

**Effects of Combination therapy of Methotrexate
with Leflunomide & Hydroxychloroquine:
Comparative Clinical Trial**

ABSTRACT

Aims: To study the role of combination therapies in the treatment of rheumatoid arthritis.

Study design: This an open-label, randomized 180-days clinical trial.

Place and Duration of Study: This study was conducted in the Department of Pharmacology and Therapeutics, BMSI and Medical unit ward 6, after approval of JPMC ethical committee, between March 2013 and May 2014.

Methodology: We included 90 patients (69 women, 21 men; age range 28-62 years) which were divided into two groups, A and B. 44 patients of group A received methotrexate (MTX) 7.5-20 mg/ week orally and Leflunomide (LEF) 10-20 mg/ day QD orally as maximally tolerated. 46 patients of group B were given MTX 7.5-20 mg/ week orally and Hydroxychloroquine (HCQ) 200 mg twice daily orally as maximally tolerated.

Results: Comparing the combination of group A with group B, group A showed highly significant improvement in mean patient's global assessment (1.4 ± 0.66) and mean pain (1.3 ± 1.11) as compared to group B (2.4 ± 1.14 , 2.2 ± 1.49). The drugs of group B showed significant improvement in mean physician's global assessment (1.7 ± 0.92) and mean morning stiffness (49.2 ± 10.59) as compared to group A (2.8 ± 0.97 , 54.4 ± 10.14). Combination treatment of group B showed significantly lower adverse effects (4.3%) as compared to group A (11.4%). Statistical analysis revealed that patients receiving both the combinations responded equally in terms of effects but group B showed significantly better in terms of adverse effects.

Conclusion: Both combinations of MTX & LEF and MTX & HCQ were well tolerated but the efficacy of MTX and HCQ was significantly superior in terms of adverse effects to the combination of MTX and LEF.

Keywords: Rheumatoid Arthritis, Methotrexate, Leflunomide, Hydroxychloroquine, Disease Modifying Anti-rheumatic Drugs.

1. INTRODUCTION

Rheumatoid arthritis is a chronic, systemic inflammatory disease that affects many tissues and organs, but mainly attacks synovial joints. The cause of rheumatoid arthritis is unknown; autoimmunity plays an important role in both its chronicity and progression. Rheumatoid arthritis is considered as a systemic autoimmune disease [1]. It affects 0.5-1% of population all over the world [2]. Studies from Nigeria, Indonesia and Africa showed lower prevalence than that reported from the western countries. The prevalence of rheumatoid arthritis in India is 0.75%. In the urban population of southern Pakistan, Karachi, its prevalence is 0.14%, whereas in northern Pakistan the estimated prevalence is 0.55% [3]. Women are three times more commonly affected than men. Onset is most frequent between ages of 40- 50 years, but people of any age can be affected [4]. If rheumatoid arthritis remain untreated, patients will become permanently disable [5]. Therefore, various treatments for rheumatoid arthritis are available. Analgesics and anti-inflammatory drugs, including steroids, are used to suppress the symptoms, while disease-modifying antirheumatic drugs (DMARDs) are required to inhibit the underlying immune process and prevent long-term damage [6]. One of the new approaches has been the combinations of DMARDs. The increase in the use of combination therapies is due to the fact that monotherapy with DMARDs is often ineffective. Although, the use of combination therapies has increased, but it is not known that which combination therapy is most useful [7]. Methotrexate is on the World Health Organization List of Essential Medicine [8]. Multiple mechanisms are involved for the treatment of rheumatoid arthritis: the inhibition of enzymes involved in purine metabolism, leading to accumulation of adenosine; inhibition of T cell activation, suppression of intercellular adhesion molecule expression by T cells; increasing CD95 sensitivity of activated T cells; inhibition of methyltransferase activity, leading to (de)-activation of enzyme activity relevant to immune system function; selective down-regulation of B cells; inhibition of the binding of Interleukin 1 beta to its cell surface receptor [9].

