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# Interleukin-6 levels in early and late rheumatoid arthritis: their comparison and correlation with clinical and laboratory indicators of disease activity.

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## ABSTRACT

**Background**: An inflammatory condition called rheumatoid arthritis (RA) is characterized by persistent inflammation that damages the cartilage and joints. It can result in different levels of osteoarthritis and cause varying degrees of disability and it has been discovered that RA is significantly influenced by interleukin-6 (IL-6). As demonstrated by numerous clinical studies, Tocilizumab, a humanized anti-IL-6 receptor monoclonal antibody, and the first-in-class IL-6 inhibitor, has demonstrated exceptional efficacy in RA. **Aim:** We aimed to evaluate the level of IL-6 for prediction, diagnosis, and staging for rheumatoid arthritis patients, and reducing joint discomfort and swelling is the main goal of rheumatoid arthritis treatment. **Material and methods:** The study included 105 patients (aged 20 - 74 yr) divided into three groups. Group I: Involved 30 healthy controls, Group II: involved 15 patients with early RA, and Group III: involved 60 patients with late RA. Routine clinical tests such as Alt, Ast, Creatinine, Urea, Tlc, Hgb, Plt, RF, ESR, and CRP were assayed for all patients. In addition, serum Interleukin-6 levels were quantified using sandwich ELISA. The absolute values of investigated markers were statistically analyzed using the SPSS program. **Results:** The mean IL-6 level was (8.75 ± 0.56 pg/ml), (17.76 ± 5.32 pg/ml) and (32.71 ± 10.08 pg/ml) for G1, G2 and G3 respectively. Our study found the levels of IL-6 in the serum of patients with late RA and early RA were extremely significant than the normal group (p=0.0001) **Conclusion:** IL-6 has a greater sensitivity and specificity than other inflammatory markers, making it useful for early detection of RA.

**Key Words:** Rheumatoid Arthritis, IL-6, RF, CRP, ESR.

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## INTRODUCTION

A systemic autoimmune disease linked to a persistent inflammatory process, rheumatoid arthritis (RA) can harm not just joints but also extra-articular organs such as the heart, kidney, lungs, digestive tract, eye, skin, and nervous system [1,2].

To prevent long-term joint damage and enhance function, disease-modifying antirheumatic medication (DMARD) treatment should begin as soon as RA is diagnosed to reduce such damage [3, 4]. As the "anchor medicine" in the treatment of RA, methotrexate (MTX) is frequently recommended to be started early according to

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clinical practice standards [5, 6]. There don't seem to be many clinically relevant hepatic adverse effects linked to MTX. Given these findings, it may be worthwhile rewriting the present MTX monitoring guidelines to encourage less frequent monitoring, particularly for patients who do not have any risk factors for liver disease [7].

Chronic inflammation combined with toxicity or exposure to drugs are the main causes of the numerous renal problems linked to RA. Membranous nephropathy, immunoglobulin A nephropathy, minimal change disease, pauci-immune glomerulonephritis, analgesic nephropathy, interstitial nephritis, mesangial proliferative glomerulonephritis, and AA amyloidosis are the most frequently seen renal disorders in people with RA who have undergone kidney biopsies [8, 9].

The incidence of renal illness may have changed over time due to changes in RA treatment patterns. In the past, more widely used agents like d-penicillamine and gold salts were directly connected to renal illness and proteinuria [10,11]. On the other hand, RA patients who receive cyclosporine medication can suffer from dose-related nephrotoxicity [12, 13]. Biologic medicines, such as inhibitors of tumor necrosis factor α, have become a successful treatment in recent years. Kidney illness remains a disease- and treatment-related characteristic of RA, as evidenced by numerous case reports pointing to a connection to glomerulonephritides and etanercept [14]. Lastly, renal injury is known to be caused by long-term maintenance anti-inflammatory therapy with cyclooxygenase 2 inhibitors or nonsteroidal anti-inflammatory medications (NSAIDs) [15].

The quintessential cytokine, interleukin (IL)-6, has redundant and pleiotropic functional action. Cardiotrophin 1 (CT-1), leukemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF), oncostatin M (OSM), cardiotrophin-like cytokine factor 1 (CLCF1), IL-6, IL-11, IL-27, and IL-31 are among the cytokines that use the common IL-6 signal transducer gp130 [16]. Over the past decade, Interleukin-6's (IL-6) multifaceted role in biological activities has been better understood during the past ten years [17,18]. Many diseases, especially inflammatory conditions like rheumatoid arthritis (RA), have been linked to the initiation or progression of IL-6 dysregulation [19,20], as evidenced by increased RA disease activity has been linked to elevated IL-6 levels in serum, synovial fluid, and other tissues [21,22].

## Material and Methods

### A- Patients, controls, and sample collection

The recent study involved 105 patients. Ethical committee approval was taken from the Faculty of Pharmacy in Minya University **(MPEC 240301)** and informed consent was approved for all patients from Minya University Hospital. Samples were collected in the period from December 2023 to October 2024.

The following patients were included: (1) patients were suffering from early Rheumatoid arthritis (2) patients were suffering from late Rheumatoid arthritis.

The samples were classified into three groups: **Group I (control)** involved thirty patients who appeared to be healthy; and this sample was excluded from liver disease, chronic kidney disease, and diabetes. **Group II (early RA)** consisted of fifteen patients with symptom duration < 3 months as early disease. **Group III (late RA)** included sixty patients with symptom duration > 3 months as chronic disease.

10 ml of venous blood from each patient was taken and divided into 2 ml fresh blood into an EDTA tube was analyzed to make a CBC test, 1.6 ml of fresh blood was into a sodium citrate tube to make an ESR test, and then the serum was separated by centrifugation for 20 minutes at 1000 x g to measure liver function tests, kidney function tests, blood inflammatory marker tests, and serum interleukin-6 levels. Serum was subsequently refrigerated at -80˚C. The following procedures were applied to all patients and controls: complete clinical assessment and taking a full history.

### B- Biochemical Examination

Standard laboratory testing was measured including CBC, Alt, Ast, Creat, Urea, ESR, CRP, and RF according to routine methods.

### 1- Assessment of Serum IL-6 by ELISA

Using a human interleukin-6 assay kit all serum samples were analyzed for IL-6 using ELISA technique as directed by the manufacturer. In summary, serum samples and prepared standards were added to the relevant ELISA plate wells in 100 µl increments, and the assay was conducted. A micro test plate spectrophotometer (Abcamm CA, USA) was used to detect the absorbance at 450 nm. Human IL-6 was used as a standard to quantify IL-6 using a calibration curve. Every standard or sample underwent duplicate analysis.

