# ANTINUCLEAR ANTIBODIES IN FAMILIAL MEDITERRANEAN FEVER

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*SUMMARY: Two previous studies investigating double-stranded DNA titers in patients with familial Mediterranean fever uncovered conflicting results, in order to testify the reproducibility of the results revealed by one of these studies a high prevalence and correlation of double-stranded DNA antibodies with disease activity. We searched these antibodies using a commercially available enzyme immunoassay and also screened the fluorescent antinuclear antibodies by using substrate slides prepared with HEp-2 cell line to see whether there would exist any kind of antinuclear antibodies whatever. According to the cut-off value obtained by adding three standard deviations to the mean of healthy controls (n=36) there was not any statistically significant increase in double-stranded DNA values of patients with active disease (n=21). The percentage of fluorescent antinuclear antibodies in FMF group, healthy controls and lupus patients taken as positive controls (n=19) were 23.8%, 8.3%, 100%, respectively. The fluorescence intensity was extremely weak in negative controls and in FMF patients except one and could be defined as 'trace' when compared to that of lupus patients which was brightly luminescent. So, the prevalence of double-stranded DNA antibodies have not increased and there was not any significant difference between the percentage of fluorescent antibodies of FMF group and that of negative controls, although it appeared relatively high. This may be the result of female preponderance of our subjects, nonspecific binding of conjugate and subjective nature of assay deciding in borderline situations.*

*Key Words: Anti-nuclear antibodies, familial Mediterranean fever.*

## INTRODUCTION

Familial Mediterranean fever (FMF) is an inflammatory disease of unknown etiology which carries the potential risk of lethal amyloidosis. It is characterized by recurrent attacks of fever, abdominal pain which is a sign of peritonitis, arthritis, maculopapular skin eruptions of lower extremities and sometimes the inflammation of serous membranes other than peritoneum. The disease is prevalent among Sephardic and Ashkenazic Jews, Armenians and people of Arabic or Turkish descent. It has been postulated both that it may or may not be an autoimmune disease. Recent studies performed in FMF patients revealed suppressor T-lymphocyte anomalies (1), defects in lipoxygenase pathway of arachidonic acid metabolism and the absence of normal inhibitor of C5a (2).

Antibodies to deoxyribonucleic acid (DNA) are substantially important in diagnosing systemic lupus erythematosus (SLE) (3) and evaluating its clinical activity. The antibodies to double-stranded DNA (ds-DNA) which are formed against the configurational epitopes

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on ribose phosphate backbone is more specific than the single-stranded (ss-DNA) variety which is directed against the free base moieties. A recent study reports that ds-DNA detected by enzyme immunoassay (ELISA) closely correlates with disease activity defined according to the scoring system of Lupus Activity Index (LAI) proposed by the European Consensus Study Group for Disease Activity in SLE (4).

Fluorescent antinuclear antibody test (FANA) detected with indirect fluorescent microscopy is a practical and useful screening assay for the first step evaluation of a variety of auto antibodies directed against the nuclear elements of the cell. Although it may be seen strongly positive in almost all of the patients with SLE and, in a limited extend, even in the first degree relatives of them (5), it is not specific for SLE at all and can be seen both in patients inflicted by other autoimmune diseases and weakly in healthy individuals also (6). There may be a crude relation between the defined patterns of appearance in fluorescent microscopy (perinuclear, diffuse, speckled, nucleolar, anti-centromer and cytoplasmic) and the coexistence of certain collagen diseases.

The limited studies previously performed to determine the prevalence of antinuclear antibodies among FMF patients have yielded conflicting results (7,8). In this study, we aimed to test the reproducibility of the outcome of a previous investigation which stated a high prevalence of anti-ds-DNA antibody occurrence and correlation with active disease insults in FMF patients and compared to healthy controls. Additionally, we searched FANAs in the sera of the same subjects to see whether there might have been any kind of antinuclear antibody whatsoever.

## MATERIALS AND METHODS

21 active FMF patients consulted to the Clinics of Çukurova University Hospital and diagnosed according to their physical and laboratory examinations and were recruited for the study. 19 SLE patients with active disease diagnosed according to 1982 revised ARA criteria and activity confirmed by LAI score were added as positive controls. 36 healthy volunteers constituted the negative controls. The mean ages of FMF patients, lupus and healthy controls were 29.6, 34.2, 33.3 respectively.

