# Urine leukotriene B4 in familial Mediterranean fever

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**ABSTRACT**

**Objective.** *To determine urinary leukotriene B4 (LTB4) levels and their role in FMF.*

**Methods.** *Urinary LTB4 levels were studied using a commercial ELISAkit in 12 FMF patients during abdominal attacks, and 20 FMF patients during remission.*

**Results.** *Urinary LTB4 levels in FMF patients during attacks were comparable to those during remission, but higher than normal levels (p=0.03).*

**Conclusions.** *These findings suggest a persistent activation of the leukotriene pathway in FMF. Whether elevated LTB4 levels are the cause or the effect of inflammation is yet to be determined.*

## Introduction

Familial Mediterranean fever (FMF) is an autosomal recessive disease caused by mutations in the MEFV gene, and prevails in populations of Mediterranean extraction, including Jews, Armenians, Arabs, Druze and Turks (1-3). The disease is characterized by recurrent episodes of acute serositis, mainly peritonitis, pleuritis and arthritis, caused by neutrophil influx to serosal membranes. High levels of a variety of acute phase proteins are typically found during the attacks. Timely diagnosis of FMF is of major importance because, if left untreated, patients may develop AA-amyloidosis, which affects the kidneys as well as other organs.

Leukotrienes are inflammation-associated stable molecules derived from the degradation of arachidonic acid by lipooxygenase (4). One of these, leukotriene B4 (LTB4), was found to be a potent chemo-attractant that induces a pronounced and rapid influx of neutrophils, comparable to that found in acute FMF attacks (4). This arachidonic acid derivative is produced by and large in endothelial cells and neutrophils, and exerts its inflammatory effect locally, in the vicinity of its production. LTB4 is selectively inactivated in human neutrophils by cytochrome P450. However, most of the LTB4 produced passes to the circulation and is excreted unchanged in the urine (5-7).

The fact that neutrophils are the main cellular component of FMF exudates makes it conceivable that LTB4 may have a role in FMF. The purpose of the present study was to determine if LTB4 in urine is a useful marker of inflammation in FMF.

## Methods

*Patients and controls*

To determine urine LTB4 levels in FMF, sterile and fresh urine samples were collected during August and September 2002 from consecutive FMF patients in remission, who were visiting the outpatient FMF clinic for routine examination, or from consecutive patients during an acute FMF abdominal attack, for which they were referred to the emergency medicine department. All patients fulfilled the criteria for diagnosis of FMF (8). None received any medication other than colchicine for at least one month prior to the examination.

Normal urine LTB4 levels were studied in 18 urine samples from healthy (by their statement) volunteers who were workers in our institute, adjusted to the FMF cohort with respect to age and sex, and who were not taking any medications. It may be noted that the patients with FMF attacks and healthy controls were participating in another study, undertaken to determine the usefulness of urine LTB4 in the differential diagnosis of right inferior fossa acute abdominal pain (Bentancur *et al*., in preparation).

All urine samples were immediately studied with Combur test sticks, Miditron Senior II urine analyzer (both from Roche Diagnostics), and light microscopy for glucose, protein, leukocytes, and red blood cells. The presence of glycosuria, proteinuria, leukocyturia or hematuria (more than 5 cells per high power field) led to the exclusion from the study. All samples were then aliquoted into polypropylene tubes and stored in -80°C until examined.

*Determination of urinary LTB4*

Samples were restored to room temperature in a stirring bath, acidified to pH 3.5 by the addition of 50 mLof 2N HC1 per ml of urine and centrifuged for 20 minutes at 3000G to eliminate particles. The samples were then assayed for the LTB4 level using a kit based on a competitive binding enzyme-linked immunosorbant assays (ELISAs), according to the manufacturer's instructions (R&D Systems Inc., MN, USA). The average percentage of recovery for the assay was 96.9% (range 81.8 – 107.7%). The assay is typically sensitive to LTB4 levels of 19.4 pg/ml or more. The assay performers were blinded to the subgroup origin of the studied samples. The LTB4 levels were normalized to the creatinine concentrations assayed in all urine samples in the chemical pathology laboratory of the hospital.

*Statistical analysis*

Statistical analysis was performed using the analysis of variance (ANOVA) test and the $\chi^2$ method.

