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Hematological Parameters and Their Correlation with C-Reactive Protein Levels in Rheumatoid Arthritis Patients Receiving Methotrexate and Folic Acid in Thi-Qar City

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Abstract

Introduction. Rheumatoid arthritis can be defined as a chronic inflammatory disease affecting multiple areas of the body, with the disorder directly affecting the synovial joints.
Purpose. This study aimed to assess hematological parameters and their correlation with C-reactive protein Levels in rheumatoid arthritis patients receiving methotrexate and folic acid in Thi-Qar City.
Materials and methods. RA patients (n=40) and healthy controls (n=20) underwent CRP titer and CBC tests. This investigation revealed that ESR and CRP levels were elevated in patients relative to healthy controls. Red blood cell and hemoglobin levels were reduced in patients relative to healthy controls. In contrast, WBC counts were elevated in patients with rheumatoid arthritis.
Results. When performing the Pearson r-test on the vital signs, it shows no statistically significant relationship between high CRP levels and RBCs, HBs, and Lymph, while it shows a relationship with Mono, PLT, and WBCs. Anemia may occur in patients with rheumatoid arthritis, as demonstrated by diminished RBC and Hb levels. Some inflammation continues even after treatment with methotrexate. CRP was significantly high and closely associated with monocytes and marginally associated with WBC.
Conclusion. There were no relationships between CRP and RBC, lymphocytes or Hb. WBC, monocytes, and other standard parameters blood tests can offer a valuable perspective on the extent of an illness and the effectiveness of treatment.
Keywords: rheumatoid arthritis, ESR, CBC, CRP, folic acid

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Гематологические показатели и их корреляция с уровнями С-реактивного белка у пациентов с ревматоидным артритом, получающих метотрексат и фолиевую кислоту, в городе Ти-Кар

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Резюме

Введение. Ревматоидный артрит можно определить как хроническое воспалительное заболевание, поражающее различные области тела, при этом заболевание непосредственно затрагивает синовиальные суставы.
Цель. Оценка гематологических параметров и их корреляции с уровнем С-реактивного белка (СРБ) у пациентов с ревматоидным артритом, получающих метотрексат и фолиевую кислоту, в городе Ти-Кар.
Материалы и методы. Пациентам с ревматоидным артритом (n=40) и здоровым лицам (n=20) были проведены анализ титра СРБ и общий анализ крови. Данное исследование показало, что уровни СОЭ и СРБ были повышены у пациентов с ревматоидным артритом по сравнению со здоровыми лицами. Уровень эритроцитов и гемоглобина был снижен, а количество лейкоцитов, напротив, было повышено у пациентов с ревматоидным артритом по сравнению со здоровыми лицами.
Результаты. При использовании r-критерия Пирсона для оценки жизненно важных показателей статистически значимой связи между высоким уровнем СРБ и количеством эритроцитов, гемоглобина и лимфоцитов не выявлено, однако выявлена связь с количеством моноцитов, тромбоцитов и лейкоцитов. Анемия может возникать у пациентов с ревматоидным артритом, о чем свидетельствует снижение уровня эритроцитов и гемоглобина. Некоторое воспаление сохраняется даже после лечения метотрексатом. Уровень СРБ был значительно повышен и тесно связан с моноцитами и в малой степени с лейкоцитами.
Заключение. Связи между СРБ и количеством эритроцитов, лимфоцитов или гемоглобина не выявлено. Уровень лейкоцитов, моноцитов и другие стандартные параметры крови могут дать ценную информацию о тяжести заболевания и эффективности лечения.
Ключевые слова: ревматоидный артрит, СОЭ, ОАК, СРБ, фолиевая кислота

■ INTRODUCTION

Rheumatoid arthritis can be defined as a chronic inflammatory disease affecting multiple areas of the body, with the disorder directly affecting the synovial joints. Clinical symptoms of RA include fever, subcutaneous nodules, symmetrical joint pain, fatigue, shoulder pain, and weight loss [1, 2].

