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Hematological and Biochemical Profiles in Rheumatoid Arthritis Patients Undergoing Methotrexate, Rituximab, and Combination Therapy

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ABSTRACT

Objective: *Rheumatoid arthritis is one of the most prevalent chronically inflammation and systemically autoimmune illnesses. Non-steroidal anti-inflammatory medications (NSAIDs), corticosteroids, biological and synthetic disease-modifying antirheumatic pharmaceuticals (DMARDs), and immunosuppressive medications were all included in the guidelines for the treatment of rheumatoid arthritis. The aim of study Longitudinal assessment of WBC, Hb, platelets, ALT, AST, creatinine, urea, and ESR during taking Methotrexate (MTX) and Rituximab (RTX) treatment each alone and in combination for RA patients.* ***Method:*** *The study involved RA patient all taken treatment for 4 years divided into three subgroup: group (A) include 30 cases who were taken methotrexate only, group(B) include 20 cases were taken RTX only and group(C) include 10 cases who were taken combination of MTX and RTX for 4 year. In addition to the control group involved 60 healthy individuals.* ***Results:*** *Our findings reveal a significant decrease in hemoglobin (Hb) levels in RA patients undergoing these treatments (p=0.03) compared to controls(11.21,11.45, 11.21 ±SD vs. 12.56±0.96). Conversely, there was a significant increase in platelet counts (p=0.002) in treated RA patients (298.71 ,266.89,308.5±SD) compared to controls (248.96±51.54). No significant differences were observed in white blood cell (WBC) counts (p=0.26). Regarding kidney function, a significant increase in both creatinine (p=0.002) and urea (p=0.003) was noted in RA patients receiving treatment (0.69, 0.71, 0.8±SD for creatinine ; 28.5 26.88−32.34±SD for urea) when compared to controls (0.59±0.19 for creatinine; 24.63±5.51 for urea). However, liver function parameters, including ALP and AST, showed no significant differences (p=0.8 and p=0.15, respectively). Finally, a significant elevation in Erythrocyte Sedimentation Rate (ESR) (p=0.004) was observed in treated RA patients (35.63,52.81, 37.5 ±SD) versus the control group (17.7±8.47), indicating heightened inflammatory activity.* ***Novelty:*** *Longitudinal assessment of hematological, liver, kidney, and inflammatory biomarkers in RA patients over a four-year period under MTX, RTX, and combined treatment is rarely reported, especially with this specific comparative design including a healthy control group.*

INTRODUCTION

One characteristic of rheumatoid arthritis (RA), a systemic and inflammatory autoimmune disease, is the primary compromise of peripheral joint synovial membranes. The prevalence of RA ranges from 0.5% to 1% of the population, with women and individuals aged 30 to 50 years being the most commonly affected[1,2]. RA is distinguished by the symmetrical manifestation of both large and small joints, with the joints of the hands & feet being particularly affected. The continuous and destructive nature of the condition can lead to substantial functional constraints, including the loss of capacity to work and a lower quality of life, unless a diagnosis is acquired at an early stage of the disease and therapy results in clinical improvements[3]. The criteria of (2010

