*HK J Paediatr (new series)* 2011;**16**:3-8

# *Original Articles*

## Serum Vitamin B12 Status in Children with Familial Mediterranean Fever Receiving Colchicine Treatment

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**Abstract** ***Aims:*** The aim of this study was to determine the effect of colchicine on serum vitamin B12 status in children with Familial Mediterranean Fever (FMF). ***Settings and Design:*** The Tel Hashomer criteria had been used to establish the diagnosis of FMF. Colchicine treatment was initiated by age based dosage schedule to each patient. ***Methods and Material:*** This study was cross sectional. Serum vitamin B12 levels were measured before and during colchicine treatment in 42 children with FMF. ***Results:*** Twenty-two girls and 20 boys, with a mean age of 11.7±4.5 years, were included in the study. The mean duration of colchicine treatment was 2.5±2.6 years. The daily colchicine doses ranged between 0.0077 to 0.0741 mg/kg. There was a significant difference between first and control visit mean serum vitamin B12 levels, 418 pg/ml and 240 pg/ml respectively ($p<0.0001$). Control visit serum vitamin B12 levels had no statistically important correlation to the duration of colchicine treatment ($r=-0.287$, $p=0.065$) but there was a significant correlation to the daily colchicine doses. ($r=+0.349$, $p=0.025$) ***Conclusions:*** The present study showed that long term administration of colchicine led to a decrease (subnormal) in the serum vitamin B12 levels. Therefore, serum vitamin B12 levels should be evaluated regularly in FMF patients receiving colchicine treatment.

**Key words** Colchicine; Familial Mediterranean fever; Vitamin B12

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*Received August 6, 2010*

### Introduction

Familial Mediterranean Fever (FMF) is an autosomal recessive autoinflammatory disorder characterised by recurrent episodes of fever, serosal inflammation and rash. Although its attacks are self-limited, some patients develop AA type amyloidosis, which leads to renal failure. FMF is common in Turks, Sephardic Jews, Arabs, Armenians, and other groups that comprise the populations of the Mediterranean and Middle East basins.[1,2]

The diagnosis of FMF is based on a characteristic clinical course, family history, and the physician's experience.[3,4] Despite genetic testing, there is no specific laboratory test.[5-8] Colchicine was used for treatment of FMF since 1972 and it has been clinically proven to reduce the frequency of attacks and prevent the development of amyloidosis in

FMF.[4,9,10] Nonetheless, colchicine has several side effects including gastrointestinal disturbances (nausea, vomiting, and diarrhea), transient alopecia, azospermia, reversible bone marrow suppression, myopathy, neuropathy and chromosomal abnormalities.[11-13]

The dosage Colchicine for prevention of attack and amyloidosis changes by age. It can be administered orally 0.5 mg per day in children younger than 5 years of age, 1 mg per day for children between 5 and 10 years of age and 1.5 mg per day for children older than 10 years of age.[10,14] A recent study from Turkey proposed that prescribing colchicine treatment according to body weight and surface area would be more appropriate in children with FMF. However the mean colchicine dose was calculated to be more than in age based dosage.[15]

Vitamin B12 functions as a cofactor in an essential reaction in lipid and carbohydrate metabolism, and is also essential for protein biosynthesis, purine and pyrimidine synthesis, methylation reactions and for folate metabolism.[16] Vitamin B12 is found primarily in foods of animal origin. Dietary vitamin B12 is absorbed and bound to an intrinsic factor that is a small glycoprotein, secreted by gastric mucosa.[17] This Vitamin B12-intrinsic factor complex is absorbed in the ileum after recognition by specific ileal receptors. Clinical manifestation of vitamin B12 deficiency in children includes neurologic/psychiatric, haematologic and gastrointestinal features.[18-21]

Colchicine can induce the malabsorbtion of vitamin B12 by reversible reduction in quality of intrinsic factor-vitamin B12 receptor in the intestinal mucosa. This effect is dose related.[22-24]

The aim of this study was to determine the effect of colchicine on vitamin B12 status in children with FMF.