The most important symptoms observed in RA patients, which significantly impact the patient's quality of life and mental health, include symmetrical inflammation in small joints, pain, swelling, deformity, morning stiffness, and limited movement [3]. Treatment focuses on anti-inflammatory effects [4]. The therapeutic aspect aims to stop the increase in inflammation or joint injury, and treatment begins with traditional medications such as methotrexate. Or other medications are used, such as anti-cytokine drugs such as anti-tumor necrosis factor (TNF) or interleukin-6 (IL-6), anti-specific cell receptor antagonists, or JAK inhibitors [5, 6].

There are several laboratory tests to diagnose and assess disease activity, including ESR and CRP tests [3]. Chronic diseases, including chronic inflammatory diseases such as rheumatoid arthritis, can cause anemia due to chronic inflammation. This can be due to impaired red blood cell formation and erythropoietin response [7].

Some studies assessed the correlation between platelets (PLT), red blood cells (RBC), platelet ratio (RPR) and hemoglobin-platelet ratio (HPR) as regards the disease activity. Little is known about the significance of the PLT and parameters of RBC in differentiating active from inactive disease [8]. Some studies have indicated that red blood cells (RBCs) and hemoglobin (Hb) are important biomarkers for predicting the severity of autoimmune diseases [9].

This study aimed to assess hematological parameters and their correlation with C-reactive protein levels in rheumatoid arthritis patients receiving methotrexate and folic acid in Thi-Qar city.

■ MATERIALS AND METHODS

Study design

This study was conducted in Thi Qar Governorate, Iraq, on patients with rheumatoid arthritis (RA) diagnosed according to the 2010 ACR/EULAR criteria. Samples were collected AL-Nasiriyah Teaching Hospital, specifically, between October 17, 2024, and January 3, 2025. This study included 60 samples, including 40 patients and 20 controls. As shown in Table 1.

The mean age of patients ranged from 22 to 70 years. This study aimed to measure disease activity in RA patients receiving methotrexate and folic acid, by measuring the erythrocyte sedimentation rate (ESR) and blood cell levels using a complete blood count and correlation between hematological markers.

Blood sample collection

Five milliliters of blood samples were extracted from each patient and a healthy participant via venipuncture using sterile syringes. The blood from each sample was partitioned into two aliquots. A three-milliliter sample was placed in a sterile gelatin tube, allowed to clot at room temperature for a minimum of 30 minutes, and subsequently centrifuged at 2,500 rpm for 10 minutes. The isolated serum was aliquoted into three

Eppendorf tubes and preserved at –20 °C until required for analysis of CRP therefore preventing repetitive thawing and refreezing. Two milliliters were aliquoted into a tube containing ethylenediaminetetraacetic acid (EDTA) at a concentration of 2 mg/mL for measurement of Erythrocytes Sedimentation Rate (ESR) and Complete blood count (CBC).

Complete blood count (CBC)

To perform CBC test after venous blood is drawn and EGTA transfer tube the reading was done on Sysmex 5 DIFF machine. The test gives an in-depth report of its finding in 1–2 minutes and the result is printed.

Estimation of human C-reactive protein (CRP)

The level of human C-Reactive Protein (CRP) was determined with the Hs-CRP + CRP Fast Test Kit (Immunofluorescence Assay) designed for The Semi-Quantitative Measurement of CRP Concentration in serum plasma or Whole Blood Samples. Test Device: The test was carried out using Getein1800 Quantitative immunofluorescence analyzer that permissible for automated.

Reading of test

The measurement is based on changes in CRP concentration in Inflammatory or infectious conditions or tissue damage. Hs-CRP analysis Can also be used for the early detection of cardiovascular diseases.

Principle of the test

The principle of this test is based on immunofluorescence technology, using a monoclonal antibody against human CRP linked to fluorescent Molecules. When the sample is added to the test strip, the CRP in the Sample binds to the fluorescent antibody, forming a complex. The Complex then travels through the nitrocellulose strip and binds to another Antibody immobilized on the analytical line. The generated fluorescent Signal is read and quantified by a quantitative immunoassay.

Test procedure

Steps for conducting the analysis:

– Insert the SD card for the current batch.
– Remove the test strip from the packaging and place it on a clean, Horizontal surface.
– Use a pipette to add 10 µL of the serum/plasma sample to the diluent.
– Mix the sample well, then withdraw 60 µL of the mixture and place it in the sample area on the strip.
– Wait for 10 minutes.
– Insert the strip into the device, and the result is automatically read and Displayed on the screen in mg/L.

