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BEYOND THE JOINTS IN RHEUMATOID ARTHRITIS: EFFECTS OF ADALIMUMAB ON HEMATOLOGIC AND LIPID INDICES

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ABSTRACT

Introduction: Tumor necrosis factor alpha (TNFα) is a multifunctional cytokine which plays a pivotal role in the pathogenesis of rheumatoid arthritis (RA). Apart from its well recognized pro-inflammatory properties, it is known to interfere with lipid metabolism and erythropoiesis.
Materials and Methods: We evaluated the effects of adalimumab on hematologic, lipid and inflammatory parameters using data from patients on adalimumab 40 mg fortnightly from 2 centers in Malaysia. Mean changes in laboratory values from baseline to Weeks 4, 12 and 24 were compared using paired T test and Wilcoxon signed-rank test.
Results: We studied 18 patients with RA who were on adalimumab 40 mg fortnightly. The inflammatory markers i.e. erythrocyte sedimentation rate and C reactive protein showed significant changes as early as at week 4 compared to baseline with p values of 0.003 and 0.005, respectively. From a baseline of high disease activity with a mean Disease Activity Score using 28 joint counts (DAS 28) of 5.3, there was a steady improvement in the disease activity and remission was achieved at week 24 with a DAS 28 of 2.4. The hemoglobin level improved at week 12 (p=0.013) and this was sustained till week 24. As opposed to previous studies, the LDL level significantly decreased at week 12 (p=0.015) and this change persisted till week 24 (p=0.001). The total cholesterol showed a similar pattern as the LDL.
Conclusions: The pharmacodynamics of adalimumab therapy in rheumatoid arthritis extend beyond the joints with favorable effects on haemoglobin and lipid profile.

Keywords: adalimumab, rheumatoid arthritis, anemia, lipid profile

INTRODUCTION

Adalimumab, also known by the trade name of Humira, is the third TNF inhibitor, after infliximab and etanercept, and the first fully human (100 % human peptide sequences) anti-TNFα monoclonal antibody. Since year 2008, FDA has approved the use of adalimumab in the treatment of rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, Crohn's disease, moderate to severe chronic psoriasis and juvenile idiopathic arthritis (Brekke and Sandlie, 2003). This relatively new form of biologic therapy was genetically engineered through phage display technology. The structure, function

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and terminal half-life of adalimumab is indistinguishable from human immunoglobulin Gl (IgGl). There is high specificity and affinity for TNFα (dissociation constant = 6 x lo-" mol/L) with sparing of similar cytokines such as TNF beta. Adalimumab exerts therapeutic effects through the blockade of TNFα from binding to subunits ~55 and ~75 of TNFα cell surface receptors (Weisman et al., 2003).

TNFα is mainly produced by monocytes and macrophages. TNFα are critical orchestrators in several rheumatic diseases by stimulating endothelial cells to express adhesion molecules that promote migration of leukocytes into affected joints; accelerating the production of metalloproteinases by synovial macrophages, fibroblasts, osteoclasts, and chondrocytes. Downregulation of these proinflammatory cytokine halts the inflammatory process involved (Weisman et al., 2003).

Rheumatoid arthritis (RA) is a chronic autoimmune disease mainly characterized by symmetrical synovitis, with potential risk of progressive erosions and deformities of joints. Epidemiologic research shows that some 1 % to 2 % of the adult population worldwide are affected by RA (Boyer et al., 1991). Patients with RA were found to have higher levels of TNFα in the serum and synovial fluid compared to healthy subjects (Tetta et al., 1990). As a multifunctional cytokine, apart from its proinflammatory properties, TNF-alpha through complex molecular mechanisms interferes with several physiological homeostasis. Researchers have speculated that TNFα derived adipokines, enhances insulin resistance and inhibits erythropoietin production (Mehran et al., 1995; DeRienzo and Saleem, 1990). It is therefore not surprising that beyond articular involvement, RA confers increased risk of cardiovascular disease. Apart from clinical remission of arthritis, accumulating evidence assigns TNF blockade to provide additional benefits to the general health of patients.

Unlike infliximab and etanercept, the changes in key laboratory parameters with adalimumab among patients with RA have not been well studied. In this analysis among the subset of RA patients, we evaluated the effect of adalimumab over time on hematologic, inflammatory and lipid indices.

