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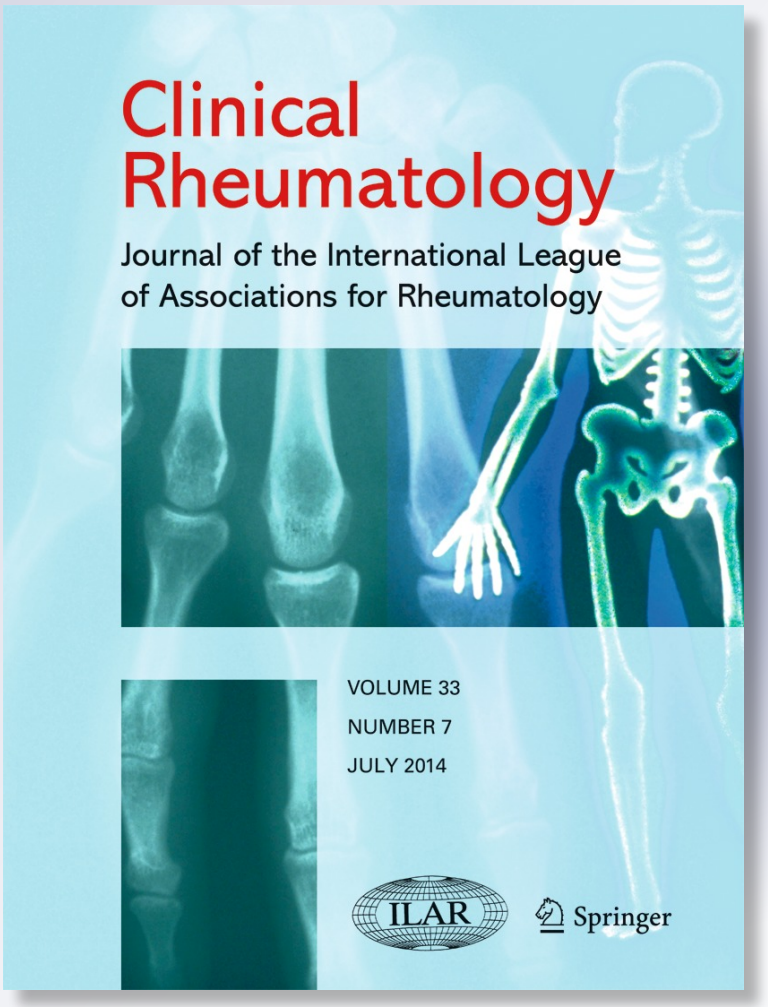

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ORIGINAL ARTICLE

Diagnostic validity of colchicine in patients with Familial Mediterranean fever

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Abstract Although response to colchicine has been proposed as one of the diagnostic criteria in patients with Familial Mediterranean fever (FMF), the validity of this response has not been validated. The aim of this study was to assess the efficacy of the response to colchicine and to evaluate the extent of the effect of placebo. A double-blind randomized placebo-controlled trial with a cross-over design was conducted. The frequency of FMF attacks, the disease score, physical examination, and acute phase reactants were assessed at 0, 3, and 6 months. Blood samples were collected for complete blood count (CBC), erythrocyte sedimentation rate (ESR), levels of serum C-reactive protein (CRP) and serum amyloid A (SAA), and *MEFV* mutation analysis in 79 patients with a preliminary diagnosis of FMF. Patients were randomly allocated to receive either drug A or drug B in a double-blind fashion. The designated drug was switched at 3 months. Patients taking colchicine had less frequent FMF attacks (median 0) and lower FMF disease score (median 0) when compared to those on placebo (median 1 and 3, respectively) (p=0.002 and p=0.007, respectively). In genetically confirmed FMF patients, median attack number and median disease score was 0 under colchicine treatment, whereas these parameters were significantly higher in the placebo group (median 2 and 8, respectively) (p=0.007 and p=0.02, respectively) suggesting that colchicine is more effective than placebo in reducing attacks and disease score. Positive and negative predictive values were 70.2 and 37.5 %, respectively. During the placebo period, patients had less FMF attacks when compared to that of the pre-study period (median 2 vs 6, respectively) (p<0.001). The high false positive rate raises concerns for considering the colchicine response test as diagnostic for FMF. The role of placebo on the attacks of periodic fever syndromes needs to be further investigated.