### C- Statistical analysis

The statistical program SPSS 18 was used to analyze the data. The result was shown as the standard deviation plus the mean (M ± SD). Chi-square analysis was used to compare the two groups' quantitative variables. A P value <0.05 was considered statistically significant, while a p value <0.001 was considered highly significant. The ROC curve was applied to determine the test's sensitivity and specificity, as well as the optimal cut-off value for the diagnostic biomarker under consideration. Furthermore, the area under the curve (AUC) was calculated to assess accuracy.

## RESULTS

**Demographic data of selected patients and controls:**

The study comprised 105 patients, aged between 20 and 74 years, with 12 men (11.4%) and 93 females (88.6%). **Group I** (control) comprised 30 subjects who appeared healthy, with ages with a mean value was 35 years. **Group II** (early RA) comprised 15 patients; their ages with a mean value was 38 years. **Group III** (late RA) comprised 60 patients; whose ages with a mean value was 48 years. All examined groups' mean age and gender values did not differ statistically (P > 0.05) as described in ***Table (1)*** and ***Figure (1)***. ***Table (2)*** and ***Figure (2,3,4)*** describe the laboratory results for Hematological parameter Levels, liver function tests Levels, and Kidney function tests Levels for each of the 105 patients in the various groups. The mean Hb, TLC, Plt, Ast, Alt, Creat, and Urea values revealed no statistically significant differences (P>0.05) among significant RA patients (G2-G3) and Healthy control (G1). ***Table (3)*** and ***Figure (5,6(b,c))*** describe the laboratory results for blood inflammation tests (ESR and CRP) and Rheumatoid Factor (RF) values revealed that highly Significant changes (P < 0.0001) in early RA and late RA compared with the control group.

### 3.2. Immunochemical identification and quantification of interleukin-6 in human serum

The concentration of interleukin-6 in serum was quantified using the sandwich ELISA technique**.** ***Figure (6a)*** and ***Table (4)*** show the ELISA-measured serum levels of interleukin-6 in the patient groups and the healthy control group.

The interleukin-6 cutoff of ELISA was determined by calculating the mean ELISA OD ± 3SD of 30 sera samples from G1 (control), to determine if a tested sample is positive or negative. The best cutoff level was set at concentrations of 15 pg/mL. By applying cut-off for all 105 samples tested by ELISA, RA patients were predicted, with a high degree of sensitivity, specificity, NPV, and PPV, and efficiency (> 85%). ***Table (4)*** by using the area under the ROC curve (AUC), *the* diagnostic value of the IL-6 was evaluated. The AUC of IL-6 (pg/mL) was 0.965 (P < 0.0001) in comparison with CRP, ESR, and RF as viewed in ***Figure (7).*** By using IL-6 cut-off = 15 Pg/mL, significant IL-6 was expected, moreover, the sensitivity, specificity, and efficiency were evaluated as 93.33%, 96.6%, and 94.2 %; respectively by using the same cut-off as shown in ***Figure (8,9).***

**Table (1):** Number, Age, and gender of healthy, early RA, and late RA samples

| Samples | Healthy | Early | Late |
|---|---|---|---|
| **No.** | 30 | 15 | 60 |
| **Age:** | Mean 35 | Mean 38 | Mean 48 |
| **Gender:** | Male: 5 / Female: 25 | Male: 1 / Female: 14 | Male: 6 / Female: 54 |

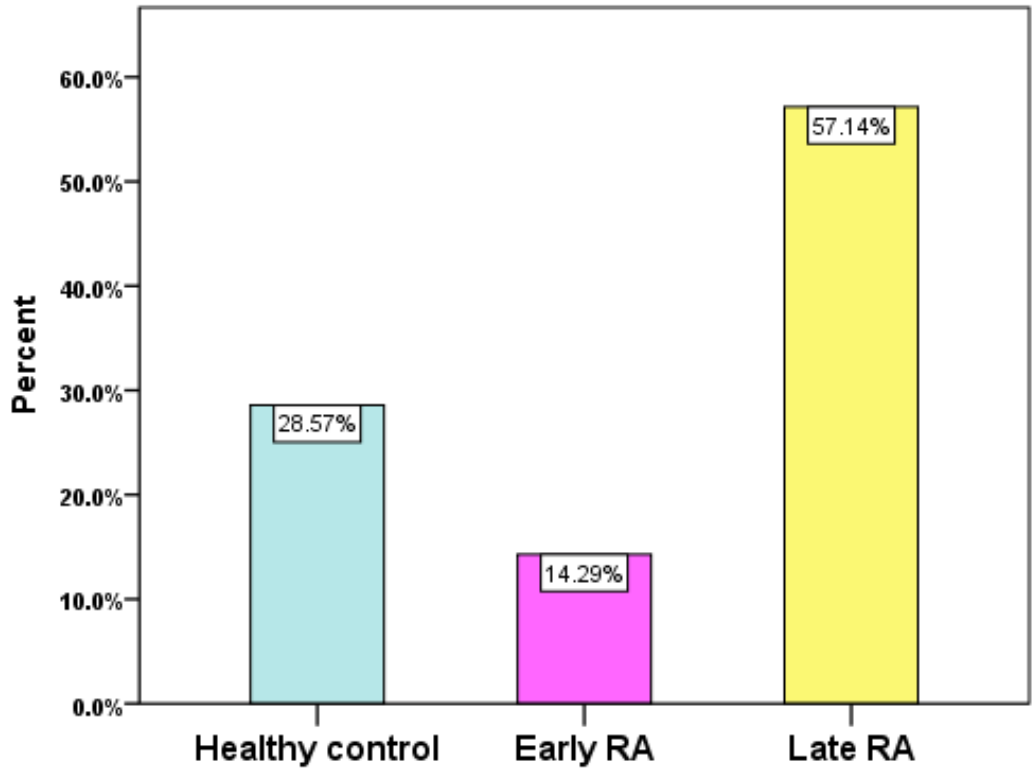



**Figure (1). Pathology distribution in patients.** 28.57 % were healthy (G1), while 14.29 % were early rheumatoid arthritis (G2) and 57.14% (G3) were late rheumatoid arthritis

