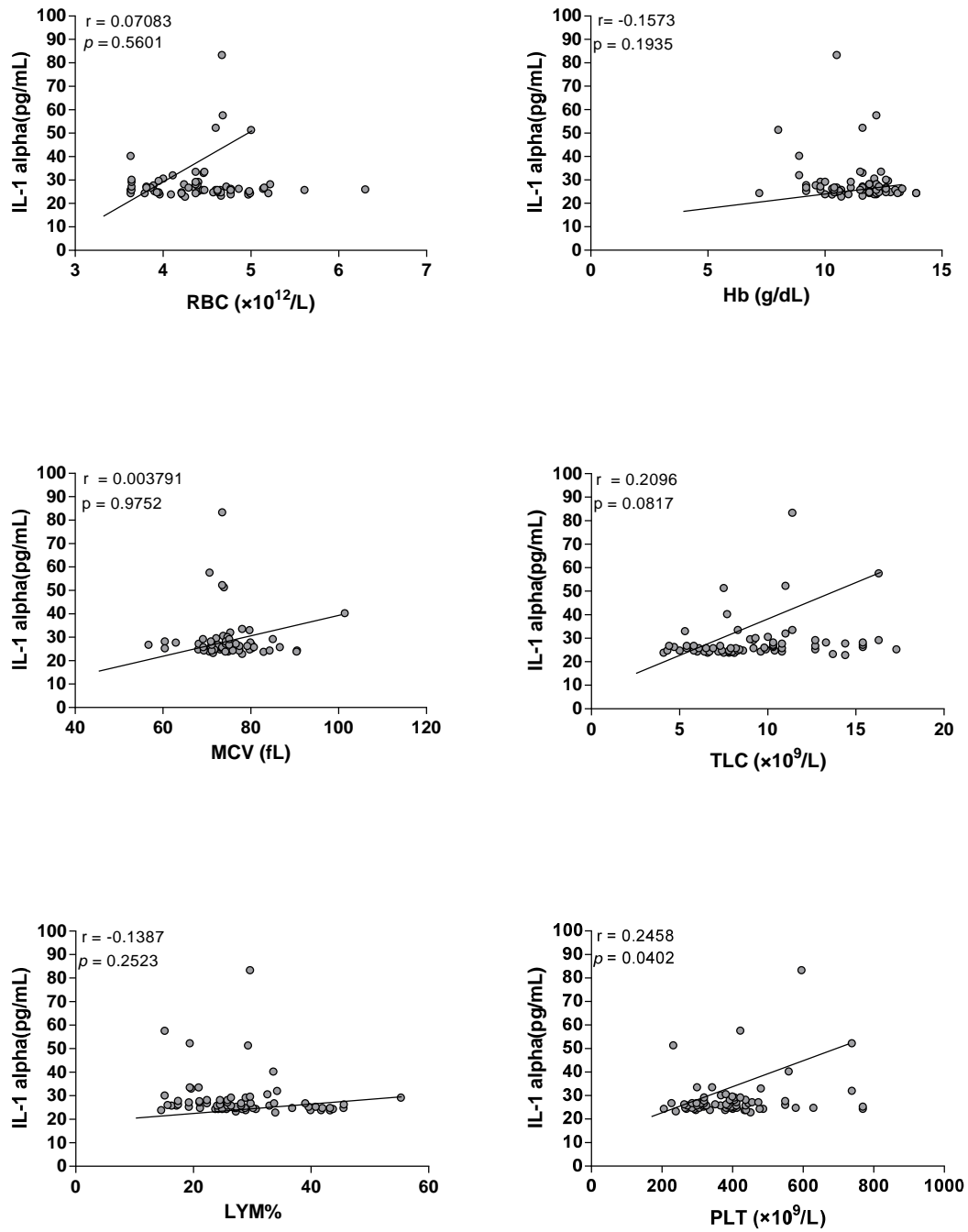
Pearson correlation test was conducted.