## Subjects and Methods

The study was conducted as a cross sectional study to assess the effect of colchicine treatment on serum vitamin B12 levels.

Familial Mediterranean fever patients who were followed at the Department of Pediatrics at an University Hospital from January 2005 to May 2008, were evaluated. The Tel Hashomer criteria had been used to establish the diagnosis of FMF.[3] Of the following criteria, the first three are major and the second three are minor, namely: I) recurrent febrile episodes accompanied by serositis; II) AA type amyloidosis; III) response to continuous colchicine therapy; IV) recurrent febrile episodes; V) erysipelas-like erythema; and VI) FMF in a first-degree relative. Two major criteria, or the combination of one major and two minor criteria, were required for a "definite" diagnosis. One major and one minor criterion were required for a "probable" diagnosis. Colchicine therapy was initiated by age based dosage schedule to each patient.

The 12 known FMF mutations were investigated in the patients. Genomic DNA was extracted from 5 ml of whole blood, obtained from the patients according to standard procedures. The patients were studied using a reverse-hybridisation, test strip-based assay (FMF StripAssay; ViennaLab Labordiagnostika, Vienna, Austria) that allows detection of the 12 most frequent *MEFV* mutations: p.E148Q (c.442G>C) in exon 2; p.P369S (c.1105C>T) in exon 3; p.F479L (c.1437C>G) in exon 5; and p.M680I (c.2040G>C), p.M680I (c.2040G>A), I692del (c.2076>2078del), p.M694V (c.2080A>G), p.M694I (c.2082G>A), p.K695R (c.2084A>G), p.V726A (c.2177T>C), p.A744S (c.2230G>T), and p.R761H (c.2282G>A) in exon 10.

The 42 subjects were recruited from the medical records of patients who were diagnosed with FMF and had a vitamin B12 result from their initial blood tests. Patients, being treated with colchicine, were also included in the study. Patients, who were receiving colchicine before initial vitamin B12 test, were not participated in the study. For assessing both initial and control visit vitamin B12 tests, patients were asked to fast overnight (*i.e.* about 8-12 hours) prior to their appointment at the outpatient clinic in our university hospital between May to July 2008. In our facility, blood samples were collected between 8:00 and 11:00 AM after a 12-hr fast, placed in a cooled container and immediately transported to the laboratory, where the plasma was separated by centrifugation within two hours of sampling. Serum folic acid and serum vitamin B12 levels were measured with the Centaur Bayer, which is based on a chemiluminescent enzyme-labelled immunometric assay.[25] The cut-off levels and reference ranges for vitamin B12 and folic acid levels are 200 pg/ml [normal range (NR), 200-950], 2.5 µg/l (NR, 2.5-20), respectively.[26,27]

Patients with a history of liver, gastrointestinal, metabolic, endocrinological, nutritional, and renal diseases and those who had received vitamin supplements for the past three months were excluded from the study. The patients' clinical and demographic information, including fever, abdominal pain, arthritis, erythema, amyloidosis, family history of FMF, and response to colchicine therapy, were obtained by a questionnaire. The disease severity was determined by calculating the Tel Hashomer severity score, based on information collected from medical records and,

if necessary, from an interview by the same (S.O.) physician. The study was performed according to the principles of Helsinki and was approved by the local ethic committee. Informed consent was obtained in all cases, from parents/guardians.

The Kolmogorov-Smirnov test was used to evaluate whether the distribution of variables was normal. One-way analysis of variance (ANOVA) and Kruskal-Wallis analysis of variance were used for the comparison of continuous data with normal and non-normal distributions, respectively. Continuous variables are presented as means ± standard deviations. Categorical variables were compared using a chi-square test. Categorical variables are presented as counts and percentages. A p-value <0.05 was considered significant. Analyses were performed using commercial software (SPSS 16.0 demo).