Keywords Colchicine · Diagnosis · Familial Mediterranean fever · Placebo

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Introduction

Familial Mediterranean fever (FMF) is the most common hereditary autoinflammatory disease. It is associated with mutations in the Mediterranean fever (*MEFV*) gene-encoding pyrin [1, 2]. Pyrin is a component of a multimolecular complex, the inflammasome, which regulates inflammation in the human body. The disease is most prevalent among non-Ashkenazi Jews, Arabs, Turks, and Armenians with a high carrier frequency in these populations [3]. The disease is characterized by recurrent bouts of fever, sterile peritonitis, arthritis, and pleuritis accompanied by elevated acute phase reactants including erythrocyte sedimentation rate, C-reactive protein, and serum amyloid A [4, 5]. Colchicine is recommended for the treatment of FMF: colchicine prevents attacks and renal amyloidosis and suppresses inflammation in the majority of the patients (*grade I evidence*) [4].

The diagnosis of FMF is a clinical one. Mutation analysis in *MEFV* gene confirms the diagnosis. Genetic analyses are expensive and may not exclude the disease completely

especially in regions, like Turkey, where very high rates of FMF carrier (1/5 individuals) are present [3, 6]. Therefore, *MEFV* analysis is not a prerequisite for the diagnosis of FMF. In areas where the disease prevalence is high, colchicine treatment can be used for a short period to confirm the diagnosis in a patient with periodic fever. Actually, response to colchicine is one of the diagnostic criteria in adult patients [7]. Furthermore, experts in the field of autoinflammatory disorders tend to use colchicine empirically in patients with periodic fever who fail to fit in a certain category of disease. However, to date, no prospective controlled study has been conducted to formally test the efficacy of colchicine.

The aim of the present study was to assess the validity of using response to colchicine as a diagnostic criterion. Another aim of the study was to understand the extent of the effect of placebo in the attacks of patients with periodic fevers.

Patients and methods

Study design

A double-blind randomized placebo-controlled trial with a cross-over design was conducted by two tertiary Pediatric Rheumatology referral centers in central Anatolia between the years 2008 and 2012 (Fig. 1). Patients met the new pediatric diagnostic criteria were included in the study [8]. Patients who had fever associated with other conditions were excluded (i.e., urinary tract infections, malignancy, etc.). Blood samples were collected for complete blood count (CBC), erythrocyte sedimentation rate (ESR), levels of serum C-reactive protein (CRP) and serum amyloid A (SAA), and *MEFV* mutation analysis (strip assay for 12 common mutations). Then the patients were randomly allocated to receive either drug A or drug B in a double-blind fashion. Each patient was evaluated at 3 months according to the number of FMF attacks, physical examination, and acute phase reactants as well as an arbitrary scoring system that has been developed using six parameters for this study to evaluate the severity of FMF attacks. These were fever and arthritis (0: absent, 1: present), abdominal pain, chest pain and arthralgia (0, absent; 1, mild; 2, moderate; 3, severe or restricting to bed), and duration of attack (1, 1–24 h; 2, 25–48 h; 3, 49–72 h; and 4, more than 72 h).

At the end of the first 3 months, drug A was switched to drug B and vice versa. At the end of second 3-month period, the same clinical and serological parameters were re-evaluated. Side effects of both drugs were also noted in each 3-month period. The score of each FMF attack was summed and disease scores were determined for the drug A and drug B periods. Clinical and laboratory parameters including number of FMF attacks and disease severity scores were compared between these periods. At the end of the study, number/severity of FMF attacks and laboratory results including *MEFV* analysis were re-evaluated for each patient.

After completion of the whole study, drug labels were unmasked. Drugs A and B were colchicine and placebo, respectively.

Subjects were ascertained and enrolled in the study after obtaining informed consent, in accordance with human subject research protocols approved by the Hacettepe University in Ankara (HEK 07/118-35).

Statistical analyses

The carry-over period and group effects were analyzed using nonparametric methods for the analysis of cross-over studies [9]. Mann-Whitney *U* and Wilcoxon tests were used for statistical analyses, where appropriate, and median and interquartile ranges (IQR) were presented as the descriptive statistics. A type 1 error level cut-off of 5 % was used to infer statistical significance.

Results

Patient characteristics

A total of 79 patients (39 girls, 40 boys) were enrolled in the study between the years 2008 and 2012. Fourteen patients were excluded from the study due to the following reasons: noncompliance to the treatment and visits ($n=12$), withdrawal of consent ($n=2$). Sixty five patients completed the study and were included in the final statistical analysis. Median age of the patients was 9.0 years (IQR 5–12.5 years). Demographic features of the study cohort are given in Table 1. At the beginning of the study, most common symptoms were recurrent abdominal pain (90.8 %) and recurrent fever (86.2 %). Attacks characterized by recurrent abdominal pain (50 out of 59 patients; 84.7 %), recurrent fever (45 out of 56 patients; 80.3 %), recurrent arthralgia/arthritis (24 out of 34 patients; 70.5 %), and recurrent chest pain (nine out of 14 patients (64.2 %) occurred every 2–4 weeks. The remaining patients suffered from attacks with intervals longer than 4 weeks.