MATERIALS AND METHODS

Data collection

We reviewed the computerized medical records of patients with RA treated with adalimumab from 2 tertiary centers in Malaysia i.e. Putrajaya Hospital and Universiti Kebangsaan Malaysia Medical Centre (UKMMC) between January 2009 and February 2011. The study protocol was approved by the respective Ethics Committee of both centers. Patients' demographic data, clinical records, comorbidities, concomitant medications and laboratory parameters data including full blood count, albumin, erythrocyte sedimentation rate (ESR), C reactive protein (CRP) and lipid profile were obtained by going through the patients' medical charts. The patients' details and parameters at the time of initiation of adalimumab, after 4 weeks, 12 weeks and 24 weeks were recorded and analyzed.

The patients in our study received a standard dose of 40 mg of adalimumab fortnightly in combination with either oral methotrexate 10 to 25 mg weekly or leflunomide 20 mg daily. All patients were switched to adalimumab after failure to achieve remission with combination of conventional disease modifying anti-rheumatic drugs (DMARDS). 3 of the subjects had received infliximab prior to switching to adalimumab for better disease control.

DAS 28

In our clinical practice, the Disease Activity Score using 28 joint counts (DAS 28) is used to monitor the disease activity of rheumatoid arthritis. The final score is calculated based on swollen joint count, tender

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joint count, visual analog score (0-100 mm) and the level of ESR (erythrocyte sedimentation rate) or CRP (C reactive protein). A DAS 28 of less than 2.6 defines remission whereas a value of more than 5.1 confirms high disease activity (Riazzoli et al., 2010).

Inclusion and exclusion criteria

Inclusion criteria were as follows: patients with rheumatoid arthritis who were treated with adalimumab 40 mg fortnightly between January 2009 and February 2011. Exclusion criteria were as follows: patients who had the following during the study period a) started on lipid lowering agents or had escalation of the dose of lipid lowering agents, b) started on steroids or had escalation of the dose of steroids and c) received blood transfusion or erythropoietin therapy.

Laboratory assessment

ESR and CRP were measured by protein latex particle enhanced immunoturbidimetric assay (Roche Diagnostics). The normal CRP value was < 0.50 mmol/L. Rheumatoid factor (RF) by ELISA (IgM isotype) were assessed in all patients. Total cholesterol, HDL cholesterol and triglyceride levels were determined by enzymatic methods. These parameters were measured on a Modular P analyzer (Roche Diagnostics). LDL-cholesterol levels were calculated by using the Friedewald formula. Measurement of serum albumin is by using a dye binding technique using bromocresol green (BCG). Both centers used the same diagnostic assays to evaluate the laboratory parameters.

Statistics

Analysis of data was performed using the SPSS version 17.0 statistical package. All results are presented as mean ± SEM or median (range). A p value was considered significant at < 0.05. The changes in the pre and post treatment parameters with adalimumab was analysed using the paired Student's T-test for normally distributed variables and Wilcoxon signed-rank test for variables which were skewed.

RESULTS

Demographics and baseline characteristics

Demographics and baseline characteristics of the patients are shown in Table 1. In total, 18 patients were studied. The vast majority of the patients were females (88.9 %). The mean age of the patients was 52.1 ± 9.1 years. Most of the patients (72.2 %) had seropositive disease. In general, at the time of commencement of adalimumab, the patients had a high disease activity with a mean DAS 28 of 5.3 ± 0.9. The 8 patients with dyslipidemia were on statin therapy but there were no changes in the dose of the lipid lowering agent throughout the study period.

Table 1: Demographic data and baseline characteristics of patients

Parameter	n=18
Age	52.06 ± 9.09
Female	16 (88.9 %)
Duration of RA	4.24 ± 3.13
Rheumatoid factor positive	13 (72.2 %)
DAS	5.30 ± 0.90
Concurrent DMARD	
Methotrexate	13 (72.2 %)
Leflunomide	5 (27.8 %)
Prednisolone	3 (16.7 %)
Diabetes mellitus	3 (16.7 %)
Dyslipidemia	8 (44.4 %)

*Data presented as n (%) or mean ± standard deviation

Changes in inflammatory markers and disease activity

Figure 1 illustrates the improvement in acute phase reactants and disease activity over time with adalimumab. Both inflammatory markers i.e. ESR and CRP dropped drastically with treatment. These changes were found significant as early as at week 4 with p values of 0.003 and 0.005, respectively. The mean ESR decreased significantly

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from 82.6 ± 28.2 to 56.8 ± 25.0 whereas the CRP dropped from a median of 3.2 to 0.3 after 24 weeks of treatment. Albumin, a negative acute phase protein, increased steadily from baseline with significant p values of 0.019, 0.002 and 0.001 at weeks 4, 12 and 24, respectively. The mean DAS 28 from high disease activity (5.3) improved to moderate disease activity (4.2) at week 4 followed by low disease activity (2.9) at week 12 and remission (2.4) was achieved at week 24.

