Research Paper

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The Influence of Anti-CD20 Therapy on Some Immunological Parameters in patients with Rheumatoid Arthritis

Medical Science

KEYWORDS : Rheumatoid arthritis, Rituximab, Autoantibodies, Cytokines.

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ABSTRACT

Background: Rheumatoid arthritis (RA) is a chronic systemic immune mediated disease that results in significant morbidity and crippling effects. Recently a prominent role for B cells in the pathogenesis of RA has been confirmed, and it has been shown that anti-B cell therapy is a powerful tool in the treatment of RA.

Aims of the study: To assess the effect of rituximab (anti-CD20) treatment on the levels of autoantibodies [rheumatoid factor (RF-IgM) and anti-cyclic citrullinated peptide (ACCP- IgG)], and cytokines profile (IL-1α, IL-8, IL-17 and GM-CSF) in patients with RA.

Subjects and Methods: This study included 65 patients with RA, of these, 35 were patients without treatment, represent untreated patients group, the remaining group consist of 30 patients were treated with rituximab therapy, represent treated patients group, and 25 healthy individuals as controls group. ACCP and cytokines levels in serum were measured by enzyme linked immunosorbent assay, while serum RF was estimated by agglutination test.

Results: Compared to healthy control group untreated patients group have shown significantly higher ($p<0.01$) seroprevalence of (RF and ACCP), and higher levels of IL-1α, IL-8, IL-17 and GM-CSF.On the other hand, patients group treated with rituximab showed significant ($p<0.05$) decrease in seroprevalence of RF and ACCP, and in levels IL-1α and IL-17, but not in levels of IL-8 and GM-CSF when compared to untreated patients group. Interestingly, in treated patients group significant correlations of serum IL-1 α and IL-17 levels were found with each of DAS and RF.

Conclusion: The current data indicate that anti-CD20 therapy not only neutralizes the effects of B-cell, but it also down-regulates the proinflammatory interleukins like IL-1α and IL-17 in RA patients. In addition these findings showed that cytokines can be used to predict the response to rituximab in patients with refractory RA.

Introduction

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic synovial inflammation and subsequent tissue damage. It affects approxmatly 1-2 % of world's population. Hypertrophy and inflammation of the soft tissues around synovial joints is the common phenomenon occurring in RA (1). The etiology of RA is not known but it is classified as one of the autoimmune diseases, there is a prominent immunological dysfunction in the joints and many other tissues by accumulation of chronic inflammatory cells including T and B-lymphocytes, monocytes and macrophages (2, 3). In recent times an eminent role for B cells in the pathogenesis of RA has been confirmed (4), Edwards and Cambridge reported that the RA syndrome and, in particular, extraarticular disease is associated with the production of the autoantibodies known as rheumatoid factor (RF) and anti-cyclic citrullinated peptide (ACCP) (5). Clinical observations suggest that the role of B-cells and their progeny in the pathogenesis of RA goes beyond autoantibodies production to include autoantigen presentation and thus help in T cell activation (6, 7).

The activation and infiltration of T cells and macrophages in the synovium lead to production of cytokines (8, 9). A large number of cytokines are active in the joints of patients with RA. It is now clear that these cytokines play a fundamental role in the processes that cause inflammation, articular destruction, and the comorbidities associated with RA. The most prominent of these are tumor necrosis factor (TNF), interleukin-1 (IL-1), IL-6, IL-8, IL-10; IL-17 and Granulocyte macrophage colony stimulating factor (GM-CSF) (10). IL-1α in vitro induces cytokine production by synovial mononuclear cells, prostanoid and matrix metalloproteinase release by fibroblasts (11). IL-8 which is involved in cellular recruitment, GM-CSF involved in macrophage development. IL-17 which has pleiotropic effects on multiple cell types including osteoblast expression of RANK leading to osteoclast activation (12).