**RBC:** red blood cells,

**Hb:** hemoglobin,

**MCV** mean cell volume,

**TLC:** total leukocytes counts.



**Fig.2: Relationship between interleukin-1 alpha and hematological biomarkers in rheumatoid arthritis patients**

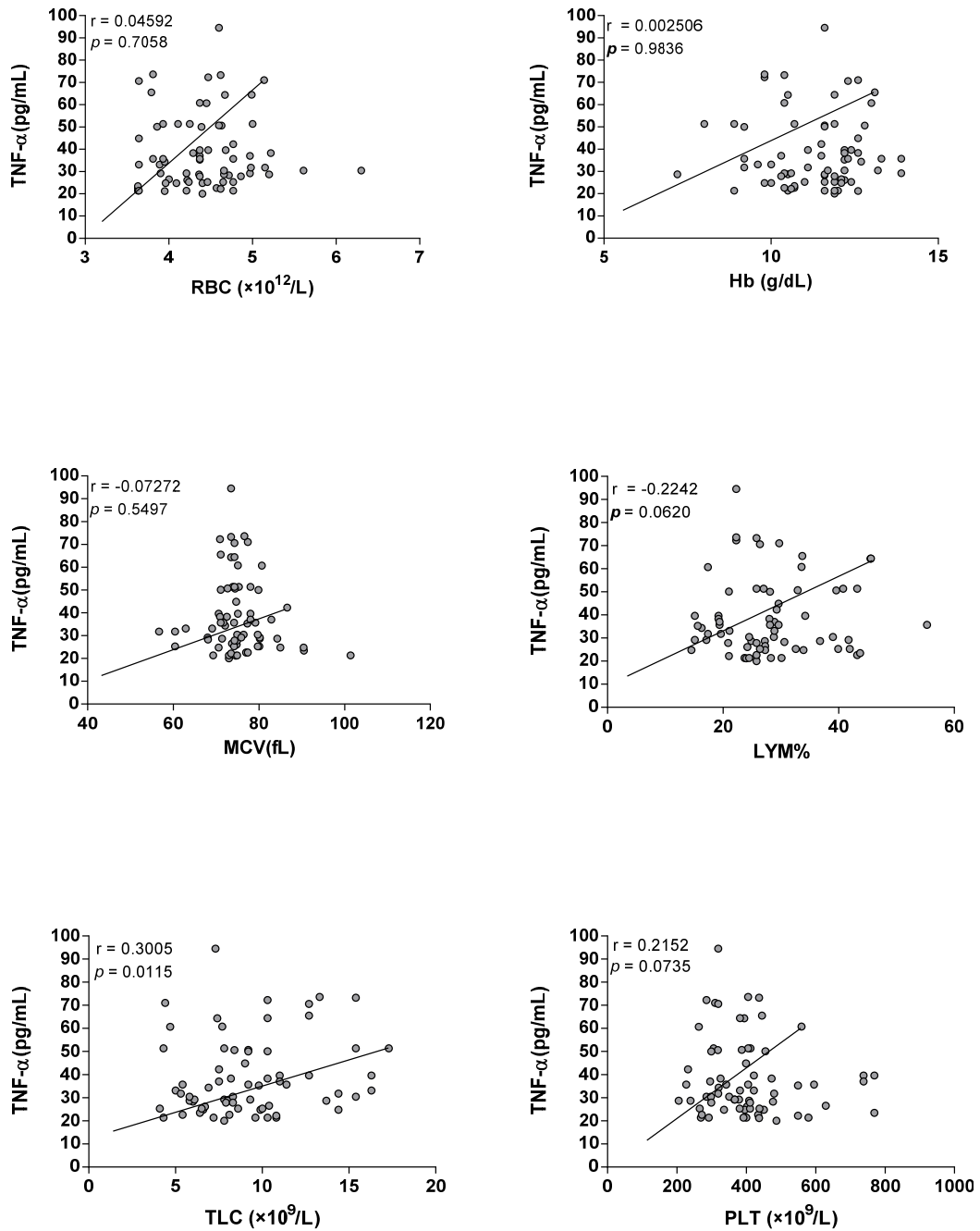
Pearson correlation test was conducted.