Result calculation

The device calculates automatically, and the CRP concentration is Displayed directly. The measurement range is 0.5 to 200 mg/L. Results Are classified according to the reference values specified in the user Manually.

Statistical analysis

The data were analyzed using both parametric and non-parametric statistical methods depending on the type of variable. The Independent Samples t-test was applied to compare mean values between patients and healthy controls, including hematological parameters, ESR, and CRP levels. This test is suitable for evaluating differences between two independent groups under the assumption of normally distributed data and equal variances. For categorical data, such as sex distribution between rheumatoid arthritis patients and the control group, Fisher's Exact Test was employed. When the number of observations is small or when there are low expected cell frequencies, this test can be especially important because an exact probability rather than an estimated probability determined from the normal distribution is obtained. For all analysis a p-value less than 0.05 was considered significant while results with a p-value less than 0.001 were considered highly significant. Insignificant results were observed if the p-value was more than 0.05.

■ RESULTS

This study included 40 patients with daytime rheumatoid arthritis and 20 Control subjects. Of the patient group 40, 35 were female (58.3%) and 5 were male (8.3%). In the control group, 16 were female (26.7%) and 4 were male (6.7%). The p-value was 0.464, as shown in Table 1.

The mean age of female patients was 44.1 years, while that of males was 13.3 years. In the control group, the mean age of females was 41.3 years, while That of males was 12.2 years, as shown in Figure 1.

Comparing blood cell levels in rheumatoid arthritis patients with healthy controls

The results showed a slight increase in white blood cell (WBC) levels (10^3/uL) in patients compared to healthy controls, with a mean of (P=0.48) and a degree of freedom (DF=57), where the mean WBC in patients were (Mean=7.32).

When measuring red blood cells (RBC) (10^6/uL), the results showed a decrease in the level of red blood cells in patients compared to healthy controls, with the mean RBC count in patients being (Mean=4.29), while in healthy controls it was (Mean=4.82) with a degree of freedom (DF=58), with a mean of (P=<0.001). The results showed an increase in lymphocyte levels in patients, with a mean of 2.31 [10^3/uL], while the mean value in the control group was 1.53 [10^3/uL], with a degree of freedom (DF) of 58 and a P value of 0.002.

Table 1
Sex distribution analyzed by Fisher's exact test between rheumatoid arthritis patients and control group

Sex		Group		Total	p-value
		Patients	Controls		
Female	Frequency	35	16	51	0.464^{NS}
	Proportions	58.3%	26.7%	85%	
Male	Frequency	5	4	9	
	Proportions	8.3%	6.7%	15%	
Total	Frequency	40	20	60	
	Proportions	66.7%	33.3%	100%	

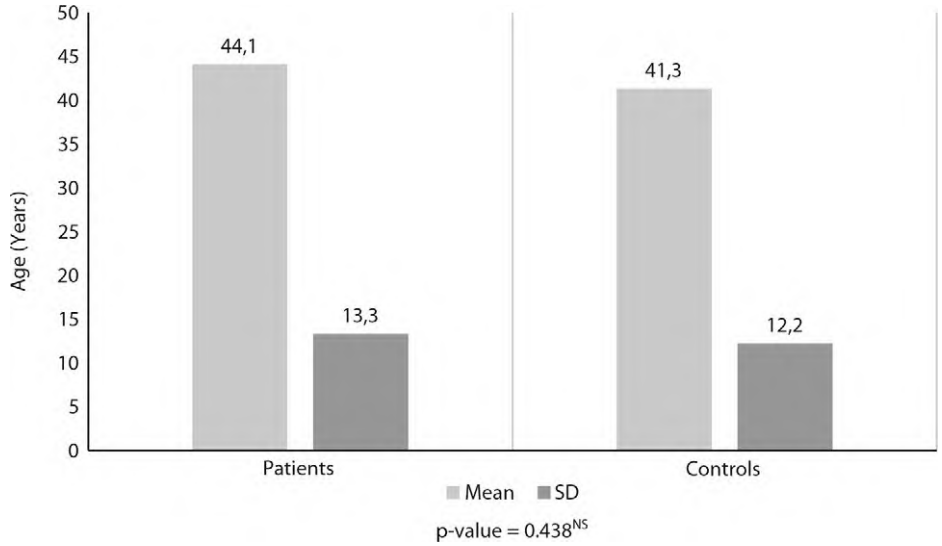

Fig. 1. Distribution of rheumatoid arthritis patients and control group according age (years)

When comparing the results for monocytes, it was noted that their levels were higher in patients, with a mean of 1.66 and a mean of 0.53 in the control group, with a degree of freedom (DF) of 39.41 and a P value of 0. 005.As for platelet levels, the results did not reveal a significant difference between patients and healthy controls.