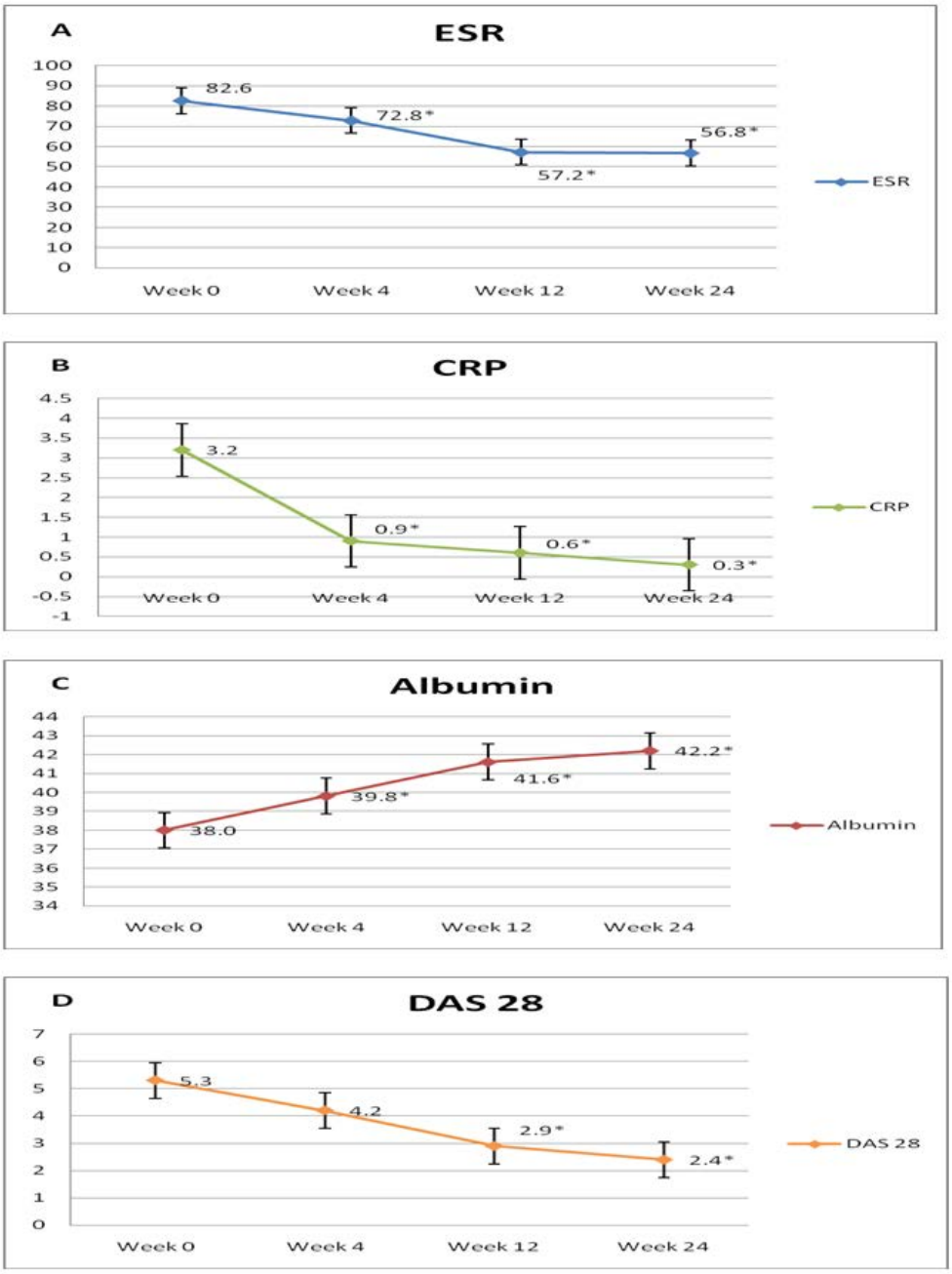

Figure 1: Changes in mean ESR (A), CRP (B), Albumin (C) and DAS 28 (D) with adalimumab therapy at baseline, week 4, week 12 and week 24.
*Statistically significant changes from baseline (week 0)