The identification and diagnosis of RA early in the disease course is becoming increasingly important because early and intensive treatment has been demonstrated to prevent joint damage, to preserve joint function, and to improve work participation of the patient (13). The blockade of activated immune system mediators those that might trigger joint inflammation and damage has already led to the development of highly efficient biological treatments of disease (14). Several biological therapeutic approaches have been introduced for use typically in patients refractory to conventional agents such as disease-modifying anti-rheumatic drugs (15). Anti-CD20 antibody or rituximab is a monoclonal antibody that selectively targets CD20-positive B-lymphocytes. Rituximab successfully depletes CD20-expressing B-cells, which have several functions in the immune response by a mixture of apoptosis, antibody-dependent cell-mediated cellular cytotoxicity and complement-dependent cytotoxicity (16). It is used for the treatment of patients with RA with an inadequate response or tolerance to tumor necrosis factor inhibitors. This therapy works by turning off a part of the immune system that is not working properly in autoimmune diseases. It has reduced signs and symptoms of RA, and manages to slow down the joint destruction (17). The present study was undertaken to determine whether or not the clinical response to anti-CD20 therapy of RA patients lead to changes in the seroprevalence of autoantibodies (RF and ACCP) and in the circulating levels of cytokines (IL-α, IL-8, IL-17and GM-CSF).

Subjects and Methods

Blood samples were collected from 65 patients with RA, their age ranged from 23 to 68 years, and 25 healthy controls were age and sex matched with patients group Their age ranged from 20 to 63 years, , and all of them had no history or clinic evidence of RA. In this study we have divided the RA patients into two groups according to treatment; the first group consists of 35 untreated patients (without any treatment), while the second group consists of 30 treated patients (treated with rituximab).

The pateints were from attendants seeking treatment in the Rheumatology and Rehabilitation center at Baghdad Teaching Hospital in Medical city in Baghdad from June 2012 till April 2013. The diagnosis of each case was established by clinical examination done by a specialist rheumatologist and confirmed by laboratory and radiological investigations.

Serum samples were separated from the whole blood and stored at -20ºC until used. Serum RF- IgM was estimated by agglutination test (RF-Spinreact S.A. / Spain), whereas ACCP- IgG, IL-α, IL-8, IL-17and GM-CSF have been estimated by using commercially available enzyme-linked immunosorbent assay and performed as recommended in leaflet with kits (ACCP-Human GmbH. Wiesbaden/ Germany; IL-α, IL-8 and GM-CSF-Cusabio/ China; IL-17- Ray Bio/ USA).

Data were analyzed using the statistical package for social sciences (SPSS v13).The statistical significance of difference in mean of a quantitative normally distributed variable was tested by T-test and ANOVA test. Non-normally distributed variable, as shown by histograms and Smemirnove-Kolmogorove test are described by median and the non-parametric tests of significance were advocated for use. The statistical significance of difference in median between 2 groups was tested by Mann-Whitney test. Correlation among different parameters was calculated by the Spearman correlation coefficient test, P value less than the 0.05 was considered statistically significant.

Results

The demographic characteristics of patients groups and controls group included in this study are presented in (Table 1). No statistically significant differences (p>0.05) in age or gender existed among studied groups. The presence of autontibodies in control group and untreated patients group are shown in (Figure 1). The seroprevalence of RF and ACCP in patients were significantly higher (P<0.001) than control group (71% IU/ml; 80% RU/ml vs. 12% IU/ml; 8% RU/ml respectively).

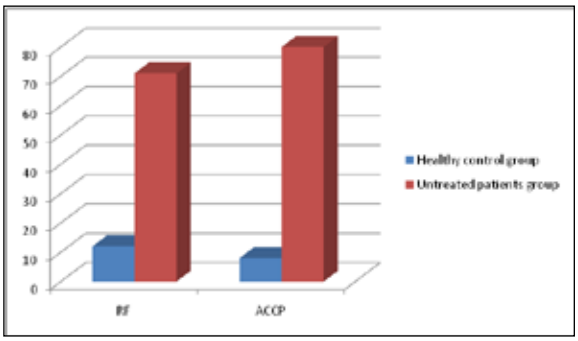
In addition our results revealed that the median serum levels of IL-1α, IL-8, IL-17 and GM-CSF were significantly elevated (P<0.001) in untreated RA patients (106.0; 888.0; 635.0 and 55.60 pg\ml, respectively) than that in control group (20.10; 288.0; 210.0and 15.30 pg\ml respectively), as clearly shown in (Figure 2).

The other important result in this study was effect of rituximab on the clinical and immunological parameters which included in the current study. Table 2 shows that the seroprevalence of RF and ACCP, and serum levels of DAS, IL-1α and IL-17 significantly fell (P<0.05, P<0.01) in patients treated with rituximab as compared to those patients who have received no treatment (40%; 43%; 4.15±0.75; 77.0 and 417.5 vs. 71%; 80%; 5.23±0.89; 106.0 and 635.0 respectively). On the other hand there were no significant changes (P>0.05) in the levels of circulating IL-8 and GM-CSF between two groups of patients (888.0; 55.0 pg/ml vs. 812.0; 52.0pg\ml, respectively). Meanwhile, our results indicate that the seroprevalence of RF and ACCP, and serum levels of IL-1α and IL-17 were remained significantly elevated (P<0.001) in patients treated with rituximab as compared to healthy control, as shown in (Table 3). Interestingly, in treated RA patients group, significant correlation of serum IL-1 α and IL-17 levels were found with each of DAS and RF, (Table 4).