Leflunomide is an immunosuppressive disease-modifying anti-rheumatic drug (DMARD) [10]. Its uses include active, moderate to severe rheumatoid arthritis and psoriatic arthritis. Mechanism of action of leflunomide is inhibition of pyrimidine synthesis [11]. Hydroxychloroquine is a weak base that can pass through the lipid cell membrane and specially concentrate in acidic cyto-plasmic vesicles which increases pH of these vesicles in macrophages or other antigen-presenting cells that limits the association of autoantigenic peptides with class II MHC molecules in the compartment for peptide loading and/or the subsequent processing and transport of the peptide-MHC complex to the cell membrane [12]. Hydroxychloroquine is used for the treatment of malaria, systemic lupus erythematosus, rheumatic disorders like rheumatoid arthritis and Sjögren's Syndrome, and porphyria cutanea tarda, post-Lyme arthritis., anti-spirochaete activity [13].

With this background, the purpose of this study was to compare the effects of combination therapies, methotrexate with leflunomide and hydroxychloroquine in patients of rheumatoid arthritis.

2. METHODOLOGY

2.1 Study Design

2.1.1 Grouping of Patients:

Patients of Rheumatoid arthritis of either sex, 30-60 years old, with 6-months history of active disease, and at least 3 of the following 4 features: erythrocyte sedimentation rate (ESR)>28 mm/hour, duration of morning stiffness 45 minutes, 8 tender joints, and 3 swollen joints, despite monotherapy with methotrexate since 6 months were included in the study. 110 patients were enrolled, divided into two groups, A and B, with 55 patients in each group. Randomization was done by allocation ratio 1:1 and it was blocked at every sixth patient i.e. first three patients were given methotrexate and leflunomide; next three patients were given methotrexate and hydroxychloroquine [14]. Out of these, 90 patients completed the study, 44 patients in group A and 46 patients in group B. Group A (n=44) was treated by methotrexate 7.5-20 mg/ week orally and leflunomide 10-20 mg QD orally as maximally tolerated. Group B (n=45) were treated by methotrexate 7.5-20 mg/week orally and hydroxychloroquine 200 mg twice daily orally as maximally tolerated.

2.2. Evaluation of Patients:

The enrolled patients were evaluated every 7th day until 30th day, then every 30th day. If there was no improvement in symptoms at the 60th day of evaluation, it was considered as an ineffective treatment. If they improved, they were evaluated every 30th day for the duration of next 90 days and then after 90 days. Efficacy was assessed by patient's global assessment, physician's global assessment, erythrocyte sedimentation rate, morning stiffness, Numeric pain scale scoring, number of tender joint count and number of swollen joint count.

2.2.1 Pain Assessment of Patients:

The pain of the patients was assessed by patient's global assessment. It was measured by visual analogue scale (VAS) from 0cm (no pain) to 10cm (severe pain) which was marked by the patient. VAS was horizontally placed on which patient was asked to mark from 0 cm to 10 cm [15]. (Figure-1).

Figure-1: Visual Analogue Scale

0cm	5cm	10cm
No pain		Worst possible pain

Pain assessed by physician's global assessment [16]. Physicians scored pain on a six-point scale of global assessment of arthritis. This scale consists of:

- 0= none- No pain.
- 1= Mild- slight, tolerable pain.
- 2= Moderate- pain causing discomfort.
- 3= Severe- unbearable pain.
- 4= Very severe pain.
- 5= Worst possible pain

Numeric Pain Scale determined pain according to following score: 0-none, 1-3-mild, 4-6-moderate and 7-10- severe [19]

2.2.2 ESR measurement of Patients:

ESR determines degree of non-specific inflammation in the body. It is governed by balance between prosedimentation factors, mainly, and factors resisting sedimentation, namely negative charge of erythrocytes (zeta potential). When an inflammatory process is present,

the high proportion of fibrinogen in the blood causes red blood cells to stick to each other. The red cells form stacks called 'rouleaux,' which settle faster, due to their increased density.

