



## **Effects of Combination Therapy of Methotrexate with Sulfasalazine and Hydroxychloroquine: Comparative Clinical Trial**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author QUAB designed the study, performed the statistical analysis and wrote the protocol. Author KMM reviewed the final draft. Author MHD edited the final draft. Author AA managed the analyses of the study and wrote the first draft of the manuscript. Author HF managed the literature searches. Author FH supervised and verified the analysis. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Aims:** To study the role of two 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.

**Methodology:** Eighty-nine patients were enrolled (69 women, 20 men; age range 28-62 years). A and B were the groups assigned to the patients. MTX 7.5-20 mg/ week orally and SSZ 10-20 mg / day orally as maximally tolerated were prescribed to the 55 patients of group A. MTX 7.5-20 mg/ week orally and HCQ 200 mg twice daily were prescribed to the 54 patients of group B..

**Results:** When we compared group A with group B, group A showed major progress in mean

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swollen joint count ( $1.9 \pm 0.97$ ) as compared to group B ( $2.7 \pm 1.78$ ). Group B showed major progress in mean physician's global assessment ( $2.7 \pm 0.92$ ) as compared to group A ( $3.8 \pm 1.22$ ). For that reason, our study showed that patients receiving both the combinations responded equally in terms of efficacy but the combination of MTX and HCQ is better tolerated than the combination of MTX and SSZ.

**Conclusion:** Both combinations of MTX & SSZ and MTX & HCQ were equally effective but the combination of MTX & HCQ was superior in terms of tolerability than the combination of MTX and SSZ.

**Keywords:** Rheumatoid arthritis; methotrexate; sulfasalazine; hydroxychloroquine; disease modifying anti-rheumatic drugs; clinical trials.

## 1. INTRODUCTION

Rheumatoid arthritis is a chronic, systemic inflammatory disease which effects many tissues and organs, but mainly effects synovial joints. Autoimmunity plays an important role in both its chronicity and progression though its cause is not known. It is considered as a systemic autoimmune disease [1]. It affects 0.5-1% of population all over the world [2]. The prevalence of the disease in the western countries is more than Nigeria, Indonesia and Africa. Its prevalence in India is 0.75%. In northern Pakistan its prevalence is 0.55% but in the urban area of southern Pakistan i.e. Karachi, its prevalence is 0.14% [3]. Women are three times more commonly affected than men. The onset of the disease is most common between the ages of 40-50 years but People of any age can be affected [4]. If it remain untreated, it causes permanently disability [5]. Therefore, several treatments for rheumatoid arthritis are available. Analgesics and anti-inflammatory drugs are used to reduce the symptoms, including steroids, but, disease-modifying antirheumatic drugs (DMARDs) are required to inhibit the underlying immune process and delay the long-term damage [6]. One of the new methods has been the combinations of DMARDs. Monotherapy with DMARDs is often ineffective that is why there is the increase in the use of combination therapies. Even though, the use of combination therapies has increased, but it is not known which combination therapy is the most useful [7]. To solve this problem, we compared two combinations of DMARDs; methotrexate with sulfasalazine, and methotrexate with hydroxychloroquine. Methotrexate is on the World Health Organization List of Essential Medicine [8]. There are several mechanisms through which Methotrexate acts in the treatment of rheumatoid arthritis like, accumulation of adenosine through the inhibition of enzymes involved in purine metabolism;

