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Research Article

Correlation between Serum Levels of Factor I, CD59, Interferon-gamma, and Interleukin-6 with the Response to Rituximab in Iraqi Patients with Rheumatoid Arthritis

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Abstract

Background: Rituximab is a chimeric IgG1 kappa immunoglobulin that has been genetically modified to incorporate human constant region sequences together with murine light- and heavy-chain variable region sequences. People use it to treat rheumatoid arthritis and certain malignancies. ***Objective***: The study aimed to assess the potential association between the serum levels of Factor I, CD59, interleukins (IL)-6, and interferon-gamma (IFN)-γ and the response to Rituximab treatment in Iraqi rheumatoid arthritis patients. ***Methods***: A cross-sectional study was conducted at the rheumatology center at Baghdad Teaching Hospital. Ninety adult patients who have been diagnosed with rheumatoid arthritis and are receiving Rituximab intravenous infusions were included. The enrolled patients were divided into a responder group (45 patients) and a non-responder group (45 patients). The response to Rituximab was assessed according to the 28-joint Disease Activity Score (DAS28). ***Results***: The serum levels of Factor I and CD59 were significantly higher in the non-responders group in comparison to the responders group. In addition, the serum IL-6 and IFN-γ levels were significantly elevated in the non-responders group in comparison to the responders group. The estimated marker serum levels showed a strong, significant correlation with the 6-month change in DAS28. ***Conclusions***: In Rituximab nonresponder RA patients, serum levels of Factor I, CD59, Factor H, IL-6, and IFN-γ are higher, and they have good potential to be used in the assessment of the response to Rituximab therapy.

Keywords: CD59, Factor I, Factor H, Rheumatoid arthritis, Rituximab.

العلاقة بين مستويات العامل الأول و CD59 والأنترفيرون-غاما والأنترلوكين-6 في المصل مع الاستجابة لريتوكسيماب في المرضى العراقيين المصابين بالتهاب المفاصل الرثوي

الخلاصة

الخلفية: ريتوكسيماب هو غلوبولين مناعي خيمري تم تعديله وراثيا لدمج تسلسلات المنطقة البشرية الثابتة جنبا إلى جنب مع تسلسلات المنطقة المتغيرة ذات السلسلة الخفيفة والثقيلة. يستخدم لعلاج التهاب المفاصل الرثوي وبعض الأورام الخبيثة. **الهدف**: تقييم الارتباط المحتمل بين مستويات العامل الأول، CD59 ، IL-6 ، وإنترفيرون جاما في المصل والاستجابة لعلاج ريتوكسيماب في مرضى التهاب المفاصل الرثوي العراقيين. **الطريقة**: أجريت دراسة مقطعية في مركز الروماتيزم في مستشفى بغداد التعليمي شملت تسعين مريضا بالغا تم تشخيص إصابتهم بالتهاب المفاصل الرثوي ويتلقون حقن ريتوكسيماب في الوريد. تم تقسيم المرضى المسجلين إلى مجموعة المستجيبين (45 مريضا) ومجموعة غير المستجيبين (45 مريضا). تم تقييم الاستجابة لريتوكسيماب وفقا لدرجة نشاط المرض المكونة من 28 مفصلا DAS28. **النتائج**: كانت مستويات العامل الأول و CD59 أعلى بشكل ملحوظ في مجموعة غير المستجيبين مقارنة بمجموعة المستجيبين. بالإضافة إلى ذلك ، كانت مستويات IL-6 و IFN-γ مرتفعة بشكل ملحوظ في مجموعة غير المستجيبين مقارنة بمجموعة المستجيبين. أظهرت مستويات العلامات المقدرة ارتباطا قويا وكبيرا مع التغيير لمدة 6 أشهر في DAS28. **الاستنتاجات**: في المرضى غير المستجيبين، تكون مستويات العامل الأول و CD59 و Factor H و IL-6 و IFN-γ أعلى، ولديهم إمكانية جيدة لاستخدامه في تقييم الاستجابة لعلاج ريتوكسيماب.