2.2.3 Morning Stiffness:

The patients of rheumatoid arthritis who had morning stiffness, of ≥ 45 minutes were included and evaluated [17]. In baseline, most of the patients gave history of morning stiffness which persisted for two hours. Sometimes it lasted throughout the day. It was observed noticeably in the joints of fingers and hand; wrist, elbow, knee, ankles, feet, shoulder, hip, and jaw were also affected in different enrolled patients.

2.2.4 Tenderness and Swelling:

Tenderness and swelling were assessed as present or absent. Shoulder, elbow, wrist, metacarpophalangeal, proximal and distal interphalangeal joints and knee were examined [18].

2.2.5 Monitoring of toxicity:

Before enrolment for the study, following investigations were done for all the patients: ECG, X-ray of chest and hands, liver function test, complete blood cell counts, ESR, urine D/R (Detailed Report) and at every follow-up visit. Patients were excluded from the study if their laboratory results were deranged. Concurrent therapy with systemic corticosteroids was continued if dosage remained stable throughout the study period and patient took no more than 10 mg of prednisone (or its equivalent) per day. We also permitted non-steroidal anti-inflammatory drugs.

2.3. Statistical Analysis:

The data analysis was done by SPSS version 16.0. The results were given as Mean and Standard deviation (SD) for quantitative variables (age, duration of diseases, pain score, ESR, laboratory investigations etc.) and percentage/proportion for categorical qualitative variables (gender, complaints, ECG and x-ray findings, efficacy and side effects etc.). Efficacy and side effects were compared among treatment groups by Chi-square test. An analysis of variance (ANOVA) was used to compare the average change (mean \pm SD) in outcome over treatment period among the two groups.

3. RESULTS

Group A was randomly dispensed MTX and LEF, and B was treated by MTX and HCQ for six-month duration. At baseline, the difference in the age of the patients, disease duration, rheumatoid factor positivity, percentage of females, and percentage of steroid usage in two treated groups were non-significant. The mean MTX dosage ranged from 16.0 to 17.0 mg/week. The mean LEF dosage ranged from 16.0 to 17.0 mg/day. The dosage of HCQ remain constant throughout the study. At the end of study period, that is 6 months, there was insignificant decrease in mean tender joint count, mean swollen joint count in group B as compared to group A. When mean patient's global assessment scale (for pain and quality of life) was compared, the decrease in the parameter was highly significant in group A than in group B and when mean physician's global assessment scale (for pain and quality of life) was compared, decrease in group B was highly significant. At the same time, there was non-significant decrease in mean erythrocyte sedimentation rate in both groups A and B. A highly significant decrease was seen in the mean morning stiffness in group B when compared to group A. A significant decrease in mean joint pain in group A was observed when compared to group B (Table-1, Figure-2)

Table 1. Comparison of Group A (Methotrexate & Leflunomide) and Group B (Methotrexate & Hydroxychloroquine)

	MTX & LEF Vs MTX & HCQ	p-value
Tender joint count (maximum 38)		
Baseline (day 0)	14.5±7.22	
6 months	13.9±5.49 5.8 ± 3.71 5.0 ± 4.42	>0.05 >0.05
Swollen joint count (maximum 38)		
Baseline (day 0)	11.3±4.59 9.2±3.31	>0.05
6 months	2.9 ± 1.71 2.7 ± 1.78	>0.05
Global assessment – Patient's (0-10 scale)		
Baseline (day 0)	5.2±0.76 5.9±0.97	>0.05
6 months	**1.4 ± 0.66 2.4 ± 1.14	**<0.01
Global assessment – Physician's (0-10 scale)		
Baseline (day 0)	4.6±1.23 4.8±0.92	>0.05
6 months	2.8 ± 0.97 **1.7 ± 0.92	**<0.01
ESR (mm/ hour)		
Baseline (day 0)	87.2±13.10 83.6±25.32	>0.05
6 months	56.5 ± 8.15 52.7 ± 16.74	>0.05
Morning stiffness (minutes)		
Baseline (day 0)	82.8±15.89 79.6±15.81	>0.05
6 months	54.4 ± 10.14 **49.2 ± 10.59	**<0.01
Pain (0-10 scale)		
Baseline (day 0)	5.4±1.26 6.1±1.18	>0.05
6 months	** 1.3 ± 1.11 2.2 ± 1.49	**<0.01