**Table (2):** Liver function, kidney function, and hematological parameters of samples in three groups

| Hematological parameters | G1 Control | G2 Early RA | G3 Late RA | **P value |
|---|---|---|---|---|
| 1) Hb (g/dl) | 13.08 ± 1.82 | 11.78 ± 1.44 | 11.21 ± 1.37 | 0.06 |
| 2) TLC (×$10^3$µl) | 6.67 ± 1.70 | 7.16 ± 1.98 | 6.03 ± 1.72 | 0.05 |
| 3) PLt(*$10^3$µl) | 258.23 ± 58.98 | 300.40 ± 84.57 | 274.55 ± 86.73 | 0.05 |
| **Biochemical Marker** | **G1 Control** | **G2 Early RA** | **G3 Late RA** | **P value** |
| 1) ALT (IU/L) | 18.06 ± 2.81 | 19.13 ± 4.13 | 20.66 ± 6.39 | 0.08 |
| 2) AST (IU/L) | 18.6 ± 3.46 | 19.20 ± 3.56 | 21.13 ± 6.13 | 0.05 |
| 3) Creatinine (mg/dl) | 0.77 ± 0.18 | 0.78 ± 0.16 | 0.90 ± 1.16 | 0.05 |
| 4) Urea (mg/ml) | 22.30 ± 4.60 | 23.86 ± 4.61 | 27.31 ± 6.93 | 0.06 |

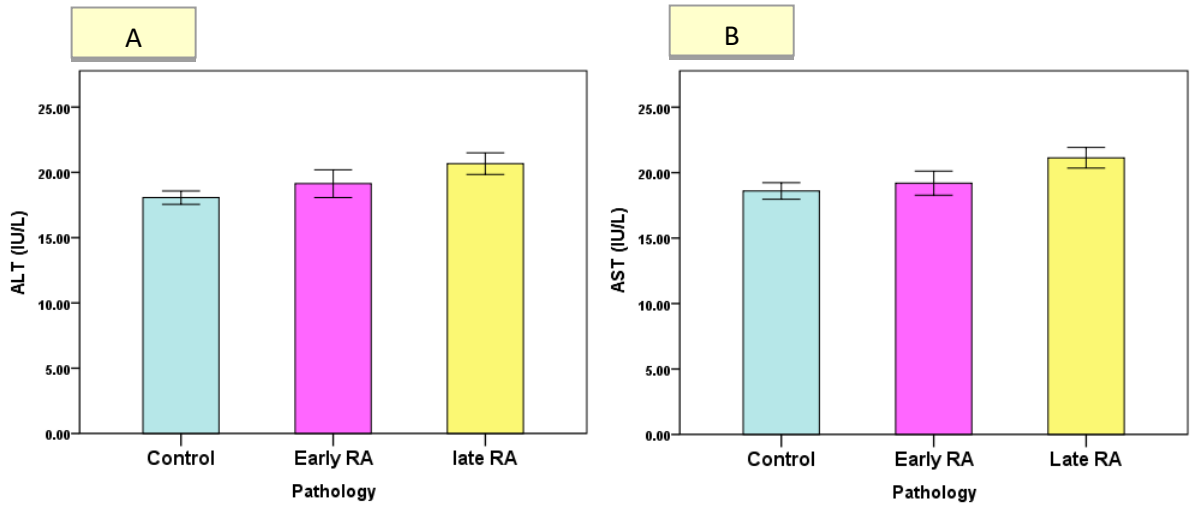



**Figure (2). Bar charts represent mean ±SD of (A): ALT (IU/L), (B): AST (IU/L) levels between three groups.** The overall significance of differences between each of the three groups was assessed using a t-test. No Significant changes (P > 0.05) were seen among RA patients and normal controls.

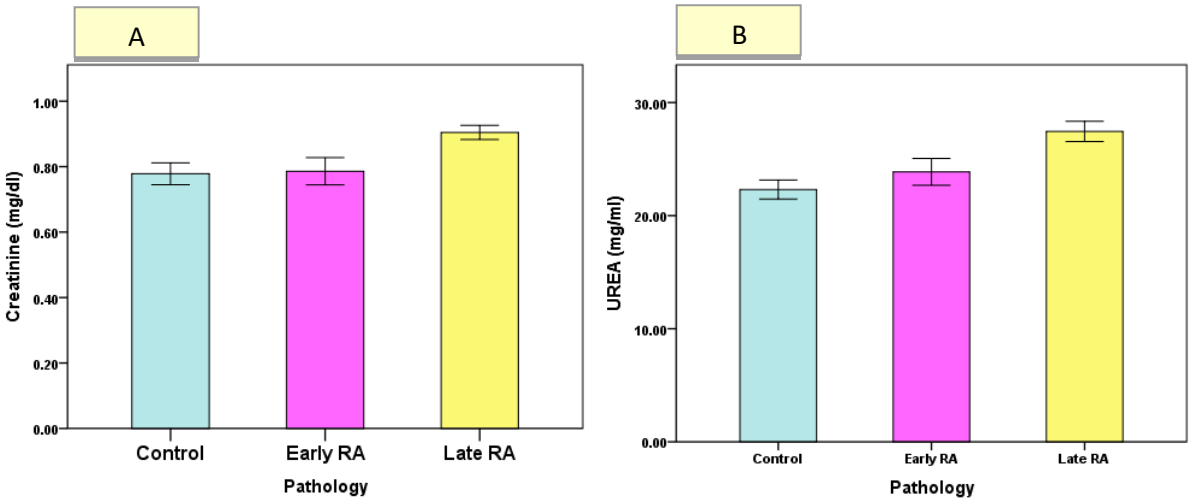



**Figure (3). Bar charts represent mean ±SD of (A): Creatinine (mg/dl), (B): Urea (mg/ml) levels between three groups.** The overall significance of differences between each of the three groups was assessed using a t-test. No Significant changes (P > 0.05) were seen among RA patients and normal controls.