The mean was 267.30 in patients and 285.15 in healthy controls, with a P value of 0.384, meaning there was no significant difference in the values. As for hemoglobin levels, a decrease was observed in patients compared to healthy individuals, where the average was (Mean=11.69), while in healthy individuals (Mean=13.69), and the value was (P=<0.001). As shown in table 2.

Table 2
Comparing blood cell levels in rheumatoid arthritis patients with healthy controls

Marker	Group	No.	Mean	SE	t	DF	P-value
WBC (10^3/uL)	Patients	40	7.32	0.39	2.023	57	0.48*
	Controls	20	6.07	0.32			
RBC (10^6/uL)	Patients	40	4.29	0.08	–3.868	58	<0.001**
	Controls	20	4.82	0.04			
Lymph (10^3/uL)	Patients	40	2.31	0.15	3.308	58	0.002*
	Controls	20	1.53	0.13			
Mono (10^3/uL)	Patients	40	1.66	0.37	2.952	39.41	0.005*
	Controls	20	0.53	0.02			
PLT (10^3/uL)	Patients	40	267.30	1.13	–.876	58	0.384NS
	Controls	20	285.15	1.46			
HB (g/dL)	Patients	40	11.69	0.24	-4.839	57	<0.001**
	Controls	20	13.69	0.32			

Measuring levels as acute phase proteins (CRP) in RA patients and control groups

Comparing CRP levels between patients and healthy controls showed higher levels in patients compared to healthy controls. The mean value in patients was (Mean=25.30 mg/L) and in healthy controls was (Mean=8.55 mg/L) at a significance level of (P=0.032*) and a degree of freedom of (DF=2.195). As shown in Table 3.

Relationship between C-reactive protein (CRP) and hematological parameters

The Pearson's correlation coefficient analysis revealed a moderate and statistically significant positive relationship between CRP and WBC (r=0.002, P=0.468), as well as a relatively strong and significant positive correlation between CRP and monocytes (r=0.001, P>0.560). Conversely, no significant relationships were seen between CRP and both RBC and lymphocytes (P<0.05). C-reactive protein (CRP) levels showed a strong positive correlation with.

Platelet count (PLT) (r=0.727, P=0.021) and a moderate positive Correlation with monocyte count (r=0.560, P=0.000), both of which were Statistically significant, whereas no significant correlation was observed Between CRP levels and lymphocyte count (r= –0.068, P=0.678) in Patients with rheumatoid arthritis.

■ DISCUSSION

Analyzing Descriptively: According to the descriptive analysis, there was a definite prevalence of females in the study population, with the bulk of the sample being females (87.5% of cases and 80% of controls). According to recent research, women are more likely than males to have rheumatoid arthritis (RA), with a female-to-male ratio of 2:1 to 4 : 1 [10, 11].

The results of the study showed that the mean ages of females and males were 44.1 years for rheumatoid arthritis at its peak age group, while it was 13.3 years in boys. Both women and men in control group had an average age of 41.3 years and 12.2 years, respectively. This spread of ages pointed out where the big proportion to woman patients

Table 3
Measuring levels of acute phase proteins (CRP) in RA patients and control groups

Marker	Group	No.	Mean	SE	t	DF	P-value
CRP (mg/L)	Patients	40	25.30	2.26	2.195	58	0.032*
	Controls	20	8.55	2.19			

Table 4
Pearson correlation coefficients (r) and Significance (P) between CRP as inflammatory and hematological parameters in rheumatoid arthritis patients

Parameters	r	P value ≤0.05	Result
CRP with WBCs	0.468	0.002	Significant positive correlation
CRP with RBCs	0.063	0.699	No significant positive correlation
CRP with HB	-0.164	0.318	No significant negative correlation
CRP with PLT	0.727	0.021	Significant positive correlation
CRP with Mono	0.560	0.000	Significant positive correlation
CRP with Lymph	–0.068	0.678	No significant negative correlation

were middle-aged (40s ~ 50s); it is much like other studies that women tend to contract a rheumatoid arthritis in forties and fifties [12, 13]. In addition, healthy control group age range is usually selected to match that of ill patients, so that demographic comparisons can be fair comparison as it was done in the present study [14]. This age distribution highlights the importance of including age as a variable in clinical trial design and analysis of patient populations, in particular immunologic and inflammatory variables which could vary by age.