MEFV mutation analysis

None of common mutations was detected in 30.8 % of patients. Twenty patients (30.8 %) were homozygous with leading M694V mutation. Fifteen patients (23 %) were compound heterozygous and 10 patients (15.4 %) were heterozygous. Overall, the phenotype of 35 patients (53.8 %) could be definitely confirmed by mutation analysis (homozygosity or compound heterozygosity). Results of *MEFV* mutation analyses are given in Table 2.

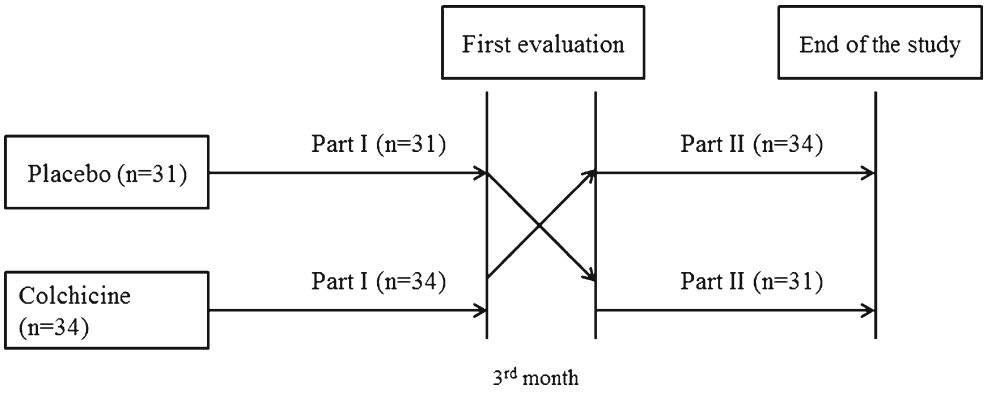

Fig. 1 Allocation of the patients in the study

Colchicine versus placebo

At the beginning of the study, 31 patients were allocated to receive placebo and 34 patients to colchicine. After 3 months, the drugs were switched (Fig. 1). Patients taking colchicine had less frequent FMF attacks (median 0) and FMF disease score (median 0) when compared to placebo (median 1 and 3, respectively) (p=0.002, p=0.007; respectively). ESR was significantly lower in patients on colchicine arm compared to those on the placebo arm (median 9 vs 16 mm/h, p=0.032). However, serum CRP and SAA levels were not different between the two arms (Table 3).

The effect of colchicine was also investigated in patients with a confirmed diagnosis of FMF (i.e., patients with homozygous and compound heterozygous mutations). Again, patients on colchicine had less frequent FMF attacks (median 0) and score (median 0) when compared to patients on placebo (median 2 and 8, respectively) (p=0.007, p=0.02; respectively). Serum CRP and SAA levels between the two groups were not statistically different; however, ESR was lower in patients receiving colchicine (12 vs 21 mm/h, p=0.049) (Table 3).

The placebo effect was assessed in patients with homozygous or compound heterozygous mutations. During the placebo period, the frequency of the FMF attacks (median 2) was significantly less when compared to that of the pre-study period (median 6) (p<0.001). Pre-study period was also compared with patients who received placebo in the first 3 months of the study. Patients who initially received placebo, again, had less frequent FMF attacks when compared to that of the pre-study period (p=0.007). Laboratory parameters of the pre-study period were compared those with placebo period. There was no statistical difference in SAA levels between these two periods. However, the ESR and serum CRP levels of the patients were less when compared to those of the pre-study period (Table 4).