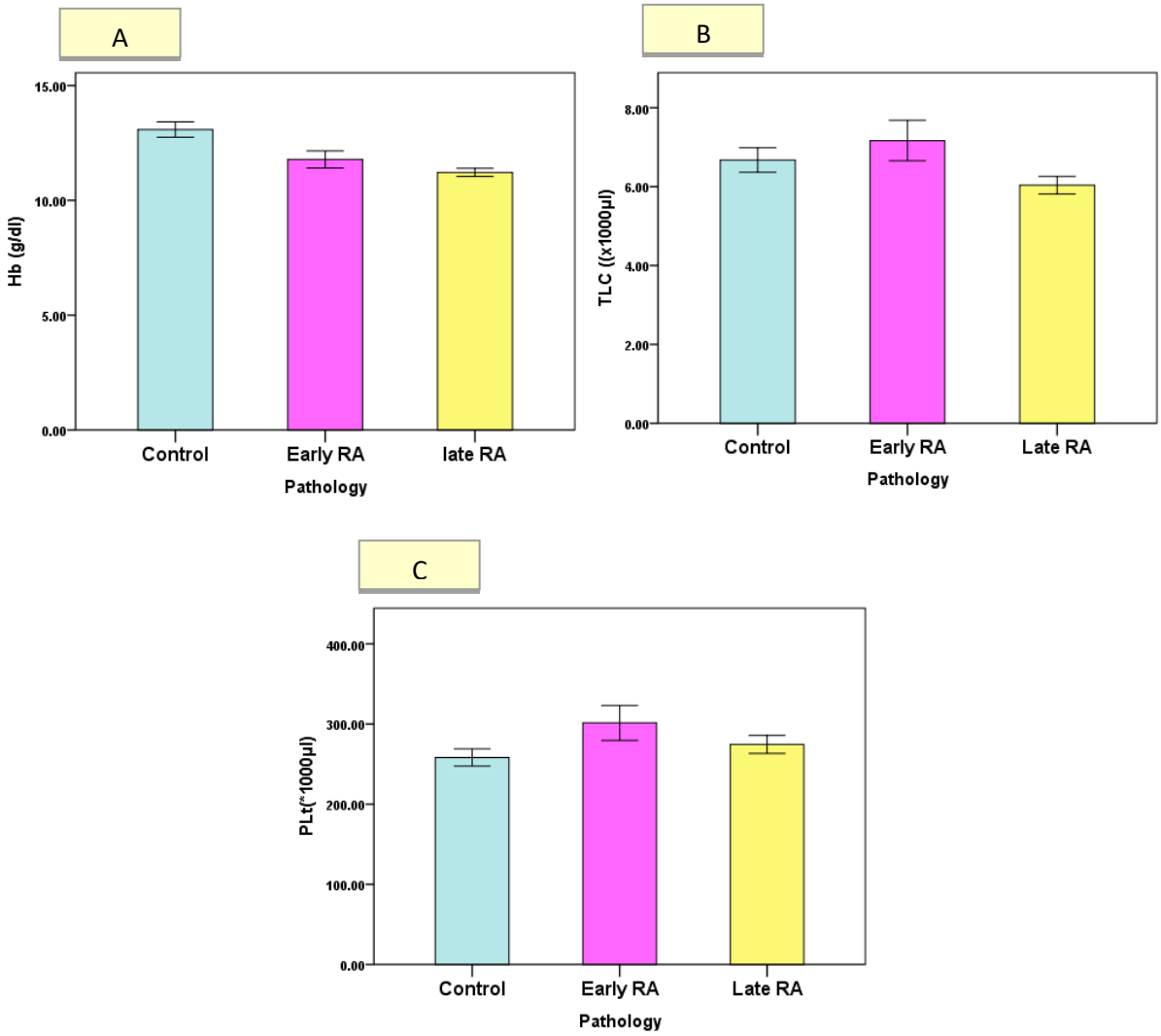



**Figure (4). Bar charts represent mean ±SD of (A): Hb (g/dl), (B): TLC (×$10^3$µl), (C): PLt(*$10^3$µl) levels between three groups.** The overall significance of differences between each of the three groups was assessed using a t-test. No Significant changes (P > 0.05) were seen among RA patients and normal controls in all hematological markers. HB: hemoglobin, TLC: Total Leukocyte Count, PLt: platelets

**Table (3):** Concentration of ESR, CRP and RF of samples in three groups

| Markers | G1 Control | G2 Early RA | G3 Late RA | **P value |
|---|---|---|---|---|
| **1) I$^{st}$ hour ESR (mm/hr),** | 5.96 ± 1.27 | 46.73 ± 16.29 | 32.88 ± 17.72 | 0.0001 |
| **2) 2$^{nd}$ hour ESR (mm/hr)** | 10.8 ± 1.98 | 82.46 ± 24.13 | 61.40 ± 85 | 0.0001 |
| **3) CRP (mg/l)** | 4.06 ± 0.78 | 32.53 ± 3.00 | 19.6 ± 2.12 | 0.0001 |
| **4) RF(u/ml)** | 4.6 ± 1.06 | 25.66 ± 2.81 | 19.8 ± 1.23 | 0.0001 |

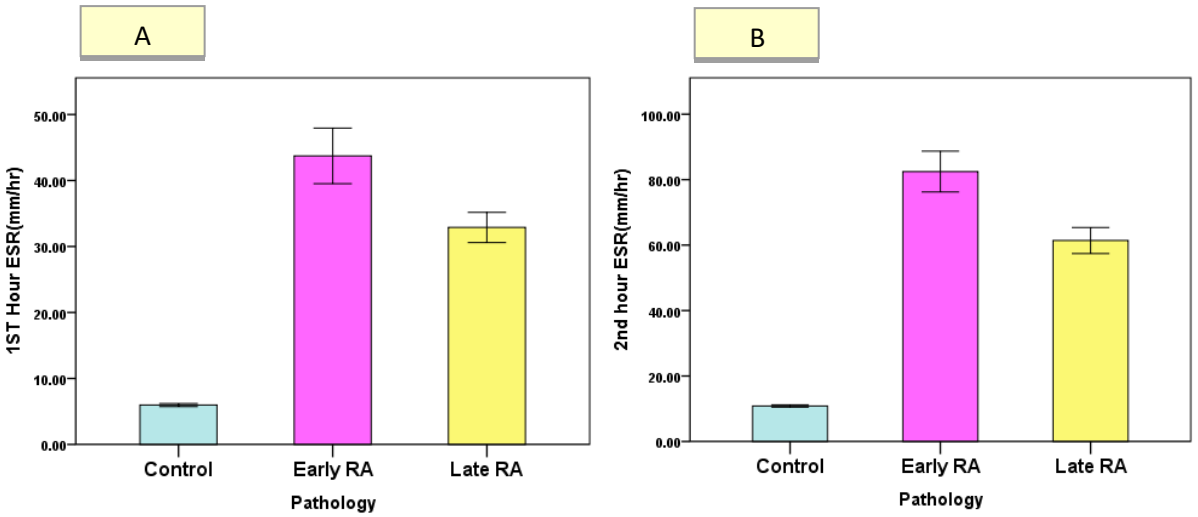



**Figure (5). Bar charts represent mean ±SD of (A): I$^{st}$ hour ESR (mm/hr), (B): 2$^{nd}$ hour ESR (mm/hr) levels between three groups.** The overall significance of differences between each of the three groups was assessed using a t-test. there are high significant differences ($P < 0.0001$) were seen among RA patients and normal controls in ESR level. 2$^{nd}$ hour ESR: second hour Erythrocyte Sedimentation Rate. I$^{st}$ hour ESR: first hour Erythrocyte Sedimentation Rate