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Changes in hematologic parameters

After 12 weeks of adalimumab (6 injections), there was a significant rise in hemoglobin level compared to baseline (p=0.013). The difference in the mean hemoglobin level between week 4 and week 12 was 0.78 g/dL. This response was sustained even at 24 weeks (p=0.014). Adalimumab therapy had no effects on platelet and white cell count levels (Table 2).

Changes in lipid profile

At week 4, all lipid parameters i.e. low density lipoprotein (LDL), high density lipoprotein (HDL), total cholesterol and triglycerides did not show any statistically significant difference from baseline. However, at week 12, there was marked improvement in the LDL level compared to pre-treatment level (p=0.015). There was a parallel drop in total cholesterol level (p=0.015). For both LDL and total cholesterol, the effect lasted even at week 24 with p values of 0.002 and 0.010 respectively. The HDL and triglyceride parameters did not show significant changes with adalimumab treatment (Table 2).

DISCUSSION

This study clearly illustrates that the beneficial effects of adalimumab is beyond articular inflammation and damage. The suppression of TNFα had a demonstrable influence on erythropoeisis and lipid metabolism. As a relatively new anti TNF agent, there are fewer studies on adalimumab on this aspect compared to its older counter parts like infliximab and etanercept.

One of our novel findings is the improvement in rheumatoid anemia with adalimumab. TNFα acts both on multipotential progenitors and their derivatives. High levels of circulatory TNFα inhibit erythropoiesis through several mode of actions. The 4 main mechanisms involved are 1) shortened survival of erythrocytes 2) reduced capacity of iron utilization 3) suppressed colony-forming units-erythroid (CFU-e) and 4) reduced erythropoietin production which in-

Table 2: Changes in hematologic and lipid parameters with adalimumab therapy from baseline to weeks 4, 12 and 24

Laboratory results	Baseline	Week 4	p values for changes from baseline	Week 12	p values for changes from baseline	Week 24	p values for changes from baseline
Hemoglobin	10.9 ± 1.3	11.1 ± 1.1	0.068	11.8 ± 0.9	0.013	11.8 ± 0.6	0.014
White cell count	8.2 ± 1.9	8.3 ± 1.9	0.433	8.7 ± 2.2	0.436	7.7 ± 1.9	0.275
Platelet	324.9 ± 97.3	317.2 ± 102.3	0.171	340.7 ± 81.0	0.375	330.3 ± 75.4	0.881
Total cholesterol	5.4 ± 0.7	5.2 ± 1.1	0.143	4.6 ± 0.7	0.015	4.4 ± 0.9	0.010
LDL	3.7 ± 0.8	3.6 ± 0.9	0.072	2.9 ± 0.5	0.015	2.8 ± 0.6	0.002
HDL	*1.3 (1.0-2.3)	*1.3 (1.2-1.9)	*0.909	*1.2 (20.9-1.9)	*0.972	*1.2 (1.1-1.7)	*0.615
Triglyceride	1.1 ± 0.3	1.1 ± 0.7	0.569	1.1 ± 0.4	0.672	0.9 ± 0.3	0.398

All data presented as mean ± standard deviation
p values are based on paired T test.
*Data presented as median (range) & p values are based on Wilcoxon signed-rank tests

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hibits generation of glycophorin A (GPA) positive cells from CD34+ progenitors. Figure 2 summarizes the role of TNF alpha in the pathophysiology of rheumatoid anemia (Buck et al., 2009). Doyle et al. (2009) based on their large, multicenter study reported that treatment with infliximab plus methotrexate resulted in significant improvement in the hemoglobin levels compared to placebo. This is the first study to our knowledge, which has demonstrated improvement in hemoglobin level among RA patients which was evident as early as week 12 of adalimumab therapy. Results from the CHARM trial involving patients with Crohn's disease showed a significant increase in hemoglobin level only at week 26 of treatment with adalimumab (Rubin et al., 2011).