Significant p-value *<0.05, highly significant**<0.01
 MTX=methotrexate, LEF=leflunomide, HCQ=Hydroxychloroquine

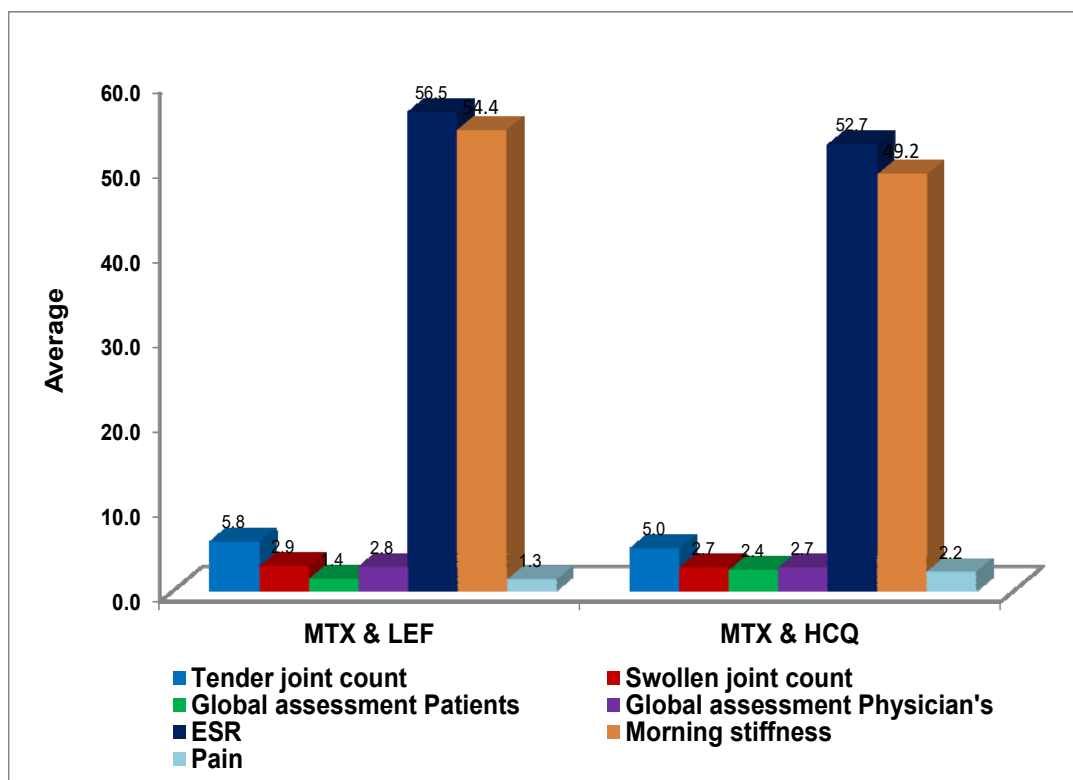


Fig. 02. Comparison of Group A (Methotrexate & Leflunomide) and Group B (Methotrexate & Hydroxychloroquine)

Table 02. The observed side effects of combination therapies in rheumatoid arthritis patients

	MTX & LEF	MTX & HCQ
No. of patients	44	46
Headache	2(4.5%)	1 (2.2%)
Rash	1 (2.3%)	-
Pneumonia	-	-
GIT distress	2 (4.5%)	
Weight loss	-	1 (2.2%)
Total	5	2
Percentage of side effects	11.4%	4.3%