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]. Sulfasalazine is a sulfa drug and a derivative of mesalazine. It is formed by combining sulfapyridine and salicylate with an azo bond [10]. Sulfasalazine is used in the treatment of inflammatory bowel disease, including ulcerative colitis and Crohn's disease, rheumatoid arthritis, psoriatic arthritis and reactive arthritis [11]. Its mechanism of action is not clearly understood. One possible mechanism is that since sulfasalazine and 5-ASA is poorly absorbed from gut so may be it has the extra-intestinal effects especially on joints by modulating local chemical mediators of the inflammatory response, especially leukotrienes, and is a free radical scavenger or an inhibitor of tumor necrosis factor (TNF). Other proposed mechanism is that, sulfapyridine, which is another metabolite of sulfasalazine is responsible for its arthritic effects that is also responsible for its side effects [10]. Hydroxychloroquine is a weak base that can pass through the lipid cell membrane and particularly accumulate in acidic cyto-plasmic vesicles which increases pH of these vesicles in macrophages or other antigen-presenting cells that restricts the binding 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. 109 patients were enrolled, divided into two groups, A and B, with 55 patients in group A and 54 patients in group B. 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 sulfasalazine; next three patients were given methotrexate and hydroxychloroquine [14]. Out of these, 91 patients completed the study, 45 patients in group A and 46 patients in group B. Group A (n=45) was treated by methotrexate 7.5-20 mg/ week orally and sulfasalazine 500 mg-1gm/day orally as maximally tolerated. Group B (n=46) were treated by methotrexate 7.5-20 mg/week orally and hydroxychloroquine 200 mg twice daily orally.

#### 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 number of tender joint count and number of swollen joint count, patient's global assessment, erythrocyte sedimentation rate, Numeric pain scale scoring, morning stiffness, physician's global assessment.

##### 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]. (Chart 1).

**Chart 1. Visual analogue scale**

0 cm	5 cm	10 cm
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 [17].

##### 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 [18]. 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 [19].

**2.2.5 Monitoring of toxicity**

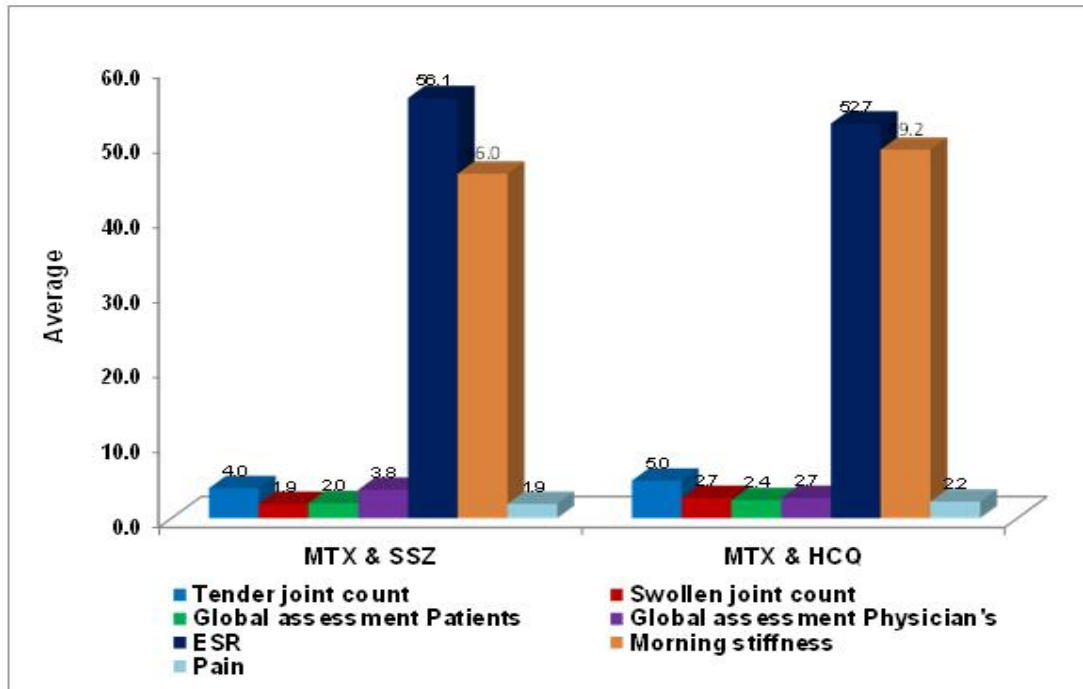
Before enrolment for the study, following investigations were done for all the patients: ECG, X-ray of chest and hands, liver function test, complete blood cell counts, ESR, urine D/R

(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.