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INTRODUCTIONRheumatoid arthritis is a chronic systemic inflammatory autoimmune disease that is caused by metabolites of arachidonic acid and various inflammatory cytokines, leading to disability and premature death. The involvement of rheumatoid arthritis in joints usually has a characteristic presentation, with synovitis occurring in symmetrical small joints [1,2]. Recently, it became clear that rheumatoid arthritis etiopathology has genetic and epigenetic elements; in addition to that, the environment could also play a crucial role [3]. In people who are genetically predisposed to the condition, environmental variables are thought to be the catalyst for the onset of rheumatoid arthritis. Thus, alterations in extrinsic factors linked to demographic traits, or birth cohort effects, may have an impact on the clinical phenotype of RA, including the age at which the ailment first manifests [4]. The

disease entails a significant interaction between the adaptive elements and the innate elements of the immune system. Irregularities in the cellular and humoral immune responses result in the development of autoantibodies [5]. Rituximab is a chimeric IgG1 kappa immunoglobulin that has been genetically modified to incorporate human constant region sequences together with murine light- and heavy-chain variable region sequences. It targets the CD20 antigen found on the surface of B cells, both healthy and cancerous. It is used to treat some cancers and rheumatoid arthritis [6]. Complement is a major part of the innate immune system, forms a barrier and acts as a true safeguard system to maintain tissue integrity [7,8]. A variety of complement regulators have been recognized as a two-edged sword in terms of health and illness, including Factor I, Factor H, C1-inhibitor, C4b binding protein, clusterin and vitronectin, complement decay accelerating factor (DAF), CD35, CD46, CD59 and complement receptor immunoglobin [9]. In rheumatoid joints, cytokines are considered to play a dual role as pro- and anti-inflammatory mediators, depending on the illness. Patients suffering from rheumatoid arthritis have many cytokines in their joints, such as IFN-γ and IL-6 [10,11]. The study aimed to assess the potential association between serum levels of Factor I, CD59, IL-6, and IFN-γ and response to Rituximab treatment in Iraqi rheumatoid arthritis patients.

METHODS

Study design and setting

A cross-sectional study was conducted under specialized physician supervision in the Specialized Center of Rheumatology at Baghdad Teaching Hospital in Baghdad, Iraq, during the period from January to November 2023. The current study included a convenient sample of ninety adult patients already diagnosed with rheumatoid arthritis according to the revised 2010 American College of Rheumatology/European League and Rheumatism classification criteria [12].

Sample selection

Inclusion criteria included patients who were receiving Rituximab as monotherapy at a dose of 1 gram vial intravenous infusions for at least six months and willingness to participate in the study. Exclusion criteria included patients taking another biological agent, patients diagnosed previously with chronic autoimmune diseases or malignancies, and patients taking steroids. The recruited patients were allocated into two groups, including group 1 (responders to Rituximab) and group 2 (non-responders to Rituximab). The selected patients were either responders to Rituximab (45 patients) or non-responders to Rituximab (45 patients). The response to Rituximab was assessed according to the DAS28. According to DAS28, the tender joint count (TJC) and swollen joint count (SJC) in 28 joints, including the shoulder, elbow, wrist, knee, metacarpophalangeal and proximal interphalangeal joints, are recorded in addition to the measure of the visual analogue scale (VAS) of 100 mm and the erythrocyte sedimentation rate, or C-reactive protein. The final value of DAS is calculated according to the following formula [13]:

$$DAS28(CRP) = 0.56*\sqrt{(TJC28)} + 0.28*\sqrt{(SJC28)} + 0.014*GH + 0.36*\ln(CRP+1) + 0.96.$$

$$DAS28(ESR) = 0.56*\sqrt{(TJC28)} + 0.28*\sqrt{(SJC28)} + 0.014*GH + 0.70*\ln(ESR).$$

A reduction of DAS28 by at least 0.6 and to a value less than 5.1 from the baseline score after 6 months of Rituximab therapy was considered indicative of clinical response. Patients who did not show such a reduction in DAS28 were considered non-responders [14].

Data collection and outcome measurements

The data collection included age (years) and gender. The investigations included hemoglobin (g/dL), white blood cell (WBC) count (cell/µL), blood urea (mg/dL), serum creatinine (mg/dL), anti-cyclic citrullinated peptide antibody (ACCP), and anti-citrullinated protein autoantibodies (ACP). In addition to Factor I, CD59, IL-6, and IFN-γ were measured by sandwich enzyme-linked immunosorbent assay analysis-specific kits.

Ethical considerations

The Ethical Committee of the College of Pharmacy, University of Baghdad, approved this study via official letter No. RECAUBCP11122023 dated 11/12/2023, in accordance with the Helsinki Declaration. Before the participation agreements were documented, all participants were informed of the study's purpose and expected benefits.

Statistical analysis

The data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 25. Categorical data was represented as frequencies and percentages, whereas continuous data was represented as mean ± standard deviation (SD). The normality of the data distribution was tested using the Shapiro-Wilk test. The non-normally distributed variables were compared using the Whitney test. For categorical variables, Pearson's chi-squared test was used. Spearman's correlation coefficient (p) was used to identify statistically significant correlations between measured parameters. p-values < 0.05 were deemed significant.