4. DISCUSSION:

Due to the advancement in pathophysiology of rheumatoid arthritis, its management is continuously evolving. Traditional DMARDs will undoubtedly remain the chosen initial treatment. Recent guidelines promote early and continued use of DMARDs [20]. Various studies demonstrate the effectiveness of combination therapy over monotherapy in the treatment of rheumatoid arthritis [14]. Most of DMARD therapies have a weakness that their comparison with active therapy have not been done. Shashikumar et al (2010) in an open-label; randomized clinical trial of 60 patients with 12 weeks duration also observed that there was no statistical significance in improvement in disease activity in the group methotrexate+hydroxychloroquine as compared with methotrexate+leflunomide. This result was comparable with our result [21]. Our study showed a highly significant lower level of adverse effects in combination therapy of methotrexate and hydroxychloroquine, Mikuls & O' Dell (2000) surveys (1995, 1997 and 1999) showed the same results that the combination of methotrexate and hydroxychloroquine is safe over the combination of methotrexate and leflunomide [22]. The associated hepatotoxicity (Combe, 2006) of MTX/LEF combination was not documented in our trial might be because of the limitation of short duration of the trial [23]. In addition, neutropenia (Scott et al, 2010) that is related to LEF and MTX was not reported [24]. Similarly, HCQ-related ophthalmoplagia was also not recognized in the present trial, perhaps, HCQ being otherwise less toxic decreases the adverse effects of MTX and also decreases the dosage of MTX (Table 02).

5. CONCLUSION

The patients of rheumatoid arthritis responded equally well in both the combinations but significantly better to the combination of methotrexate and hydroxychloroquine in terms of safety.

CONSENT (WHERE EVER APPLICABLE)

All authors declare that 'written informed consent were obtained from the patients (or other approved parties) for publication of this study and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.'

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

This randomized, open-label, clinical trial was conducted in the Department of Pharmacology and Therapeutics, BMSI and Medical unit ward 6, with the approval of JPMC ethical committee (F.2-81/2013 GENL/12001/JPMC) for six months. Written informed consent was taken from enrolled patients. All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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UNDER PEER REVIEW

APPENDIX II

CONSENT FORM (URDU)

اجازت نامہ

میں نے یہ اجازت نامہ پڑھ لیا ہے۔ میں نے اسیماں لکھن طریقے سے
ن اجازت نامہ پر اپنے معالج سے بحث کی ہے۔ میں اس میں رضا کارانہ طور پر حصہ لے رہا / رہی ہوں۔ میں اس کے
مقصود، طریقہ کار، ممکن نقصان اور فوائد کے بارے میں کافی جاننا / جانتی ہوں۔ میں یہ بھی جاننا / جانتی ہوں کہ میں معالج
اور / یا دوائی استعمال کر سکتا / سکتی ہوں کہ اگر مجھے کسی قسم کے کوئی نئے اثرات یا سبب سے ذہن میں مزید کوئی سہولت ہیں۔
میں اس کے بھی بیماری کے بارے میں نہیں جاننا / جانتی جو مجھے اس میں حصہ لینے سے روکے۔ میں اس میں حصہ لینے
لیے متفق ہوں۔

_____ : معالج کے دستخط

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APPENDIX III

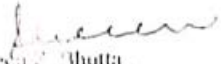
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KARACHI.75510.

Dated the 7/5 2013

✓ Dr. Qurrat-ul-ain Aukhari,
M.Phil Student
Pharmacology & Therapeutic, BMSI
Jinnah Postgraduate Medical Centre
Karachi.

Subject: Approval of Research for Ethical Committee

Please refer to your letter dated 18th April, 2013 and to inform you that the committee has examined the case and given approval to conduct proposed research under supervision of Dr. Moosa Khan, Assistant Prof. of Pharmacology & Therapeutics, BMSI, JPMC, Karachi.


Prof. Shereen Z. Bhutta
Prof. Department of Gynae and Obst & Chairperson
Institutional Review Board Committee.

Copy forwarded for information and necessary action to the Dr. Moosa Khan, Assistant Prof. of Pharmacology & Therapeutics, BMSI, JPMC, Karachi.

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