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

	<b>MTX &amp; SSZ Vs MTX &amp; HCQ</b>	<b>p-value</b>
<b>Tender joint count</b> (maximum 38)		
<b>Baseline (day 0)</b>	13.7±7.08	
	13.9±5.49	>0.05
<b>6 months</b>	4.0 ± 3.63	
	5.0 ± 4.42	>0.05
<b>Swollen joint count</b> (maximum 38)		
<b>Baseline (day 0)</b>	8.6±4.37	
	9.2±3.31	>0.05
<b>6 months</b>	** 1.9 ± 0.97	
	2.7 ± 1.78	**<0.01
<b>Global assessment – Patient’s (0-10 scale)</b>		
<b>Baseline (day 0)</b>	5.6±1.64	
	5.9±0.97	>0.05
<b>6 months</b>	2.0 ± 0.99	
	2.4 ± 1.14	>0.05
<b>Global assessment – Physician’s (0-10 scale)</b>		
<b>Baseline (day 0)</b>	5.6±1.49	>0.05
<b>6 months</b>	4.8±0.92	
	3.8 ± 1.22	**<0.01
	** 2.7 ± 0.92	
<b>ESR (mm/ hour)</b>		
<b>Baseline (day 0)</b>	86.2±18.87	>0.05
	83.6±25.32	
<b>6 months</b>	56.1 ± 10.41	>0.05
	52.7 ± 16.74	
<b>Morning stiffness</b> (minutes)		
<b>Baseline (day 0)</b>	71.6±19.06	
	79.6±15.81	>0.05
<b>6 months</b>	46.0 ± 19.06	
	49.2 ± 10.59	>0.05
<b>Pain (0-10 scale)</b>		
<b>Baseline (day 0)</b>	6.0±1.65	
	6.1±1.18	>0.05
<b>6 months</b>	1.9 ± 1.45	
	2.2 ± 1.49	>0.05

Significant p-value \*<0.05, highly significant\*\*<0.01  
 MTX=methotrexate, SSZ=Sulfasalazine, HCQ=Hydroxychloroquine



**Fig. 1.** Comparison of Group A (Methotrexate & Sulfasalazine) and Group B (Methotrexate & Hydroxychloroquine)

**Table 2.** The observed side effects of combination therapies in rheumatoid arthritis patients

	MTX & SSZ	MTX & HCQ
No. of patients	45	46
Headache	1(2.3%)	1 (2.2%)
Rash	2 (4.4%)	-
Pneumonia	-	-
GIT distress	2 (4.4%)	-
Weight loss	-	1 (2.2%)
Total	5	2
Percentage of side effects	11.1%	4.3%

### 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 ± SD) in outcome over treatment period among the two groups.

### 3. RESULTS

Group A was randomly dispensed MTX and SSZ, 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 SSZ dosage ranged from 0.7gm to 0.8 gm/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 in group B as compared to group A. When mean swollen joint count 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 patient's global assessment scale (for pain and quality of life), mean erythrocyte sedimentation rate, mean morning stiffness and mean joint pain in both groups A and B. (Table 1, Fig. 1).

#### 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. Verschueren P et al (2015) 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+sulfasalazine. 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, Fedorenko E et al showed the same results that the combination of methotrexate and hydroxychloroquine is safe over the combination of methotrexate and sulfasalazine [22]. The associated hepatotoxicity (Combe, 2006) of MTX/SSZ combination was not documented in our trial might be because of the limitation of short duration of the trial [23]. In addition, leukopenia (Scott et al, 2010) that is related to SSZ and MTX was not reported [24]. Similarly, HCQ-related ophthalmoplagia was also not recognized in the present trial (Bukhari et al 2020), perhaps, HCQ being otherwise less toxic decreases the adverse effects of MTX and also decreases the dosage of MTX (Table 2) [25].

#### 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. Both combinations of MTX & SSZ and

MTX & HCQ were equally effective but the combination of MTX & HCQ was superior in terms of tolerability than the combination of MTX and SSZ.

#### CONSENT

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

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. 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.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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