## Results

Forty-two patients (22 girls and 20 boys) with a mean age of 11.7±4.5 years were included in this study. The mean date since diagnosis was 2.5±2.6 years and disease severity score was 6.4±21. Numbers of attacks per year before and after colchicine treatment were 2.2 and 0.3 respectively.

The mean vitamin B12 levels in girls and in boys were 427 pg/ml and 408 pg/ml respectively. The mean duration of colchicine treatment was 2.5±2.6 years, same as the date since diagnosis, and there was not a statistically important difference by sex. The daily colchicine doses ranged between 0.0077 to 0.0741 mg/kg (mean colchicine dose= 0.0346±0.0139 mg/kg/day). The period between the first and second vitamin B12 testing was 16.5±9.8 months. For laboratory findings, mean corpuscular volume (MCV), red cell distribution width (RDW), vitamin B12 levels were significantly different between the first and control visit measurement, even though there was no significant difference between the first and control visit haemoglobin measurements (Table 1). Control visit serum vitamin B12 levels had no statistically important correlation to the duration of colchicine treatment (r=-0.287, p=0.065) but there was a significant correlation to the daily colchicine doses (r=+0.349, p=0.025).

On control visit examination blood tests, MCV was higher than 96 fl in one patient who was under colchicine treatment for one year (colchicine dose for this patient was 0.025 mg/kg/day). MCV was found to be lower than 80 fl in 22 patients. Seventeen (40%) patients had vitamin B12 levels less than 200 pg/ml. Of these vitamin B12 deficient patients, first and control vitamin B12, MCV, RDW test results were statistically different (Table 2). There was not enough response to colchicine treatment in N=14 (33.3%) patients, and also there was no correlation between response to colchicine treatment with control visit serum vitamin B12 levels (r=+0.117, p=0.46). Genetic test results and vitamin B12 levels at control visit were given on Table 3.

Serum folate values were only determined from control visit examination blood test results; we did not reach admission folate levels. The mean folate level was 8.25±4.05 ng/ml.

## Discussion

Colchicine has been used as a standard therapy for eliminating the attacks and preventing the deposition of amyloidosis in patients with FMF.[28] Although it has been shown that it is effective and safe, this therapy has side effects, such as gastrointestinal disturbances, azospermia, transient alopecia, neuropathy, and myopathy.[11-13,29,30] In our study, only two patients had diarrhea, side effects as mentioned above, remains had no gastrointestinal or other

**Table 1** Laboratory findings of the patients

| | Before colchicine | Upon colchicine | p |
|---|---|---|---|
| Haemoglobin (gr/dl), Mean±SD | 12.5±1.57 | 12.8±1.46 | 0.141 |
| MCV* (fl), Mean±SD | 75.5±5.95 | 85.0±6.87 | 0.003 |
| RDW** (%), Mean±SD | 14.1±1.48 | 15.1±2.84 | 0.019 |
| Vitamin B12 (pg/ml), Mean±SD | 418±179 | 240±103 | 0.000 |
| Folate (ng/ml), Mean±SD | – | 8.25±4.05 | – |

*Mean Corpuscular Volume; ** Red Cell Distribution Width

**Table 2** Laboratory findings of the vitamin B12 deficient patients at control visit (n=17)

| | Before colchicine | Upon colchicine | p |
|---|---|---|---|
| Haemoglobin (gr/dl), Mean±SD | 12.7±1.75 | 13.0±1.78 | 0.393 |
| MCV* (fl), Mean±SD | 76.3±5.46 | 86.3±6.31 | 0.000 |
| RDW** (%), Mean±SD | 13.9±1.39 | 14.9±2.1 | 0.018 |
| Vitamin B12 (pg/ml), Mean±SD | 325±135 | 151±54 | 0.000 |

*Mean Corpuscular Volume; **Red Cell Distribution Width

**Table 3** Genetic test results and vitamin B12 status at control visit

| Allel status | N | Vitamin B12 (pg/ml) (Mean±SD) | p |
|---|---|---|---|
| Homozygous | 16 | 253±74 | 0.769 |
| Compound heterozygous | 13 | 230±137 | |
| Heterozygous | 13 | 226±100 | |
| Total | 42 | 240±103 | |

system complaints.