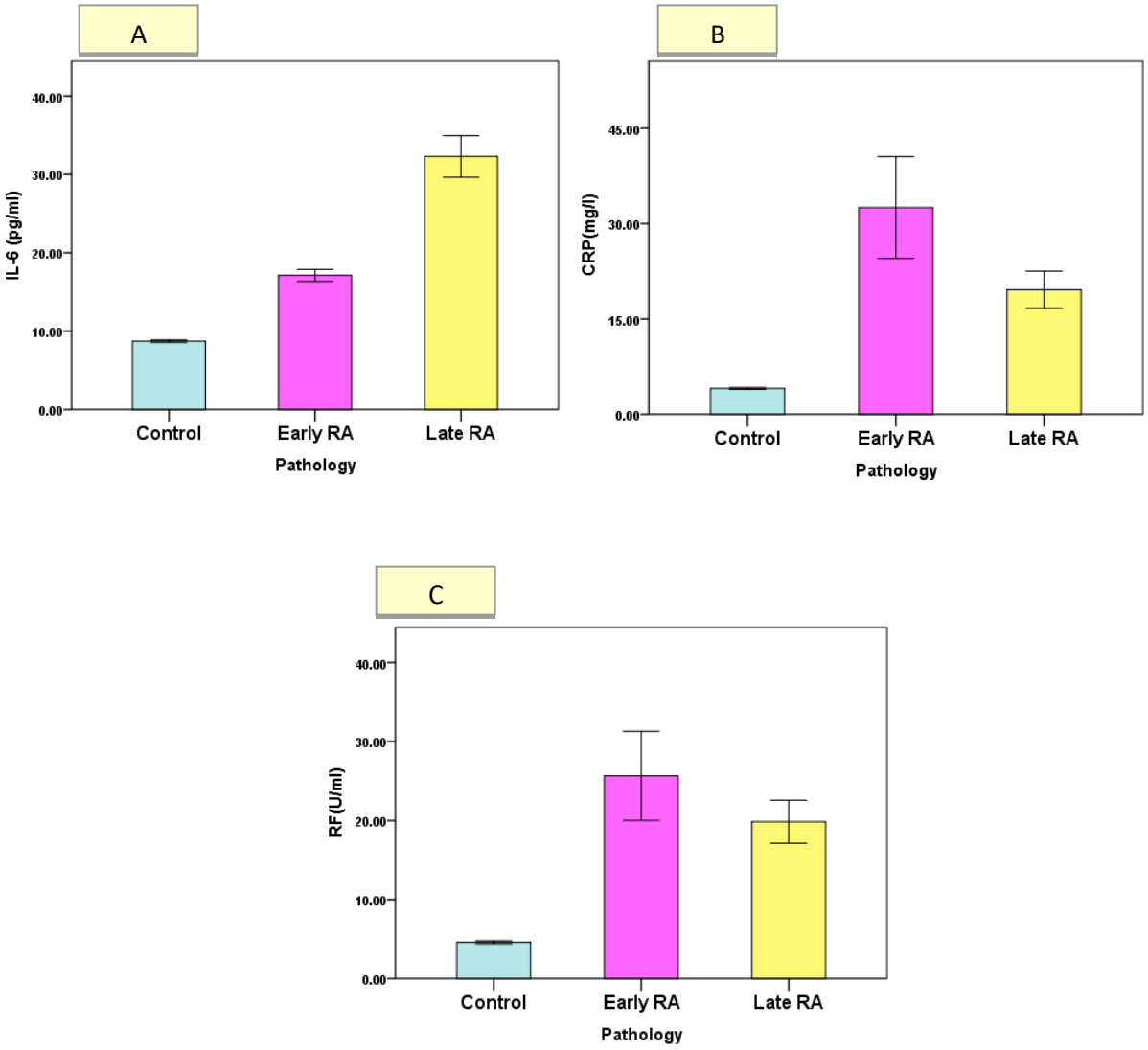
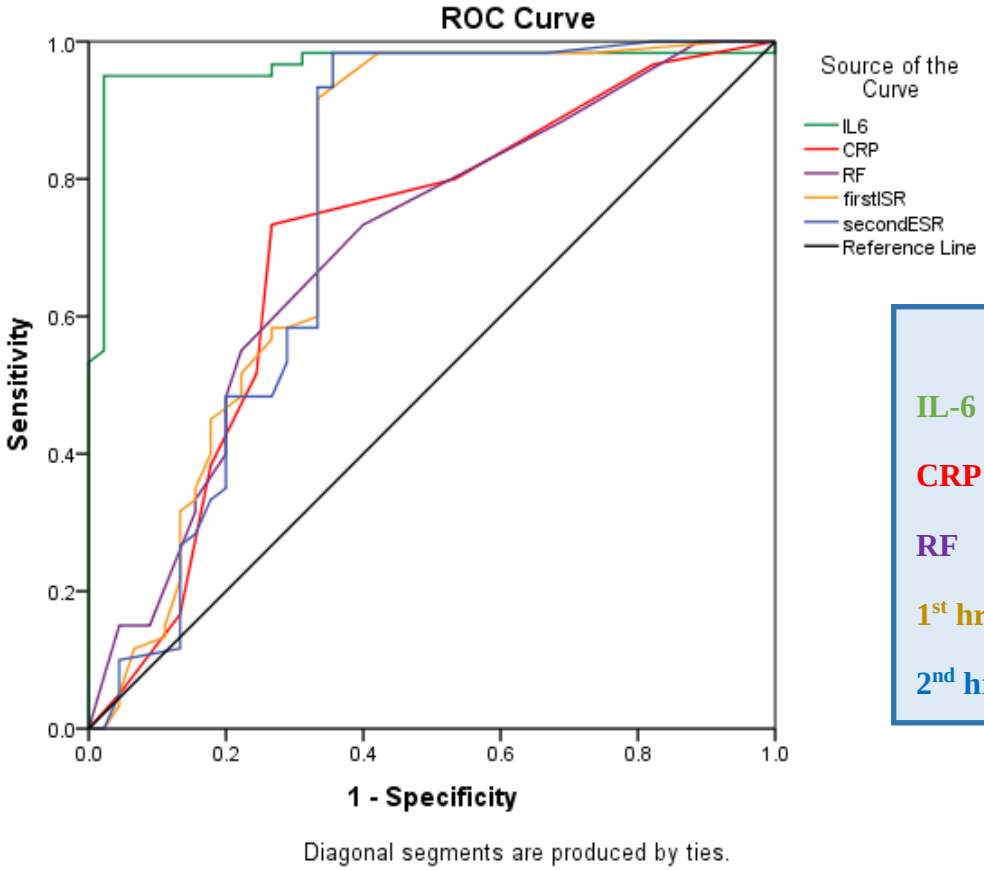