Table 1 Demographic, clinical, and laboratory characteristics of the participants at the beginning of the study

Group	
Total number of patients	65
Gender (female/male)	32/33
Median age at the time of the study (IQR)	9.0 years (5–12.5)
Median age of onset (IQR)	5.0 years (3–9.5)
Median number of attacks noted last 3 months prior to the enrollment (IQR)	3.0 (3–6)
Clinical features	%
Recurrent abdominal pain	90.8
Recurrent fever	86.2
Recurrent arthralgia	36.9
Recurrent chest pain	21.5
Recurrent arthritis	15.4
Laboratory features[a]	Median (IQR)
ESR	25 mm/h (14.5–44.5)
CRP	3.9 mg/dL (0.65–18.48)
WBC	9,500/mm^3 (7,500–11,800)
SAA	77 mg/L (16–120)

CRP C reactive protein, *ESR* erythrocyte sedimentation rate, *SAA* serum amyloid A, *WBC* white blood cell count, [a] Normal laboratory values ESR 0–20 mm/h, CRP 0–0.8 mg/dL, and SAA 0–10 mg/L.

Table 2 *MEFV* mutation analyses of the study population (n=65)

MEFV mutation	Number of patients (%)
Negative	20 (30.8)
Heterozygous	10 (15.4)
M694V	5
E148Q	3
M680I	2
Compound heterozygous	15 (23)
M694V/V726A	6
M680I/M694V	5
M694V/A744S	1
E148Q/V726A	1
M680I/V726A	1
V726A/A744S	1
Homozygous	20 (30.8)
M694V/M694V	16
M680I/M680I	3
V726A/V726A	1

Table 3 Comparison of clinical and laboratory parameters of patients receiving colchicine and placebo

	Placebo Median (IQR)	Colchicine Median (IQR)	*p*
All patients			
Disease score	3 (0–10)	0 (0–1.5)	*0.007*
Number of attacks	1 (0–3)	0 (0–1)	*0.002*
CRP (mg/dL)	0.5 (0.5–2)	0.5 (0.5–1)	0.251
SAA (mg/L)	55 (9.1–129.25)	16.5 (6–100.5)	0.700
ESR (mm/h)	16 (7–25)	9 (5–16.5)	*0.032*
Patients with homozygous and compound heterozygous *MEFV* mutation			
Disease score	8 (0–12)	0 (0–0.75)	*0.020*
Number of attacks	2 (0–3)	0 (0–1)	*0.007*
CRP (mg/dL)	0.9 (0.5–8.75)	0.5 (0.5–3.4)	0.679
SAA (mg/L)	93 (11.9–136.5)	75.5 (16.5–152.25)	0.463
ESR (mm/h)	21 (14–33.5)	12 (7–25)	*0.049*
Patients without *MEFV* mutation			
Disease score	0 (0–7.75)	0 (0–1.5)	0.28
Number of attacks	0 (0–2)	0 (0–0.75)	0.29
CRP (mg/dL)	0.5 (0–0.5)	0.5 (0.5–0.93)	0.43
SAA (mg/L)	36.5 (6.85–115.75)	10 (3–73)	0.65
ESR (mm/h)	10 (4.5–19)	6 (2.25–12.75)	0.14

CRP C reactive protein, *ESR* erythrocyte sedimentation rate, *SAA* serum amyloid A

Effects of colchicine and placebo were also analyzed in patients without mutation. Number of attacks, disease score, ESR, and serum CRP and SAA levels were comparable between two groups (Table 3).

Sensitivity and specificity of a colchicine trial in patients with a preliminary diagnosis of FMF were calculated as 88.9 and 15 %, respectively. Positive and negative predictive values were 70.2 and 37.5 %, respectively with an accuracy of 66.2 % (Table 5).

No side effect related to both colchicine and placebo occurred during the study period.

Table 4 Clinical and laboratory parameters of patients with homozygous and compound heterozygous *MEFV* mutation during pre-study and placebo periods

Parameter	Pre-study Median (IQR)	Placebo Median (IQR)	*p*
Number of attacks	6 (3–6)	2 (0–3)	*<0.001*
CRP (mg/dL)	12.8 (1.97–37.25)	0.9 (0.5–8.75)	*0.013*
SAA (mg/L)	92.5 (28.25–120.75)	93 (11.9–136.5)	0.498
ESR (mm/h)	29.0 (20–52)	21 (14–33.5)	*0.045*

CRP C reactive protein, *ESR* erythrocyte sedimentation rate, *SAA* serum amyloid A

Table 5 Sensitivity, specificity, and positive and negative predictive values of a colchicine trial in patients with a preliminary diagnosis of FMF

Colchicine response	Genetic positive[a]	Genetic negative	Total
Yes	40	17	57
No	5	3	8
Total	45	20	65

Sensitivity 88.9 %, specificity 15.0 %, positive predictive value 70.2 %, negative predictive value 37.5 %, accuracy 66.2 %

[a] Patients with homozygous, compund heterozygous, and heterozygous *MEFV* mutations