Table-1: Baseline characteristic of the studied groups.

	Study groups			P-value
	Healthy control group	Untreated patients group	Treated patients group	
Age Range	(20-63)	(23-68)	(25-66)	P>0.05^{NS}
Mean±SD	42.84±11.87	43.57±11.90	44.83±9.01	
Gender type				
Female	20 (80%)	30 (86%)	24 (80%)	P>0.05^{NS}
Male	5 (20%)	5 (14%)	6 (20%)	
Total	25 (100%)	35 (100%)	30 (100%)	

NS: not significant



Figue-1: Seroprevalence of RF and ACCP in healthy control group and untreated patients group.

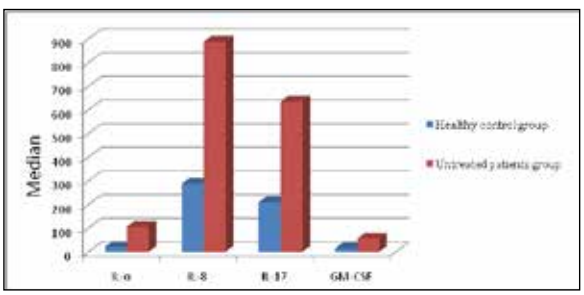


Figure-2: Median serum levels of cytokines (IL-1α, IL-8, IL-17 and GM-CSF) in healthy control group and untreated patients group.

Table 2: Differences in levels of DAS-28 and immunological parameters in two groups of patients.

	Study groups		
	Untreated patients group n=35	Treated patients group n=30	P-value
DAS			
Mean±SD	5.23±0.89	4.15±0.75	<0.05*
RF			
Positive (n %)	25 (71%)	12 (40%)	<0.05*
ACCP			
Positive (n %)	28 (80%)	13 (43%)	<0.05*
IL-α			
Median	106.00	77.00	<0.01*
Mean±SD	123.69±74.69	81.70±56.09	
IL-8			
Median	888.00	812.00	>0.05^{NS}
Mean±SD	918.80±411.28	770.13±379.05	
IL-17			
Median	635.00	417.50	<0.05*
Mean±SD	629.51±351.66	494.23±321.29	
GM-CSF			
Median	55.60	52.00	>0.05^{NS}
Mean±SD	57.79±39.37	58.06±32.32	

***Significant.**

Table 3: Differences in seroprevalence (RF and ACCP) and serum levels of IL-1α and IL-17 in treated patients group and healthy control group.

	Study groups		
	Healthy control group n=25	Treated patients group n=30	P-value
RF			
Positive	3 (12%)	12 (34%)	<0.05*
ACCP			

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Positive	2(8%)	12 (40%)	<0.05*
IL-α			
Median	20.10	77.00	<0.05*
Mean±SD	22.38±16.06	81.70±56.09	
IL-17			
Median	210.00	417.50	0.05*
Mean±SD	267.20±111.47	494.23±321.29	

Table 4: Correlation among various parameters in RA cases treated with rituximab.

Variables	DAS	RF	ACCP	IL-1α	IL-17
DAS	1	r=0.371 P=0.015*	r=0.242 p=0.041*	r=0.271 p=0.022*	r=0.468 p=0.002*
RF	r=0.371 p=0.015*	1	r=0.078 p=0.655	r=0.328 p=0.013*	r=0.560 p=0.002*
ACCP	r=0.242 p=0.041*	r=0.078 p=0.655	1	r=0.033 p=0.852	r=0.103 p=0.557
IL-1α	r=0.271 p=0.022*	r=0.328 p=0.013*	r=0.033 p=0.852	1	r=0.053 p=0.764
IL-17	r=0.468 p=0.002*	r=0.560 p=0.002*	r=0.103 p=0.557	r=0.053 p=0.764	1