RESULTS

As shown in Table 1, the mean age of responders was 48.58±12.6 years, which was significantly

younger than the mean age of non-responders (54.22±9.53 years).

Table 1: Distribution of study variables according to response

Variables		Responders (n=45)	Non-responders (n=45)	*p*-value
Age (years)		48.58±12.6	54.22±9.53	0.019
Sex	Male	5(11.1)	1(2.2)	0.203
	Female	40(88.9)	44(97.8)	
Haemoglobin (g/dL)		12.12±1.7	11.86±1.25	0.402
WBC count (cell/µL)		8.95±2.62	6.54±1.89	<0.001
Blood urea (mg/dL)		36.17±17.92	27.33±5.96	0.004
Serum creatinine (mg/dL)		0.72±0.55	0.55±0.15	0.003
ACP (IU/ml)		2.93±2.78	10.03±3.75	<0.001
ACCP (IU/ml)		12.57±7.25	38.54±13.41	<0.001
DAS28 score at the start of Rituximab		5.71±0.80	5.26±0.99	0.020
Current DAS28 score		4.12±5.6	5.62±0.8	<0.001
Change in DAS28 score		1.6±0.9	-0.36±0.7	<0.001

Values were presented as frequencies, percentage, and mean±SD. WBC: White blood cell; ACCP; Anti-cyclic citrullinated peptide antibody; ACP; Anti-citrullinated protein autoantibodies; DAS28: 28-joint disease activity score.

Most patients were females; only 5 (11.1%) were male responders and only 1 (2.2%) was a non-responder male. Hemoglobin showed no statistically significant differences between the two study groups. The WBC count was significantly higher in responders compared to non-responders, with 8.95±2.62 cell/µL and 53.29±34.29 mm/hour, compared to 6.54±1.89 cell/µL and 37.56±29.65 mm/hour, respectively. Blood urea and serum creatinine were significantly higher in responders in comparison to non-responders, with 36.17±17.92 mg/dL and 0.72±0.55 mg/dL, compared to 27.33±5.96 mg/dL and 0.55±0.15 mg/dL, respectively. ACP and ACCP were significantly lower in responders compared to non-responders, with 2.93±2.78 IU/ml and 12.57±7.25 IU/ml compared to 10.03±3.75 IU/ml and 38.54±13.41 IU/ml, respectively. The DAS28 score before starting Rituximab was significantly higher in responders, with 5.71±0.80 compared to 5.26±0.99, while it was significantly lower after treatment, with 4.12±5.6 vs. 5.62±0.8, and there was a statistically significant decrease in the responders group. Factor I was significantly higher in non-responders (83.04±16.01 nmol/L) than in responders (30.81±18.47 nmol/L). CD59 was significantly higher in non-responders (31.22±7.18 ng/mL) than in responders (13.89±5.11 ng/mL) (Table 2). IL-6 was significantly higher in non-responders (56.03±12.24 ng/ml) than in responders (16.8±5.08 ng/ml), and IFN-γ was higher in non-responders (30.91±5.65 pg/ml) than in responders (12.02±8.49 pg/ml) (Table 3).

Table 2: Distribution of complement re-collectors according to response

Variable	Responders (n=45)	Non-responders (n=45)	*p*-value
Factor I (nmol/L)	30.81±18.47	83.04±16.01	<0.001
CD59 (ng/mL)	13.89±5.11	31.22±7.18	<0.001

Table 3: Distribution of proinflammatory mediators according to response

Variable	Responders (n=45)	Non-responders (n=45)	*p*-value
IL-6 (ng/ml)	16.8±5.08	56.03±12.24	<0.001
IFN-γ (pg/ml)	12.02±8.49	30.91±5.65	<0.001

IL-6: Interleukins-6; IFN-γ: Interferons-gamma.

The correlation study between Factor I and CD59 with the study variables revealed that the highest correlation was reported with ACCP, ACP, and the changes in the DAS28 score. WBC count, blood urea, and serum creatinine showed weak negative correlations with Factor I and CD59 (Table 4).