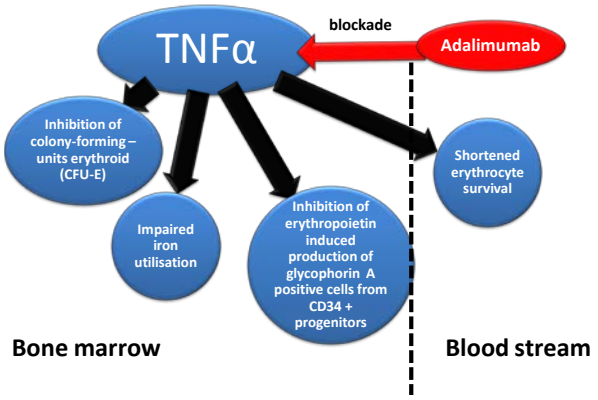

Figure 2: Role of TNFα in rheumatoid anemia. TNFα blockade by adalimumab improves anemia through its interference in above mechanisms.

Albumin is a negative acute phase protein. We believe that the significant early increase in albumin is due to the achieved control of inflammation rather than improvement in the nutritional status as suggested by a recent study (Rubin et al., 2011). In response to systemic inflammation, the liver responds by producing acute-phase reactants. At the same time, the production of a number of other proteins is reduced which are referred to as "negative" acute-phase reactants. Examples include albumin, transferrin, transthyretin, antithrombin and transcortin. Albumin is therefore an inflammatory marker. Based on this study findings, there was an inverse relationship between albumin and the other two positive acute phase reactants i.e. ESR and CRP. The physiological decrease in synthesis of albumin is to utilize amino acids for efficiently producing other acute-phase proteins (Ritchie et al., 1999). Besides, the fractional catabolic rate of albumin was found to have a positive correlation with the disease activity in RA (Ballantyne et al., 1971).

To date, the data on the effects of TNF antagonists on lipid profile has been rather inconsistent and remains controversial. For instance, there is conflicting evidence on the net effect of infliximab on lipid indices. While some studies demonstrated no changes in lipid profile, the others have shown significant rises in total cholesterol and HDL (Soubrier et al., 2008; Allanore et al., 2006; Wijbrandts et al., 2009). In stark contrast with other biologic studies, the LDL and total cholesterol levels in this study showed a decreasing trend with adalimumab therapy. The effects of adalimumab on lipid profile has not been extensively studied for comparison.

Based on our literature search, so far there are only 4 studies which have evaluated changes in lipid profile among RA patients treated with adalimumab. The number of subjects were generally small, ranging from 4 to 50 patients. In 3 of the studies, the HDL-cholesterol levels increased significantly from baseline to between 2 to 16 weeks of treatment (Wijbrandts et al., 2009; Seriolo et al., 2006; Popa et al., 2005). The remaining study showed no changes in the lipid profile (Soubrier et al., 2008).

In keeping with our results, there are in vivo studies which have documented that TNFα has the capacity to induce dyslipoproteinemia by stimulating production of LDL and triglyceride. The mechanism involved remains hypothetical and theoretical with multiple processes at different cellular lev-

els. TNFα is believed to stimulate cholesterol biosynthesis through induction of maturation of sterol regulatory element binding protein-1 (SREBP-1), increased free fatty acid production, induction of lipolysis, increase in hepatic HMG-CoA reductase activity and alteration in the expression of adipokines such as leptin and adiponectin (Chen et al., 2009).

The greatest limitation of our study is the small sample size. In most developing countries like Malaysia, the use of adalimumab is limited by its cost. This could explain the discrepancy between our results and previous studies. Besides, the atherogenic index calculated as the log of triglyceride/HDL which is a predictor of atherosclerosis and coronary artery disease was not studied (Nwagha et al., 2010). In fact, it would have been interesting to study the effects of adalimumab treatment on the apolipoprotein B/apolipoprotein A-I ratio. Emerging evidence suggests that the predictive power of the apo B/A-I ratio is superior to other individual lipid parameters such as triglyceride, LDL, HDL or total cholesterol (McGorrian et al., 2011).

In conclusion, adalimumab therapy in RA was associated with significant improvements in anemia, LDL, total cholesterol and inflammatory markers. Our findings may be partially explanatory for the well documented cardioprotective effects of TNF antagonist. The pharmacodynamics of adalimumab in relation to erythropoiesis and lipid metabolism merits further research as it has therapeutic implications.

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