RBC: red blood cells,

Hb: hemoglobin,

MCV mean cell volume,

TLC: total leukocytes counts.



**Fig.3: Relationship between TNF-alpha and hematological biomarkers in rheumatoid arthritis patients**

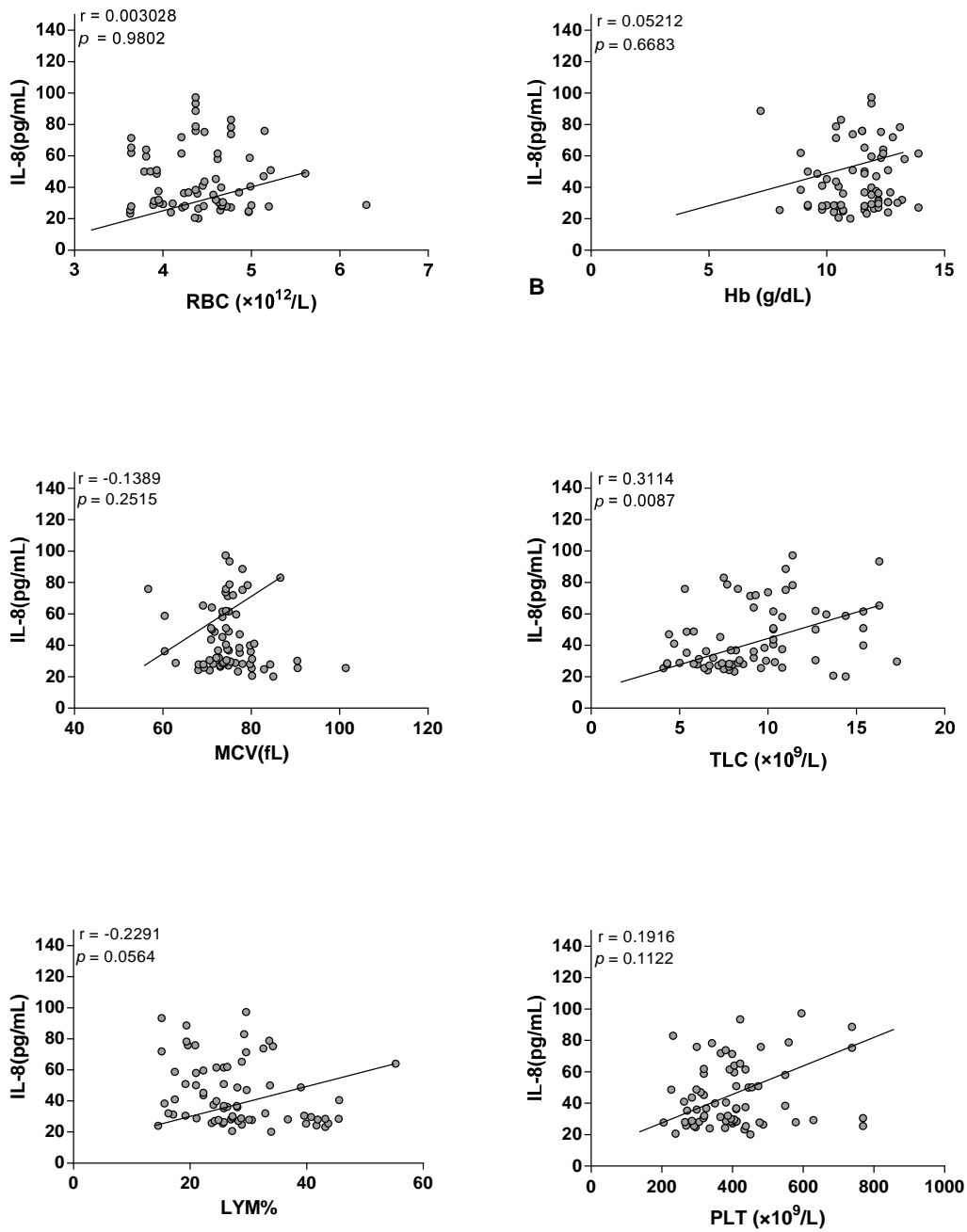
Pearson correlation test was conducted.