**Figure (6). Bar charts represent mean ±SD of (A): IL-6 (pg/ml), (B): CRP (mg/l), and (C): RF(u/ml) levels between three groups.** The overall significance of differences between each of the three groups was assessed using a t-test. High significant changes ($P < 0.0001$) were seen among RA patients and normal controls. IL-6: interleukin 6, CRP: C-reactive protein, RF: Rheumatoid Factor.

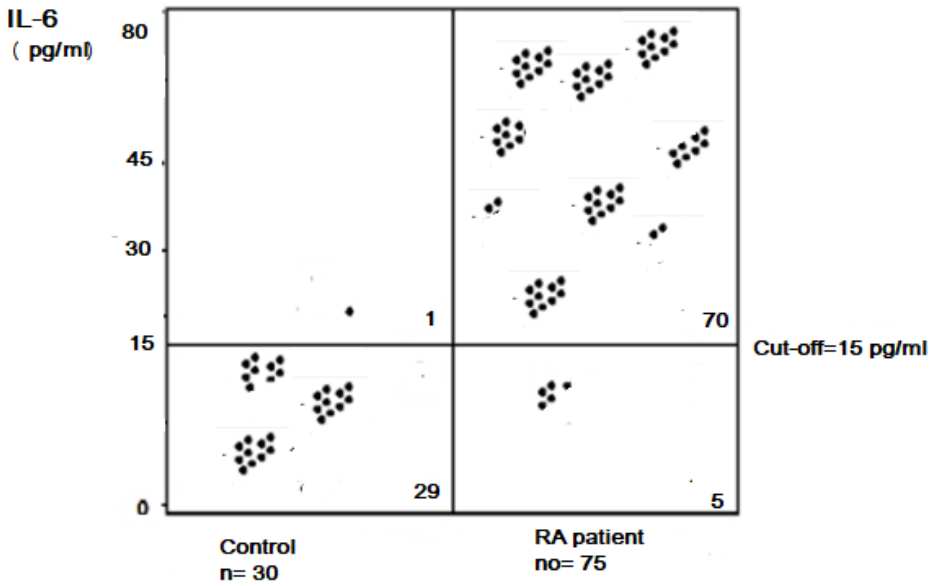
**Table (4):** Concentration of IL-6 of healthy, early RA and late RA samples

| | G1 Control | G2 Early RA | G3 Late RA | **P value |
|---|---|---|---|---|
| **1) IL-6 (pg/ml),** | 8.75 ± 0.56 | 17.76 ± 5.32 | 32.71 ± 10.08 | 0.0001 |




| AUR | |
|---|---|
| IL-6 | 0.965 |
| CRP | 0.701 |
| RF | 0.700 |
| 1st hr ESR | 0.767 |
| 2nd hr ESR | 0.759 |

**Figure (7). The AUC (area under ROC curve) of all laboratory biomarkers for discriminating RA patient from healthy control.** IL-6 showed the highest area under ROC curve among all biomarkers followed by ESR




- **Figure (8). Cut off levels of IL-6 using ELISA for control and RA samples**

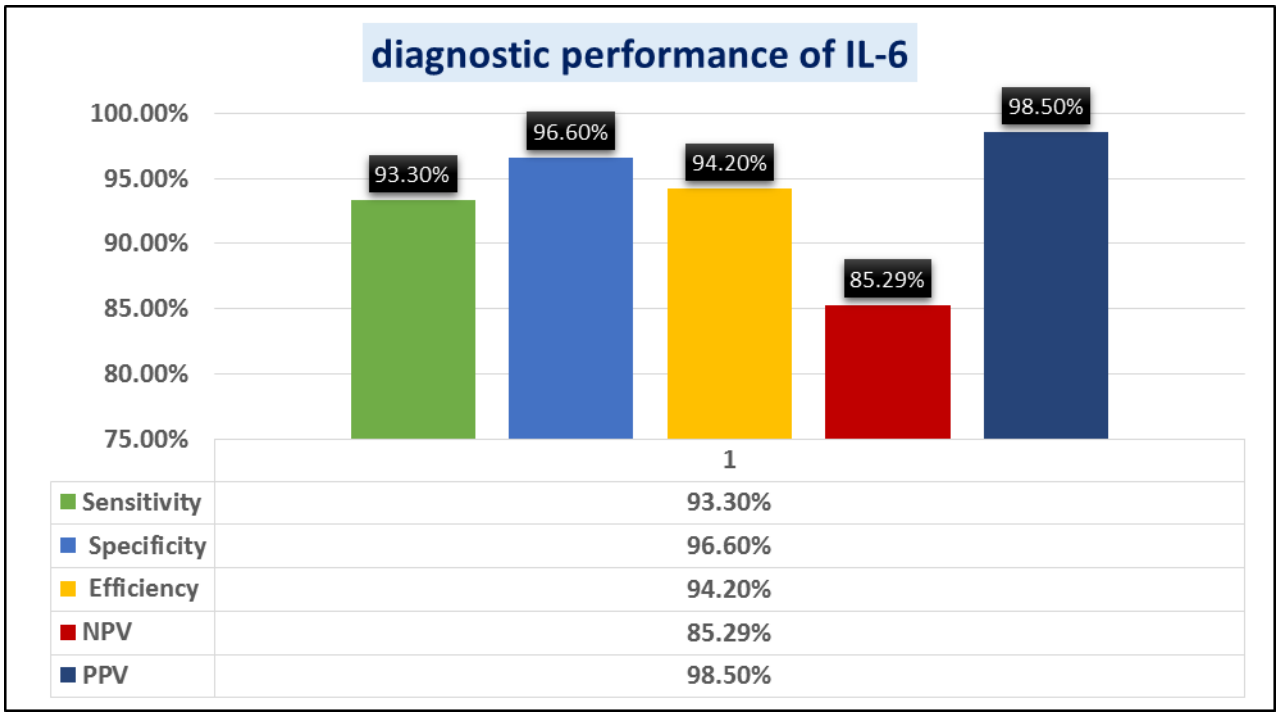



**Figure (9). Overall performance characteristics of IL-6 detection using ELISA.** By applying IL-6 cut-off = 15 pg/mL, RA patients were predicted, with a high degree of sensitivity, specificity, NPV and PPV, and efficiency (> 85%).