**Results**

A total of 35 samples were randomly collected, 12 from FMF patients (5 men and 7 women, mean age 40 ± 31 years) with acute abdominal FMF attacks, and 23 from FMF patients in remission. Three from the FMF patients in remission were excluded because of abnormal findings in the urinalysis according to the pre-defined criteria, leaving 13 men and 7 women with a mean age of 50 ± 30 years ($p > 0.05$ for both age and sex).

The mean LTB4 level in FMF patients in acute attack was 328 ± 237 pg/mg creatinine (Fig. 1). The mean LTB4 level in FMF patients in remission was comparable, 329±121 pg/mg creatinine. In healthy controls the LTB4 levels were 210±62 pg/mg creatinine, significantly lower than in patients during an FMF attack ($p = 0.03$).

**Discussion**

In the present study we found that urine LTB4 is elevated in FMF patients. This elevation was found to be unrelated to the clinically overt signs of inflammation that characterize acute FMF attacks, therefore suggesting a possible persistent activation of the arachidonic acid-derived leukotriene pathway. Our results are consistent with those of others implying a role for eicosanoides in FMF by showing that lipoxygenase products are present in the serum and synovial fluid of FMF patients (9), and that FMF attacks may be prevented or reduced in frequency by restriction of dietary fat, which is the main source of arachidonate (10).

Shohat *et al*. even went one step further and suggested that an over-expression of eicosanoides in FMF is the cause and not an effect of the inflammation, and that the primary insult in FMF lies in a defect in the lipocortin family, which includes several inhibitors of phospholipases. They speculated that the suggested defect causes a phospholipase A2-dependent release of arachidonic acid and a subsequent cycloxygenase and lipoxygenase mediated generation of eicosanoide derivatives (11). Based on this hypothesis, one may further infer that LTB4, one of the lipoxygenase products and the subject of the present study, as a powerful pro-inflammatory chemotactic mediator, induces a selective neutrophil influx followed by an enzyme release (4), which could eventually lead to the symptoms occurring during an acute attack of FMF.

Urinary LTB4 parallels plasma levels (7), suggesting that the persistent rise of urinary LTB4 in FMF is consistent with the finding in FMF that acute phase reactants (APRs) remain elevated in one-third of FMF patients (12, 13), even in the absence of symptoms. However, in contrast to other APRs in FMF, no additional rise in LTB4 was observed during acute attacks. This persistent elevation therefore provides support for Shohat *et al*.'s view that the leukotriene pathway is activated by some sort of underlying primary defect and not by inflammation. We postulate that pyrin, the protein product of the MEFV, is associated either directly or indirectly with the leukotriene pathway, and when this protein is mutated, as is the case in FMF, a rise in LTB4 occurs. A possible system for pyrin to exert such an effect is through the neutrophil cytochrome P450, which normally disactivates LTB4 (6).

Our study demonstrates that a persistent activation of the lipoxygenase system, suggestive of a state of chronic inflammation even in the absence of overt manifestations, is present in FMF patients. This finding may open new research avenues, looking at the possibility that pyrin is a leukotriene inhibitor, and studying the effect of leukotriene inhibitors, a new generation of anti-inflammatory medications, on FMF attacks and the resultant reactive amyloidosis.

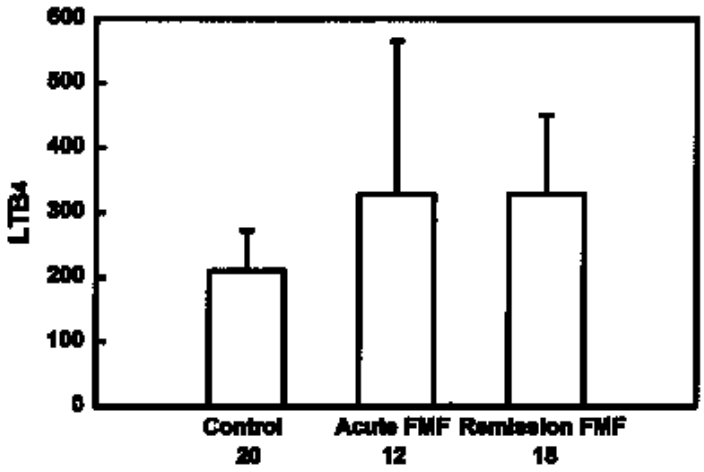


**Fig. 1.** Mean and SD of LTB4 in FMF patients in acute attack and remission, expressed in pg/kg creatinine.

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