ACR/EULAR) are organized into four categories, each with a point score: The joint Symptoms, acute-phase reactants (CRP and/or ESR), serology (containing RF and/or ACPA), and symptom duration, whether 6 weeks or longer. Any joint that seems swollen or painful upon examination is considered to have "joint involvement," and radiographic showing synovial inflammation can confirm this. [4]. Patients with early-stage RA appreciate early diagnosis and treatment because it prevents the progression of joint injury.Nonsteroidal anti_inflammatory drugs, such as ibuprofen and naproxen, are common treatments for RA because they reduce tissue inflammation, swelling, and pain, but they also have gastrointestinal side effects, such as pain, bleeding, and ulcers, which can be lessened by taking them with food or by administering other medications[5], Corticosteroids and glucocorticoids, particularly at low doses, have been proved to impact the symptoms and warning signs of arthritis as well as reduce the course of joint deterioration, DMARDs which work to stop progressive damage to cartilage, bone, and surrounding soft tissues, are also commonly used to treat RA.[6]. These treatments come in two types, such as: Chemical or synthetic DMARDs, including methotrexate, which is the most commonly utilized and a component of almost all modern RA therapy plans [7]and Biological DMARDs have transformed the treatment of RA, other types of arthritis, and immune-mediated diseases. TNF antagonists include rituximab, infliximab, adalimumab, golimumab, etanercept certolizumab, tocilizumab and abatacept [8], immunosuppressive medications. Patients with nonspecific arthritis with positive levels of RA predictive biomarkers such as anticyclic citrullinated peptides (anti-CCPs) and/or rheumatoid factors (RFs) may benefit from DMARDs[9].

Methotrexate (MTX) inhibits the formation of protein molecules, thymidylate, RNA as well,DNA. MTX's anti-inflammatory effects in RA appear to be at least largely due to adenosine metabolism changes and probable effects on the level of tumor necrosis factor (TNF) activity. Dihydrofolate cannot be converted to active tetrahydrofolate as MTX inhibits dihydrofolate reductase,which is an enzyme that regulates the metabolism of folic acid which results in immunosuppressive and toxic effects. The medicine was found to be useful in RA patients even at lower doses (15-25 mg) weekly [10].

It can also be alonge with additional DMARDs, including hydroxy-chloroquine and sulfasalazine. When this is ineffective, a biologic DMARD is frequently used in combination with methotrexate to increase efficacy. The adverse effects and clinical consequences of highdose MTX used to treat cancer can be reversed with large doses of calcium or folate [11]. However, the administration of folic acid has no affect on the efficiency of lower doses from chemical methotrexate used in RA patients, and it is almost usually included in the RA treatment regimen to lessen the adverse consequences of MTX[12]. The adverse effect profile of sulfasalazine, leflunomide, and methotrexate is comparable. All of these substances have common side effects, including rash or allergic reaction, bone marrow suppression, hepatotoxicity, gastrointestinal distress (diarrhea, nausea, and stomach pain), and a higher probability of common and sometimes dangerous illnesses. MTX & Leflunomide may result in weight loss, peripheral neuropathy, and hypertension[13]. Based on RA disease activity measures, like the variety of sore or inflamed joints or the doctor's global assessment score, several studies have demonstrated that folic acid supplementation reduces toxicity but does not change

the effectiveness of methotrexate [14]. RTX differs from other biologic DMARDs in that it binds CD20+ B cells, resulting in the inhibition of B-cell-mediated inflammation responses. Another differentiating feature of RTX is the significant gap between treatment rounds; RTX's specific decrease of CD20-positive B cells resulted in a longer duration of the therapeutic effect with each cycle of therapy[15]. According to clinical assessment, RTX retreatment is usually recommended after around six months [16].

MATERIAL AND METHODS

Patient and Control

According to the 2010 ACR/EULAR criteria, 60 cases of rheumatoid arthritis patients were examined at Merjan Teaching Hospital, Rheumatology Unit, Babylon province and Al-Sader Teaching Hospital, Medical Rehabilitation and Joint Unit, Al-Najaf Al-Ashraf province. These patients were evaluated by specialists and given a diagnosis of rheumatoid arthritis based on clinical clinical findings Materials & Methods and serological parameters. RA patient were taken treatment including methotrexate, Rituximab and combined therapy and they distributed according to the type of treatment into three groups. The first group include patient taken treatment for 4 year divided into three subgroup: group (A) include 30 cases who were taken methotrexate only, group(B) include 20 cases were taken RTX only and group(C) include 10 cases who were taken combination of MTX and RTX for 5 year. Second group involved 60 healthy individuals. Blood Samples from patients and controls was collected and transferred into three tubes as follows: one ml of blood transferred to EDTA tube in order to examination complete blood count for detection WBCs, Hb and platelets , one ml of blood transferred to ESR tube used for examination ESR by Westergren which is a manual and rapid method for estimation ESR , and finally 3 ml of blood transferred to gel tube and centrifuged at 4000 rpm for 5 minute to separate the serum for detection ALP AST, urea and creatinine.