## DISCUSSION

RA is a model immune-mediated inflammatory disease that affects several joints. When autoantibodies are found in a patient's serum, it is linked to more aggressive articular disease, a higher incidence of extra-articular symptoms, and a higher death rate. Similar to type I diabetes mellitus, rheumatism is still linked to Early mortality and chronic morbidity, leading to cardiovascular disease-related premature death [23,24], even with significant advancements in antirheumatic treatment. A considerable percentage of patients are still unable to achieve disease remission, even though the progression of radiographic joint damage has decreased over the past few decades due to improved DMARD use and the introduction of biologics [25], which can result in disability, a decline in quality of life, a diminished capacity to work, and improved health.

With a prevalence of about 1% of the global population, RA is the most prevalent and significant inflammatory rheumatic illness in terms of socioeconomic status. It is predicted that, as the population ages, this prevalence will rise by about 22% between 2005 and 2025 [26]. Because of its great frequency, irreversible joint deterioration, and frequent co-morbidities, the disease has a significant socioeconomic burden. Treatment is available within a window of opportunity early in the disease's progression. Aggressive antirheumatic therapy can alter the disease's progression during this period, reducing the progressive degradation of

joints, preventing disability, and potentially lowering the risk of cardiovascular co-morbidity [27, 28]. It is conceivable that the preclinical phases of RA present a window of opportunity for prevention.

The literature has identified many risk factors associated with an increased risk of RA or inflammatory arthritis, including infections, vaccinations, hormonal and reproductive risk factors, including breastfeeding [29, 30], the timing, number, and outcome of pregnancies [31–32], and lifestyle factors, including diet [33, 34], smoking [35-37], and obesity [35, 38]. Moreover, exposure to silica and periodontitis has been associated with an increased prevalence of RA.

Autoantibodies are among the laboratory markers that have been linked to RA disease activity and/or prognosis. [39] Although it does not track disease activity, IgM rheumatoid factor (RF) is an excellent prognostic indicator. Its specificity for RA is relatively low [40].

RFs are autoantibodies that target immunoglobulin (Ig) G's Fc region. Despite the existence of IgA and IgG RF, IgM RF is most frequently measured in clinical practice. Although RF is present in up to 80% of RA patients, it has limited specificity because it can also arise in a variety of other inflammatory conditions that result in persistent antigenic stimulation. These comprise different rheumatologic illnesses (e.g., Sjogren's syndrome, systemic lupus erythematosus), infectious diseases (e.g., Epstein-Barr virus, hepatitis C virus, and subacute bacterial endocarditis), cancer (e.g., B-cell neoplasms), and healthy people [41]. Additionally, smoking has been linked to a higher incidence of RF [42]. Between 30% and 45% of people with early RA do not have RF, while some patients may develop it later in their RA [43]. When administered to patients who have a high pre-test likelihood of developing an illness (such as those with inflammatory arthritis), RF's positive predictive value rises, as it would with any diagnostic test. It is not advised to test people who have osteoarthritis, myalgia, or nonspecific arthralgia [44]. Higher titers indicate a higher likelihood of developing RA, and RF positive raises that risk [45–46]. Although RF titers may decrease with successful RA treatment, there is no reliable correlation between RF titer fluctuations and disease activity [47]. It is not advised to monitor RF levels serially [48–49]. RF positive may raise the likelihood of responding to B-cell depleting monoclonal antibodies (like rituximab) when choosing RA therapy [50].

The rate at which erythrocytes fall through plasma while suspended in a vertical tube, or ESR, is an indirect measure of the amounts of acute-phase reactants, primarily fibrinogen. ESR levels are influenced by red blood cell size, shape, and number as well as other plasma constituents including immunoglobulins. Obesity, end-stage renal disease, nephrotic syndrome, infection, cancer, tissue damage, and systemic or local inflammatory processes can all result in elevated ESR values. Women's ESR readings are somewhat higher than men's, and they rise with age. Moreover, a variety of conditions, including heart failure, cachexia, severe leukocytosis, and aberrant erythrocyte shape, can cause abnormally low ESR levels [51]. The ESR is not a particular indicator of inflammation, which is not surprising.

The pentraxin protein family, which includes pattern recognition molecules involved in the innate immune response, includes CRP, an acute-phase reactant [52,53]. CRP can be infectious or noninfectious, and it can occur in both acute and chronic inflammatory conditions. Numerous metabolic stresses, such as atherosclerosis, obesity, type 2 diabetes, sedentary lifestyles, poor food, and even being single, are linked to low-grade CRP rise [54–55]. Compared to ESR levels, CRP levels are less affected by age, sex, and race [56]. Moreover, CRP results differ between laboratories and lack a

recognized reference range or unit of measurement [57]. The high level of pro-inflammatory cytokines in the RA synovium causes the liver to produce more CRP, which makes it a desirable option for a disease activity biomarker [58]. CRP measurement in RA is not perfect, unfortunately. For instance, in women with RA, truncal adiposity is independently linked to higher CRP levels, irrespective of articular involvement or the use of biological treatments [59].