Discussion

There is a large differential for a patient presenting with periodic fever [10]. FMF is the most common periodic fever syndrome in eastern Mediterranean countries such as Turkey [6]. Although a genetic confirmation is of the sought for, the diagnosis of FMF is mainly a clinical one, especially in certain populations where high carrier frequencies exist [11, 12]. Response to colchicine has been used as a diagnostic criterion in adults and always been regarded as a differentiating feature for FMF [7]. Response to colchicine is also an important element in the evaluation of patients who have the clinical phenotype, but only one demonstrated mutation. However, to date, no prospective controlled study has been conducted. With the present study, we showed that colchicine had a significant effect on patients with FMF and can really serve a diagnostic criterion. To reach firm conclusions, we have included only the genetically confirmed patients (i.e., those with homozygous or compound heterozygous mutations). We observed a significant reduction in clinical activity and the laboratory results among the patients receiving colchicine when compared to those receiving placebo. However, we did not observe any significant differences in these parameters of patients without *MEFV* mutation. It should be noted that small number of patients (n=20) might have hampered to reach statistical significance.

The common periodic fever syndromes have characteristic features that help in the differential diagnosis. On the other hand, we care for patients in whom we fail to give a definite diagnosis. It is common practice to put patients on colchicine if they have periodic fevers even if they lack any mutation. We already have high level of evidence that colchicine is effective in treating the disease [13]. However, its validity in diagnosis of FMF was unknown. This study provides original data in many aspects. Our results suggest that colchicine can be used a diagnostic criterion in places where high carrier rate are present. Mutation analysis is expensive and may not exclude the diagnosis completely if direct sequencing of all exons of the *MEFV* gene is not performed. In this case, colchicine trial may be an alternative of mutation analysis.

In the present study, we also observed that placebo was also significantly effective in decreasing the attacks. Actually, the term "placebo" has been known in medicine for a long time and has been used by many physicians either to treat certain diseases or to alleviate symptoms [14]. The effectiveness of placebo has been searched in almost all disciplines of medical sciences [14, 15]. A recent meta-analysis showed that there was no evidence regarding clinically important effect of placebo but that there might be a little effect on subjective symptoms such as pain [16, 17]. Lachmann et al. have conducted a study comparing efficacy of canakinumab, an anti IL1 agent, in patients with cryopyrin-associated periodic syndrome, another periodic fever syndrome with placebo [18]. All patients on canakinumab and three patients on placebo remained in remission in the second phase of the study. In addition, all patients on canakinumab and one-fourth of patients on placebo were noted to have no or minimal disease activity. In our study, patients had less FMF attacks with placebo when compared to the pre-study period suggesting the psychological effect. It is tempting to speculate that the endogenous triggers of inflammation may be somewhat halted by the placebo effect. Patients on placebo had lower ESR and CRP levels but not SAA levels when compared to the pre-study period. This may support that SAA is indeed the most sensitive marker of chronic inflammation [5, 19]. ESR and CRP may be more dependent on acute attacks, and this may explain their decrease. On the other hand, placebo has not had an effect on the sustained inflammation reflected by the SAA. Thus, this study also confirms the importance of SAA in the evaluation of these patients.

Another important finding of the study was the relative lack of efficacy of colchicine in patients who lack two mutations. It is highly unlikely for patient without any mutation in *MEFV* to have FMF. Thus, these patients probably had another periodic fever disease or else. When these patients were evaluated separately, they did not respond to colchicine.

In this study, we have also shown that in a tertiary center nearly 2/3 of the patients in whom a *MEFV* mutation is ordered to have at least one mutation in the gene. This is a high yield albeit from an eastern Mediterranean center. This confirms that the present clinical criteria used have a high sensitivity for a diagnostic yield in FMF [8].

A drawback of our study was the short period allocated for the treatment arms. We found a rather high positive predictive value (70.2 %) but a rather low negative predictive value, suggesting that colchicine trial may be beneficial in the initial consideration of FMF. On the other hand, the very high false positive rate raises concerns for the use of a colchicine trial for the diagnosis of FMF. However, one must take into account that 3 months is a short follow-up period to draw a certain conclusion.

The short period and the carry-over effect may also explain the lack of significant difference between the laboratory levels during colchicine versus placebo. However, we were not able to lengthen these periods due to ethical considerations.

In summary, besides *MEFV* mutation analysis, colchicine trial seems to be effective in diagnosis of FMF patients especially in populations with high carrier rate. The role of placebo on treatment of periodic fever syndromes needs to be further investigated.

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