Ethical Approval

The scientific research procedure has been approved by the Medical College Ethical Committee of Jabir Ibn Hayyan University for Medical and Pharmaceutical Sciences. Informed permission was obtained from every participant in the study.

Statistical Analysis

The statistics that use in the current study are (mean ±SD, the t-test (independent) and ANOVA test by SPSS program version 20. The level of significance that used was < 0.05 in all statistical analysis.

RESULTS AND DISCUSSION

Results

The results of such research illustrated in table 1 showed that significant decrease in Hb of RA patients (p value 0.03) when taken three types of treatments MTX, RTX alone and in combined (11.29 ± 2.67, 11.45 ± 1.5, 11.21 ± 4.3) respectively in comparison with control individuals (12.56 ± 0.96). Also the finding demonstrated that significant increase (p value 0.002) in platelets of RA patients when taken three types of treatments MTX, RTX alone and in combined (298.71 ± 83.11, 266.89 ± 54.89 and 308.5 ± 46.08) respectively in comparison with control (248.96 ± 51.54) but no significant differences in WBCs (p

value 0.26) between patients and control. significant increase in kidney function included creatinine and urea of RA patients (p value 0.002and 0.003) respectively when taken three types of treatment MTX, RTX alone and in combined (0.69 ± 0.2, 0.71 ± 0.15, 0.8 ± 0.07 and 28.5 ± 7.93, 26.88 ± 6.11, 32.34 ± 7.67) respectively in comparison with control group (0.59 ± 0.19 and 24.63 ± 5.51) respectively. On the other hand, no significant differences in liver function parameters included ALP and AST (p value 0.8 and 0.15) between patents and control. that significant increase in ESR of RA patients (p value 0.004) when taken three types of treatments MTX, RTX alone and in combined (52.81 ± 73.3, 35.63 ± 7.4 and 37.5 ± 13.83 respectively respect with control group (17.7 ± 8.47).

Table 1. Comparison between treatments (methotrexate, rituximab each alone, and in combination for RA patients and control group.

Treatment	Mean ± SD				P-value
	Control	MTX	RTX	MTX+RTX	
WBC	6.53^{A} ± 1.56	7.4^{A} ± 3.1	7.45^{A} ± 2.59	7.49^{A} ± 2.57	0.26
Hb	12.56^{A} ± 0.96	11.29^{B} ± 2.67	11.45^{B} ± 1.5	11.21^{B} ± 4.3	0.03
PLT	248.96^{A} ± 51.54	298.71^{B} ± 83.11	266.89^{B} ± 54.89	308.5^{B} ± 46.08	0.002
ALP	95.82^{A} ± 17.1	99.06^{A} ± 27.22	100.37^{A} ± 19.7	101.44^{A} ± 30.3	0.8
AST	21.9^{A} ± 3.82	22.48^{A} ± 5.18	23.05^{A} ± 5.66	23.7^{A}± 9.53	0.15
Creatine	0.59^{A} ± 0.19	0.69^{B} ± 0.2	0.71^{B} ± 0.15	0.8^{B} ± 0.07	0.002
Urea	24.63^{A} ± 5.51	28.5^{B} ± 7.93	26.88^{B} ± 6.11	32.34^{B} ± 7.67	0.003
ESR	17.7^{A} ± 8.47	52.81^{C} ± 73.3	35.63^{B} ± 7.4	37.5^{B} ± 13.83	0.004

*the similar letters mean non-significant.
*the different letters mean significant.