**RBC:** red blood cells,

**Hb:** hemoglobin,

**MCV** mean cell volume,

**TLC:** total leukocytes counts.



**Fig.4: Relationship between interleukin-8 and hematological biomarkers in rheumatoid arthritis patients**

Pearson correlation test was conducted.

RBC: red blood cells,

Hb: hemoglobin,

MCV mean cell volume,

TLC: total leukocytes counts.

#### 4. DISCUSSION

This research article demonstrated a significant elevation in serum interleukin-6 (IL-6), interleukin -1alpha, tumor necrotic factor- alpha (TNF- $\alpha$ ) and interleukin-8 in RA patients in contrasted with control people. This investigation supported the formerly observations which well documented that the pro-inflammatory cytokines are associated with the RA pathogenesis [14, 15]. The cytokine IL-6 elicits an intense inflammatory reaction and has a significant role in the pathogenesis of different inflammatory arthritis especially rheumatoid arthritis [16]. TNF alpha also assumes a basic part in rheumatoid arthritis pathogenesis, in perspective of that hostile to the treatment of TNF alpha is useful in monitoring incessant aggravation of rheumatoid arthritis [17]. Proinflammatory cytokines, for example, interleukin -1alpha, interleukin-8 (chemokine) and tumor necrosis factor- alpha (TNF- $\alpha$ ) mediate the irritation and subsequent attracting the inflammatory cells to the site of inflammation particularly synovia which excite bone and articular pulverization in patients with RA [18]. The present research article likewise demonstrates significant depression in erythrocytes profiles included red cells counts, hemoglobin concentration, hematocrit, and indices of red cells in all patients with rheumatoid arthritis. This results supported the previous observations which speculate that depression of erythrocytes profile or anemia is the most obvious non-joint signs of rheumatoid arthritis [9, 19]. Then again, present observations revealed that the white cell counts, lymphocytes percentages, and thrombocytes have a significant rise in RA patients contrasted with controls subjects. Cascão et al., [20] corresponded with current investigations in that the white blood cell (particularly lymphocytes) assumes an essential role in the RA initiation. Truth be told, polymorph nuclear leukocytes are the abundant leukocytes in the patients synovial fluid, then in the early stage of discuses, the polymorph nuclear leukocytes indicate altogether bring down apoptosis levels [21]. Our outcomes additionally uncovered elevated thrombocytes levels related with all each patient suffering from rheumatoid joint inflammation. Gasparyan et al., [22] have appeared in their RA contemplate thrombocytosis and thrombocytes-inferred proteins (growth factors) inside the synovia and synovial liquid. Dakhil et al., [23] is likewise demonstrated an expansion in circulating platelets as firmly identified with fiery markers, and assume a pivotal part in the onset and pathogenesis of RA disease. Nonetheless, there is certain connection was found between serum levels of interleukin-6 cytokines and white blood cell count, and lymphocytes. IL-6 has pleiotropic effect in the inflammatory response [24]. IL-6 may have double parts in inflammation; prior to inflammatory reactions and hostile to inflammation. Moreover, serum groupings of IL-6 were Contrarily connected with hemoglobin, HCT, RBC, and red cell indices. Late examinations recommend that interleukin-6 could particularly elicit growth factor erythropoietin which responsible for erythropoiesis induction in bone marrow, so stifle ordinary bone marrow in a measurement subordinate manner [25].

#### CONCLUSION

The current research article revealed elevated level of proinflammatory cytokines interleukin-8, interleukin-1alpha, interleukin-6 and tumor necrotic factor-alpha (TNF- $\alpha$ ) in rheumatoid joint inflammation patient contrasted to typical controls. The cytokines were essentially correlated with haematological biomarkers. These outcomes recommend that the cytokines may be associated with the RA pathogenesis and reflect the improvement of the ailment.

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