Table 4: Correlation between complement re-collectors and study variables

Variable	Factor I		CD59	
	r	*p*-value	*r*	*p*-value
Age	0.167	0.115	0.266	0.011
Haemoglobin	-0.051	0.631	-0.050	0.642
WBC count	-0.373	<0.001	-0.351	0.001
Blood urea	-0.301	0.005	-0.298	0.006
Serum creatinine	-0.281	0.011	-0.272	0.015
ACCP	0.672	<0.001	0.877	<0.001
ACP	0.760	<0.001	0.656	<0.001
Baseline DAS28	-0.260	0.013	-0.182	0.085
Current DAS28 score	0.505	<0.001	0.556	<0.001
Change in DAS28 score	-0.672	<0.001	-0.663	<0.001

WBC: White blood cell; ACCP; Anti-cyclic citrullinated peptide antibody; ACP; Anti-citrullinated protein autoantibodies; DAS28: 28-joint disease activity score.

The correlation study between IL-6 and IFN-γ with the study variables revealed that the highest correlation was observed with ACCP, ACP, and the changes in the DAS28 score. WBC count, blood urea and serum creatinine showed weak negative correlations with IL-6 and IFN-γ. In addition, there were highly significant positive correlations between IL-6 and IFN-γ and Factor I and CD59. As shown in Table 5, all the complement re-collectors and proinflammatory mediators had a highly statistically significant AUC for identifying responders to rituximab. Factor I (≤ 62.1 nmol/L, sensitivity = 95.6%, and specificity = 95.6%), CD59 (≤ 22.4 ng/mL, sensitivity = 97.8%, and specificity = 97.8%), IL-6 (≤ 32.0 ng/ml, sensitivity = 97.8%, and specificity = 100%), and IFN-γ (≤ 21.2 pg/ml, sensitivity = 86.7%, and specificity = 100%), as shown in Table 6 and Figure 1.

DISCUSSION

In the current study, Rituximab responders were significantly younger than non-responders, while

gender showed no statistically significant association with response.

Table 5: Correlation between proinflammatory mediators and study variables

Variable	IL-6		IFN-γ	
	r	*p*-value	*r*	*p*-value
Age	0.244	0.021	0.230	0.029
Haemoglobin	-0.093	0.385	0.049	0.645
WBC count	-0.417	<0.001	-0.416	<0.001
Blood urea	-0.271	0.012	-0.245	0.024
Serum creatinine	-0.292	0.009	-0.183	0.104
ACCP	0.820	<0.001	0.814	<0.001
ACP	0.728	<0.001	0.678	<0.001
Baseline DAS28	-0.229	0.030	-0.173	0.104
Current DAS28 score	0.572	<0.001	0.551	<0.001
Change in DAS28 score	-0.712	<0.001	-0.651	<0.001
Factor I	0.843	<0.001	0.843	<0.001
CD59	0.844	<0.001	0.818	<0.001

IL-6: Interleukins-6; IFN-γ: Interferons-gamma; WBC: White blood cell; ACCP: Anti-cyclic citrullinated peptide antibody; ACP: Anti-citrullinated protein autoantibodies; DAS28: 28-joint disease activity score.

These results were in partial agreement with the results of Narvaez *et al*. (2011), who reported that the female-to-male ratio among responders was 62 to 20, and among non-responders, it was 24 to 2, which was not statistically significant; however, they did not find a statistically significant difference in mean age [15]. In the current study, Rituximab-responders had higher levels of ACCP antigen. A number of pieces of evidence connect the presence of ACPAs to the pathogenic process of RA. One piece of evidence suggests that they are highly specific, as they rarely appear and have low titers in other autoimmune diseases [16]. The effectiveness of targeted elimination of B cells as a treatment for RA led to the idea that autoantibodies play a significant role in the development of the disease [17]. Another piece of evidence that reinforces the connection between ACPAs and RA stems from research indicating that ACPAs appear before the disease's clinical symptoms [18].

Table 6: ROC curve analysis for complement re-collectors in identifying responders

Variables	AUC	Cut-off value	Sensitivity (%)	Specificity (%)	*p*-value
Factor I	0.971	≤62.1 nmol/l	95.6	95.6	<0.0001
CD59	0.980	≤22.4 ng/ml	97.8	97.8	<0.0001
IL-6	0.999	≤32.0 ng/ml	97.8	100	<0.0001
IFN-γ	0.961	≤21.2 pg/ml	86.7	100	<0.0001

IL-6: Interleukins-6; IFN-γ: Interferons-gamma.