Discussion

Both rituximab and methotrexate (MTX) are potent immunosuppressive medications used to treat a variety of malignancies and autoimmune disorders [17]. Due to its effectiveness and long-term safety profile, MTX was recommended by EULAR as the initial therapy approach for individuals with RA. Overall, withdrawals from MTX due to toxicity are less frequent than with most other DMARDs, and while many patients have adverse events during MTX therapy, these are usually moderate [18]. An enzyme essential to the production of DNA and RNA, dihydrofolate reductase, is inhibited by the antifolate medication methotrexate. Myelosuppression, or a decrease in the bone marrow's ability to produce blood cells, may result from its mode of action [19].A serious

but uncommon side effect of MTX is pancytopenia, which is defined as a decrease in all three cell lines: red blood cells, white blood cells, and platelets. According to studies, 1.4% to 7% of people on low-dose MTX for inflammatory rheumatic illnesses may develop neutropenia. With a frequency of 0.96–1.4%, the rate of hematological side effects appeared to be lower in previous investigations [20,21]. One frequent side effect of long-term MTX therapy is hepatotoxicity. The most prevalent test indicator of hepatotoxicity is an elevated level of aminotransferases. It was seen in individuals with psoriatic and rheumatoid arthritis receiving MTX, with a frequency ranging from 7.5% to 26%[22]. There is uncertainty regarding the MTX's hepatotoxic adverse effect. In inflammatory diseases treated with MTX, folic acid supplementation is linked to a lower incidence of elevated aminotransferases [23]. There are limited studies regarding the safety of methotrexate (MTX) in patients with reduced renal function. Research indicates that while MTX is effective, it can lead to dose-dependent renal impairment, necessitating careful monitoring of kidney function in RA patients [24]. Methotrexate (MTX) has been associated with improved renal outcomes in rheumatoid arthritis (RA) patients, as evidenced by a decrease in progression to dialysis and better management of renal complications over time, particularly after the introduction of newer treatment strategies [25]. However, the overall safety profile of rituximab suggests that it is well-tolerated, with mild adverse events reported. Rituximab treatment has been associated with a decrease in inflammatory markers, which can lead to improved hemoglobin levels over time. decrease in ESR when used RTX and in combination therapy, indicating decreased inflammation, but remain high with MTX.The treatment results in significant B cell depletion, with studies reporting profound reductions in CD20+ B cells by 12 weeks.This depletion can lead to transient leukopenia; however, WBC counts typically normalize post-treatment [26]. While specific data on platelet counts are less frequently reported, the overall safety profile of rituximab indicates that it does not significantly affect platelet levels. Most adverse events are mild and related to infusion reactions rather than hematological changes [27]. While specific data on ALP, AST, creatinine, and urea are limited, the absence of significant adverse effects on liver and kidney function is reassuring for clinicians considering rituximab for RA treatment. Conversely, some studies suggest that while rituximab is effective in reducing disease activity, the long-term effects on liver and kidney function parameters remain under-researched, necessitating further investigation to fully understand its safety profile in these areas [27,28].

CONCLUSION

Fundamental Finding : The study finding demonstrated that MTX and RTX treatments in RA patients appear to influence hematological and renal markers but they do not seem to significantly affect liver enzymes or WBC counts based on this study. Additionally, biological treatment reduced inflammation of arthritis better than chemical treatment. **Implication :** Suggest using Folic acid supplementation for reducing methotrexate-induced liver toxicity and has a modest effect on gastrointestinal side effects. **Limitation** : The findings are based on this study, indicating that broader conclusions may require caution. The specific effects on liver enzymes and WBC counts

might vary in larger or more diverse patient populations. **Future Research** : Further research should investigate the long-term impact of MTX and RTX on various biological markers, particularly focusing on liver toxicity and gastrointestinal effects, and evaluate the efficacy of folic acid supplementation in diverse clinical settings.

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