The findings of the present study revealed that (Hb) had significantly decreased in patients compared to healthy control, which were corresponding to the results of Al-Mazedi et al. [15] who associated reduced Hb with hihger activity of the disease. Rheumatoid arthritis patients and healthy controls did not differ in platelet count. This is consistent with Salliot et al. [16], and for the possible folic acid-induced prevention of MTX-induced thrombocytopenia, which may attenuate platelet variations [17].

It is found that patients had more monocytes compared with healthy donors according to this study. These are in line with the findings of [18] and colleagues, who have explained that the relative increase in these cells levels may be due to a response from patients treated by methotrexate.

In the present study, a relative increase of lymphocytes levels was also detected in patients with respect to non-infected individuals. This is also in line with finding of chronic and sustained inflammation found out by Chara et al. [18]. We observed from our study that the mean haemoglobin count in patients is less than what it was reported by Farouk et al. [17]; this also indicates a reduction in RBCs. In this present study we also observed a relative increase in WBC count in the patients when compared with controls as reported and concluded by Farouk et al. [17].

It is found that there were increased levels of CRP in the patients with rheumatoid arthritis, relative to healthy controls in our study. This is consistent with the findings of [19] and [20] have documented a relative increase of CRP levels in patients versus HC. It may be associated with disease activity, and may thereby help in tracking it. Günay and Alpsoy [21] also reported a linear association between increasing ESR, other inflammatory markers, and disease activity with an elevation in white blood cells. What we found in our analysis is consistent with that.

The exact absolute correlation was obtained for WBC, monocytes and CRP (as the acute phase protein), but not for RBC, lymphocytes or Hb. The correlation between the levels of CRP and WBC was sign weak positive that means in blood samples with higher levels of CRP, the chance of getting higher values for WBC is better. This is confirmed by other studies, which have demonstrated that CRP levels increase with acute inflammation in proportion to an increased leukocytosis, indicating a higher level of innate immune activation during AP or cardiovascular disease [22, 23]. Another interesting finding of our study was the significant correlation between CRP and monocyte, which implies that CRP production might be largely dependent on monocyte activation. Pro-inflammatory cytokines, such as IL-6 and TNF-α released by monocytes, enhance the liver synthesis of CRP. CRP can also activate monocytes and promote their differentiation into macrophages, thereby enhancing the inflammatory response [24].

This reciprocal relationship emphasizes the necessity of monitoring levels of both CRP and monocytes as biomarkers of systemic inflammation in autoimmune or chronic inflammatory disease. In contrast, no significant associations of CRP with RBC,

lymphocytes or hemoglobin were discovered. These findings are congruent with earlier research indicating that CRP primarily measures innate rather than red blood cell indices or lymphocytes counts that may be confounded by a number of mechanisms such as anemia of chronic disease, adaptive immune regulation [22]. Overall, this data indicates that CRP is a good marker of systemic inflammation because it closely mirrors innate immune responses driven by WBCs and monocytes and has nothing to do with other blood elements.

■ CONCLUSION

Patients with RA may have anemia, as indicated by decreased RBC and Hb levels. There remains some inflammation even following methotrexate treatment. CRP was markedly high and strongly related to monocytes, had marginal relationship with WBC. No associations were observed between CRP and RBC, lymphocytes or Hb. If WBC with monocytes and other routine values. Blood tests may provide important information about a disease, its effect on the body and the response to treatment. The present study emphasizes the importance of methotrexate monitoring. rheumatoid arthritis patients with folic acid and investigating the. relationship between CRP and WBC or MON for evaluation of inflammation, and treatment effect. It also emphasizes the necessity of further local-level large-scale research to validate those findings and compare them with clinical data from around the world.

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