Despite its flaws, ESR and CRP tests are still used in the diagnosis and treatment of RA. Elevated ESR and CRP readings are part of the 2010 ACR/EULAR Classification Criteria for RA [60]. CRP levels of less than or equal to 1 mg/dL are included in the 2011 ACR/EULAR criteria of RA remission used in therapy studies [61]. ESR or CRP measurement is part of the Simplified Disease Activity Index (SDAI) and the Disease Activity Score 28-ESR or CRP (DAS28-ESR or DAS28-CRP), two of the six RA disease activity markers that the ACR has authorized for use in clinical practice [62]. Although it does not state that measures that incorporate laboratory data are preferable to those that do not, the 2015 ACR Guideline for the Treatment of RA, which is frequently utilized in clinical practice, promotes the use of these disease activity measures. Furthermore, routine ESR and CRP monitoring in all RA patients is not expressly advised by the guidelines [63]. These treatment guidelines are presently being updated, with a fall 2021 release date planned. Elevations in ESR and CRP have been linked in numerous studies to radiographic and functional outcomes in RA patients [58,64]. While CRP may be better in later stages of the disease because of its reduced vulnerability to other factors like immunoglobulin levels and anemia, elevated ESR is believed to be a better predictor of these outcomes in early RA [35]. Nevertheless, around 40% of RA patients have normal ESR and CRP [65–66]. Moreover, readings may be constant even in patients who had baseline increases even after receiving treatment that improves their clinical condition [67]. It's interesting to note that CRP and ESR levels can differ as well [51]. In large observational research that included almost 9,000 patients from a practice-based registry, 26% of patients reported variable ESR and CRP measurements, even though joint counts and global ratings indicated active RA [68]. Results may no longer be able to forecast the development of radiographic joint deterioration when they are inconsistent [69]. Lastly, since biological medications like tocilizumab, a humanized monoclonal antibody targeting the interleukin-6 receptor, correct CRP levels, the use of CRP as a trackable disease activity biomarker will be discontinued.

Since hepatocytes express significant levels of IL-6R and gp130, it was formerly believed that IL-6 primarily targets the liver. However, in recent years, it has become increasingly evident that IL-6 does not act on a single target organ [70]. IL-6 is an acute-phase protein that exacerbates inflammation in the body. In reality, the liver is where most of its effects originate. There, it is processed and sets off an inflammatory cascade that produces 1-antichymotrypsin, fibrinogen, haptoglobin, serum amyloid A (SAA), and C-reactive protein (CRP). IL-6 also lowers albumin, zinc, and iron levels through a number of mechanisms [71].

The effects of IL-6 on the immune system are very fascinating; it is evident that both innate and acquired immunity are altered. Regarding innate immunity, IL-6 matures the inflammatory infiltrate and promotes the development of neutrophil and mononuclear cell infiltration. A chemoceptor for monocytes at the site of inflammation, IL-6 also exhibits its effects on T-cells and B-cells in the context of acquired immunity [72,73].

The development of RA is also influenced by other immunologic pathways, which may ultimately result in overexpression of IL-6. One important

inflammatory mediator in RA, for example, is the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) pathway, which determines an increase in TNF, which raises IL-6 levels. As IL-6 has been linked to cytokine release syndrome that is caused by T-cell treatment, it may potentially have a more subtle role in the development of RA. In many instances, blocking IL-6 produced positive outcomes, demonstrating its crucial role in inflammatory disorders [74,75]. Other RA-related systemic symptoms, particularly those affecting the neurological and cardiovascular systems, appear to be caused by IL-6.

RA patients typically exhibit increased levels of IL-6 and IL-6R in their serum and synovial fluid from affected joints [76]. Further demonstrating the interaction between innate and adaptive immunity are two more important cytokines that are currently being studied as possible therapeutic targets: IL-17 and granulocyte-macrophage colon stimulating factor (GM-CSF) [77]. Even though RA is typically considered a disease that primarily affects the joints, patients with this condition frequently have a number of comorbidities and are more likely to develop certain diseases: For example, cardiovascular illness is especially prevalent in this section of the population, and IL-6 appears to have a direct impact on this [78].
Psychiatric problems have been described similarly. Chronic illness patients, especially those with autoimmune diseases, are known to be more susceptible to a variety of psychological disorders, including depression. Higher IL-6 and CRP levels in this patient population appear to increase the likelihood of psychiatric comorbidities [79]. Therefore, focusing on IL-6 may help RA patients' general health.

Methotrexate is frequently started as monotherapy for RA and is typically effective at doses between 15 and 25 mg. It is also compatible with sulfasalazine and hydroxychloroquine, two other DMARDs medications. When methotrexate is ineffective, a biological DMARD is often used in conjunction with it for improved outcomes. It should be mentioned that the absorption of oral methotrexate varies greatly; this can be increased by utilizing a subcutaneous delivery route or by splitting the weekly dose [80]. Oral methotrexate is primarily eliminated by the kidneys by glomerular filtration and active tubular secretion [81], and it is typically absorbed through the small intestine's protein-coupled folate transporter [82]. About 10% of the drug's excretion is biliary, with some enterohepatic recycling, and the remainder is processed in the liver [83]. When low-dose methotrexate is consumed, peak plasma concentrations reach 1-2 hours later, and the majority of the medication is withdrawn from the body within 24 hours [84].

MTX clearance: 20–35% of the medication is secreted with the bile and metabolized or moved to other compartments, whereas 65–80% of the medication is eliminated by the kidneys (mostly during the first 12 hours after administration). While tubular secretion and reabsorption are less significant, glomerular filtration is the primary mechanism in renal elimination. A minor mechanism for the excretion of MTX, active biliary secretion becomes more significant in patients with renal failure. In the gut flora, carboxypeptidase transforms MTX that is expelled in the bile into 2,4-diamino-N10-methylpteroic acid (DAMPA) [85]. Drug toxicity is more likely to occur in patients with decreased renal function because they will have less drug clearance from plasma. Since MTX is a medication with low-to-medium protein binding and high tissue distribution, hemodialysis, and peritoneal dialysis only temporarily reduced MTX concentrations. Although MTX typically has a terminal serum half-life of 7–10 hours, certain people have longer elimination half-lives (up to 26 hours). In RA patients, MTX clearance ranges from 80 to 90 ml/min/m2 [86]. While the concentration of MTX in the serum dropped below the limit of detection 52 hours after the treatment, the

concentration in red blood cells stayed constant for 9 days [87]. By looking at just two plasma samples (at 0.5 and 2.0 hours after injection), it was possible to assess each person's clearance of MTX. Determining the ideal course of treatment is not aided by plasma MTX analyses [88].

## Conclusions

An ideal cut-off value for IL-6 of 72.80 was utilized to distinguish between RA patients and controls since the blood levels of IL-6 in RA patients were significantly higher than in healthy controls. But when it came to identifying RA patients, IL-6's sensitivity and specificity were only moderate. There was no association between IL-6 and laboratory, clinical, or demographic factors.

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## Conflicts of Interest

The authors declare that there are no conflicts of interest.

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