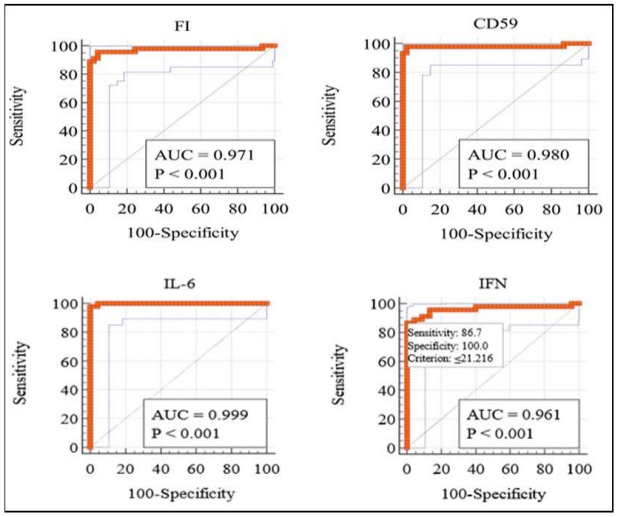

Figure 1: ROC curve of compliment re-collectors and proinflammatory mediators for identifying Rituximab responders.

In individuals without autoimmune diseases, ACPAs can be detected several years before the onset of the RA, and only a small number of people become ACPA-positive after the arthritis symptoms have appeared. Recent research has indicated a gradual emergence of these antibodies, with a notable increase in recognized epitopes occurring just before the onset of symptoms [19, 20]. However, very few ACPA-positive people will ultimately develop RA [21]. This could be explained by the multifactorial nature of RA; for example, some linking factors include obesity, tobacco use [22], roles of genes, environmental factors, and autoimmunity in the aetiology of RA, occupational exposure to silica dust, and periodontitis [23]. Since more than half of patients with RA are ACPA+ [24], those would become targets for B cells and maybe other types of arthritis. This triggers a specific antigen-antibody reaction with pathogenically significant implications [25], and since Rituximab effectively depletes circulating B lymphocytes in RA, this would limit the damage caused by this antigen-antibody reaction [26]. This explains why the levels of ACCP antigens are higher in Rituximab-treated patients in the current study. In the current study, Factor I and CD59 were significantly elevated in non-responders in comparison to responders. Hornum *et al*. (2017) studied the effects of blocking C5aR on synovial fluid leukocytes and concluded that blockade of complement system activity might improve some aspects of arthritis [27]. On another level, ACCP antigen has the potential to activate complement pathways (conventional and alternative) [28] and can activate both Fcγ-receptor-expressing cells and the complement system, which can contribute to the pathogenesis of the disease [29]. Anquetil *et al*. (2015) investigated the ability of some immunoglobulins, including IgM and IgA, to potentiate inflammation and reported that both could increase ACPA-mediated upregulation of proinflammatory cytokines in addition to complement pathway activation [3]. Hu *et al.* (2011) investigated the outcomes of blocking CD59 on sensitizing lymphoma cells to Rituximab and reported that rILYd4 (which is an hCD59 inhibitor) sensitized these cells for Rituximab-induced complement-dependent cytotoxicity in a dose-dependent manner [31]. In a different attempt to decrease CD59-related resistance to Rituximab, Ge *et al*. (2021) investigated the use of NF-κB inhibitors, which can downregulate inducible CD59 expression

and, in return, enhance the action of Rituximab [32]. In the current study, IL-6 and IFN-γ were significantly lower among responders to Rituximab. These results were in concordance with the results of another study carried out by Barahona Correa *et al*. (2018), which studied the effects of Rituximab therapy on some cytokines in patients with rheumatoid arthritis or systemic lupus erythematosus (SLE) and reported that in SLE, IL-6 and IL-8 were significantly lower after Rituximab therapy, while in RA, only IL-6 showed a significant decrease [33]. This led to the utilization of anti-IL-6 medications in the treatment of RA for over ten years and has contributed to an enhanced comprehension of the wide range of functions associated with this cytokine [34]. Regarding IFN-γ, a recent study indicates that CD8+ T cells serve as the primary source of this cytokine, and IFN-γ, in turn, activates CD4+ T cells, synovial fibroblasts, as well as monocytes and macrophages, which, when activated by IFN-γ, promote osteoclastogenesis, ultimately contributing to joint damage in RA [36].

Limitaions of the study

The most common study limitation was convincing patients to participate while they were receiving an intravenous line of their medication.

Conclusion

Serum levels of Factor I, CD59, Factor H, IL-6, and IFN-γ are higher in people with rheumatoid arthritis who don't respond to Rituximab therapy. These levels could be used to figure out how well Rituximab therapy is working for people with rheumatoid arthritis.

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Conflict of interests

No conflict of interests was declared by the authors.

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