**ds-DNA method:** For the measurement of ds-DNA values in the sera of subjects, a commercially available enzyme immunoassay (BioHyTech, Israel) was used observing the instructions of manufacturer. Briefly, multi-well plate was incubated for 30 minutes with 1/200 dilution of test sera and with enzyme conjugated anti-human immunoglobulin after which 4 successive changes of wash for each was performed, respectively. All incubations were done in 37 degrees. After a final incubation of 45 minutes with substrate (dNPP), the optical densities were read at 405 nm and a standard curve was constructed. The cut-off value was calculated by adding 3 standard deviations to the mean of healthy controls.

**FANA method:** FANAs were evaluated with indirect fluorescent antibody test by using commercially prepared slides of HEp-2 cell line (ScimedX, USA). Substrates were incubated with 30 micro-liters of 1/20 diluted test sera and 1/40 dilution of FITC conjugated polyvalent anti-human anti-sera respectively. After a brief shake with PBS (pH=7.5), a gentle wash of slides for 15 minutes with a magnetic stirrer fallowed each incubation. Finally, the slides were mounted with buffered glycerin and searched under the high power field of fluorescent microscope.

Table 1: Enzyme immunoassay and FANA results of groups.

| Average of ds-DNA values of groups (EU) | | Number of subjects above cut-off value | Number and percent of FANA positivity | n |
|---|---|---|---|---|
| FMF | 71.4 | 1(4.8%) | 5(23.8%) | 21 |
| CONTROLS | 51.2 | - | 3(8.3%) | 36 |
| SLE | - | 16(84.2%) | 19(100%) | 19 |

**Statistical method:** T-test was used in the statistical analysis of means and percentages.

## RESULTS

The results of this study are summarized in tables supplemented. As can be seen from Table 1, in only one of 21 FMF patients and in 16 of 19 lupus subjects the ds-DNA levels were above the cut-off level. On the other hand none of the healthy controls, revealed a ds-

DNA value above the cut-off level. In 11 of 19 lupus patients these values were above 500 ELISA unites which was the limit set by the apparatus. There was not any significant difference between the means of ds-DNA values of FMF patients and that of healthy control ($t = 1.73 < 2.01$, $\alpha = 0.05$).

The percentage of weak FANA positivity observed in FMF patients and healthy controls were 8.3% and 23.8% respectively and the difference was not significant ($t = 1.49 < 2.01$, $\alpha = 0.05$). If the observed fluorescence is scored according to its intensity, FANA results of lupus patients are distributed in the strongly positive end of spectrum in contrast to the healthy controls and FMF patients who showed very weak illumination which can be considered 'trace' in all subjects except one as illustrated in Table 2.

Table 2: The distribution of subjects according to scored FANA results.

| | - | trace | + | ++ | +++ | ++++ | n |
|---|---|---|---|---|---|---|---|
| FMF | 16 | 4 | 1 | - | - | - | 21 |
| CONTROL | 33 | 3 | - | - | - | - | 36 |
| SLE | - | - | 1 | 5 | 10 | 3 | 19 |

## DISCUSSION

The results of this study show that ds-DNA antibody titers are not elevated in the sera of patients with active FMF disease with respect to healthy controls. A previous study found the prevalence of anti-ds-DNA antibodies 22% and claimed a correlation of these values with febrile attacks of patients with active disease. Another study performed on a larger group of FMF patients (n=168) investigated a couple of auto-antibodies including ds-DNA and found the prevalence of these auto-antibodies 3% and no correlation with activity. Our results are in agreement with that of the latter study. Conflicting results may depend on the limited number of subjects and the standardization errors of methods used.

FANA is a subjective screening test the outcome of which differs somewhat according to the substrate used. 5% of weakly positive results are seen among healthy individuals. The incidence of this finding is higher among women and older individuals. In these cases auto-antibody titers are usually equal to or less than 1/320. Although the percentage of FANA positivities are higher in FMF patients and healthy controls in our study there is no significant difference between them statistically. Besides, the female preponderance of our subjects in FMF and healthy control group (18/3, 30/6 respectively) can explain the relatively higher percentage of FANA positivity compared the population values stated elsewhere (6). On the other hand, the intensity of immunofluorescence observed with the negative control sera and with all of FMF patients except one can be defined as 'trace' when compared to that of lupus patients which is brightly luminescent. In this respect, extremely weak positivity may be the result of nonspecific binding of conjugate and may lead to the over estimation of positive results both because of this nonspecific binding and the difficulty of decision in borderline situations.

In conclusion, although the existence of immune complexes have been observed in patients with FMF disease (9), the pathogenesis of which is controversial, the result of this study indicates no auto-antibodies against nuclear antigens in this disease and agrees with one of the limited studies performed in this field.

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