Prophylactic continuous colchicine therapy can cause altered absorption of compounds from the intestine.[30] This effect is controversial, and there are a few studies in English literature to demonstrate colchicine effects on the intestine.[20-30] Colchicine can decrease absorption of D-xylose and vitamin B12.[30] Two mechanisms can be encountered for altered absorption of vitamin B12. First; dose related, reversible reduction in the quantity of intrinsic factor-vitamin B12 receptors in the intestinal mucosa,[22-24] and the second; the inhibition of jejunal (Na+K) ATPase activity.[30] In the present study, the levels of serum vitamin B12 before and after colchicine therapy were significantly different ($p<0.01$). This result could also support previous studies indicating colchicine therapy can decrease absorption of vitamin B12.[29-31]

The symptoms and signs of vitamin B12 deficiency occurs much sooner even with a complete block in the vitamin B12 absorption. There is an abundance of total body vitamin B12 pool and about three to six years are required for a healthy person to become vitamin B12 deficient.[32] Although these data were obtained from adults, growing children need more vitamin B12 than adults and they may suffer from vitamin B12 deficiency more rapidly. Children, receiving colchicines must be followed closely. The minority of vitamin B12 deficient patients bound with insufficient durations of colchicine therapy and the interval between vitamin B12 measurements to demonstrate a significant decrease in serum vitamin B12 levels. Vitamin B12 pool of the body is supplied by food, mainly animal sources, and secondary to production by microorganisms.[33] Human cannot synthesise vitamin B12. All of our patients have still been continuing with their normal daily intake of food before and during their colchicine therapy. We considered inadequate vitamin B12 intake not to be responsible for the decrease in serum vitamin B12 levels. Nevertheless the patients' daily vitamin and trace elements intake was not under control, which leads alterations on the level of serum vitamin B12.

Once vitamin B12 deficiency is confirmed, the etiology should be searched. Causes of vitamin B12 deficiency can be divided into three groups: nutritional deficiency, malabsorbtion and other GIS causes.[34] None of our patients were on a vegetarian diet, and they did not have diarrhea, constipation, or any other GIS symptoms which indicate malabsorbtion. All patients had a serum vitamin B12 levels above 200 pg/ml at the beginning of colchicine therapy so we assumed that decrease in serum vitamin B12 value at control measurements are related to colchicine use.

The first and control visit haemoglobin measurements are not statistically different and we did not determine any anemic patient at control visit tests. MCV values were stated as increased at follow up tests and the mean MCV values were about 86.3 fl. It was under the cut off value of

macrocytic expression.[35] A recent study reported if serum vitamin B12 levels were above 200 pmol/l, MCV values were about 91-94 fl.[36] Our vitamin B12 and MCV test results corresponded with current medical literature.

Data from the present study could demonstrate a significant correlation between follow up serum vitamin B12 levels and daily colchicine doses but had no correlation with the duration of colchicine therapy. We assumed that serum vitamin B12 levels would decrease with a longer duration of therapy and higher amount of colchicine, but the mean duration of therapy in the present study was not long enough to determine colchicine's long term effects. Although colchicine is used for more than just FMF, including gout, Behcet's syndrome, scleroderma and several dermatologic syndromes,[13] there is no report in English literature that mentions colchicine's effects on serum vitamin B12 levels in human. Thus, we did not compare our results with literature.

Even though this study was constructed as a cross sectional study, there was no data for baseline comparison. Furthermore, because of a relatively low number of samples, we could not categorise the patients in subgroups, such as different duration of therapy and different colchicine doses. Further prospective cohort/clinical studies by monitoring all different factors, from the beginning and at a consistent period of follow up, that will explain more relations and correlations then present study.

In conclusion, our study showed that long term administration of colchicine led to a decrease (subnormal) in the serum vitamin B12 levels. Therefore, serum vitamin B12 levels should be evaluated regularly in FMF patients receiving colchicine therapy.

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