Discussion

The clinical data to date strongly support the premise that B cells play a major part in the pathogenesis of RA and that selective targeting of these cells may provide treatments that not only enhance our understanding of this disease but also provide substantial and prolonged clinical benefit. Rituximab is an anti-CD20 chimeric monoclonal antibody that produces a high level of sustained efficacy in RA that coincides with a profound and longlasting peripheral depletion of CD20+ B cells after treatment (18). Nowdays, Iraqi patients with RA received advanced therapy, i.e. the use of biologic agent like TNF-α inhibitor and anti-CD20 agents to control disease activity and induce remission, so this encouraged us to study the effect of rituximab treatment on the seroprevalence of (RF and ACCP) and serum levels of IL-α, IL-8, IL-17and GM-CSF in patients treated with rituximab in comparison to those patients without treatment. Although this was the main objective of the present study but nonetheless the prevalence of these immunological parameters in patients with RA was also investigated as compared to healthy controls.

The findings of the present study indicate that serum markers of B cell activation (RF and ACCP) are increase in untreated patients group as compred to healthy controls. In agreement with our study Gottenberg et al. (19) reported that RF and ACCP were elevated in patients and may be correlated with disease activity, correspondingly other recent results suggest that the presence of RF or ACCP may be a predictive factor for response to rituximab both in early RA and in established RA (20). However, the presence of autoantibodies may indicate that some forms of RA are B cell driven and sensitive to B cell depletion therapy (21). Moreover, this study observed that serum cytokines levels in RA patients were readily distinguished from those in healthy individuals, our result was similarly to previous studies (22, 23) who reported that patients with RA had increased levels of IL-1α, IL-8, IL-17 and GM-CSF. These findings support the hypothesis that RA is a complex immune-infl ammatory disorder involving the dysregulation of cellular, humoral and innate immunity, with a significant systemic signature.

The other important results in the present study were the effect of treatment with rituximab on the presence of autoantibodies and on the concentrations of cytokines in RA pateints. Consistent with the findings of previous reports (24, 25, 26), current results revealed that mean of DAS-28 and seroprevalence of RF and ACCP were significantly decreased in rituximab treated group from that in untreated group. It is well known that RA is characterized by the presence of the autoantibodies (RF and ACCP), which are produced by B-cells. Approximately 80% of patients are positive for at least 1 of them (2). Taylor et al. observed that while the specific role of these autoantibodies in the pathogenesis or progression of RA has yet to be determined, improvement of RA is frequently associated with their decline. Hence, when rituximab has been used in RA, it has been shown to improve clinical outcomes (27). Our findings were in congruence to the results of other study conducted by Hasan and colleagues (24), who mentioned that RA patients treated with rituximab were showed significantly reduction in their disease activity and their serum concentrations of RF and ACCP. The responses to rituximab seen in this study support the hypothesis that B cells are key contributors to the immunopathogenesis of RA through several potential mechanisms (28)

The present study showed also that treatment with rituximab was accompanied by significant decrease in serum levels of IL-1α and IL-17. In contrast we did not show remarkable changes in the levels of IL-8 and GM-CSF between two groups of patients. Interestingly, the reduction in IL-1α and IL-17 levels was in parallel with the decrease in DAS-28 in treated patients group, indicating that rituximab can decrease the inflammatory level of the disease, confirming the findings of previous study (24), which reported that the decrease in disease activity in RA patients after treatment with rituximab might be due to the decline in the proinflammatory interleukines production. Meanwhile van de Veerdonk et al., stated that rituximab reduced the local Th17 response in RA patients, and the decreased Th17 response was associated with strongly reduced IL-17 as well as reduced inflammation and better clinical outcome (29). It is generally accepted that RF autoantibodies, produced by B cells under influence of activated T cells, these autoantibodies are activate macrophages and T-cells, which then release inflammatory cytokines (8,30). This may be support and explain our result about the positive linear correlation between RF and each of IL-1 α and IL-17 levels in patients treated with rituximab. Coinciding with current result Thurlings et al. (31) denoted that rituximab-induced B cell depletion may cause a decrease in the activation state of T cell subsets (Th17), contributing to clinical improvement.

As discussed herein, rituximab can decrease the production of autoantibodies and some of cytokines in RA. Traditionally, we know that B-cell, which mainly secrete RF and ACCP, play an important role in the pathogenesis of RA (32). Th17, a new designated T-helper cell type marked by IL-17 secretions, has been shown to be closely related to RA (33). The results of this study indicate that rituximab not only neutralizes the effects of B-cell, but it also down-regulates the proinflamatory interleukins (IL-1α and IL-17). In addition these findings showed that cytokines can be used to predict the response to rituximab in patients with refractory RA.

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