Immunological Evaluation in Patients with Familial Mediterranean fever

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Abstract

OBJECTIVE: This study aimed to investigate T & B lymphocyte subsets and Natural Killer (NK) cells patterns in children with FMF versus normal control subjects, to estimate the immunoglobulins IgG, IgM, and IgA levels, and to scrutinize the possible use of Neutrophil / Lymphocyte ratio (NLR) as a marker for subclinical inflammation in FMF patients.

PATIENTS AND METHODS: A group of 42 patients with FMF attending the Genetics Clinic at National Research Centre were included in this study. They were 13 males and 19 females; their age ranged from 2 to 17 years old. Normal healthy subjects within the same age and sex range were included as a control group. Complete blood picture was done for all cases, and neutrophil/ lymphocyte ratio was calculated. Flow cytometer analysis was done for CD3, CD4, CD8, CD19 and CD16 using monoclonal antibodies. Immunoglobulins IgG, IgA and IgM were estimated in serum using nephelometry.

RESULTS: Positive consanguinity was present in 20 patients (47.6%). Abdominal pain was the most common manifestation followed by fever, arthritis, and red rash. CD3, CD4 and CD8 were statistically increased in patients group as compared to normal control group, while CD16 was statistically decreased.

CONCLUSION: The study suggests that quantitative measurement of CD expressions of CD3, CD4 and CD8 as well as NLR might be used as valuable markers for subclinical inflammation in FMF.

Introduction

Familial Mediterranean fever (FMF) is the most common systemic autoinflammatory disorder worldwide [1]. It is an autosomal recessive disease [2] that affects mainly the Mediterranean population [3], is characterised by recurrent self - limited fever and aseptic serosal inflammation, causing abdominal, thoracic and joints pain [4]. Children with FMF are more prone to growth retardation [5], chronic normocytic normochromic anaemia [6], and splenomegaly [7].

Malicious activation of many inflammatory pathways occurs during the attacks of FMF, in which T, B and Natural killer cells play a major role [8][9]. The interaction of these cells with endothelial cells and several inflammatory and immune mediators produced by these cells leads to the formation of atherosclerotic plaques followed by its destabilisation and vessel rupture [10].

In between attacks, inflammation continues subclinically with the associated processes of endothelial dysfunction, increased atherosclerotic changes and platelets activation. The persistent inflammation in FMF may cause endothelial dysfunction, atherothrombosis and systemic amyloidosis [11]. The Neutrophil / Lymphocyte ratio (N/L ratio) showed significant, rapid, and serial changes as immune system response to different conditions like surgical stress, systemic inflammation or severe infections [12], and can be used as a marker for predicting amyloidosis development [13].

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The most distressing complication in FMF is renal amyloidosis, leading to nephrotic syndrome and chronic kidney failure. A macromolecular complex, called inflammasome, plays a major role in the activation of IL - 1 and thus induction of inflammation and when inflammasome activity is abnormally stimulated through a mutation, it may be involved in the pathogenesis of FMF [14][15][16]. Natural killer (NK) cells are cytotoxic lymphocytes that participate in innate immunity. In addition to their cytotoxic response, these cells produce cytokines to assist the adaptive immune response [17][18].

The aim of this study was to investigate T and B lymphocytes and Natural Killer (NK) cells patterns through flow - cytometric analysis of CD3, CD4, CD8, CD16 and CD19 expression, to estimate the immunoglobulin levels IgG, IgM and IgA in FMF patients as compared to normal control subjects, and also to scrutinize the possible use of NLR as a marker for subclinical inflammation in patients with FMF.

Patients and Methods

Forty - two patients with FMF attending the Clinical Genetics Clinic at National Research Centre were included in this study. They were following up patients during the year 2015. They were 13 males and 19 females; their age ranged from 2 to 17 years old. Twenty normal healthy subjects within the same age and sex range were included as a control group.

All subjects were subjected to full clinical examination and history taking. Written consents were taken from the children parents', and the study was approved by Medical Ethics Committee of NRC (13/146). Complete blood picture was done for all cases, and neutrophil/lymphocyte ratio was calculated. Flow - cytometer analysis was done for CD3, CD4, CD8, CD19 and CD16 using monoclonal antibodies [19]. Immunoglobulins IgG, IgA, Ig M was estimated in serum using nephelometry [20].

Statistical analysis was performed using SPSS program version 13. Quantitative variables were presented as a mean and standard deviation. The unpaired t-test was used to evaluate differences between the two groups of continuous variables. Two-tailed P < 0.05 was considered statistically significant.

Results

The study group consisted of 23 males (54.8%) and 19 females (45.2%), all were diagnosed as FMF and during the attack; while the control group were normal subjects matching age and sex. The age range in the FMF study group was 2 - 17 years and 2 - 14 years in the control group respectively (Table 1). This study included 42 patients, with the clinical characteristics of FMF. Positive consanguinity was present in 20 patients (47.6 %).

Abdominal pain was the most common manifestation which usually occurs in repeated attacks and stays from 2 hours to several days followed by fever, arthritis, and erysipelas-like rashes. All the patients were treated with colchicine after being identified by molecular diagnosis.

Table 1: Age and sex of patients and control group

	Patients n = 42	Control n = 20
Age range (years)	2-17	2-14
Sex (male/female)	23/19	11/9

CD3, CD4, and CD8 were statistically increased in patients' group as compared to normal control group; p-value were 0.001, 0.002, 0.004, respectively (Table 2). While CD16 has statistically decreased, the p-value was 0.007 as compared the control group.

Table 2: Cellular expression of CD3, CD4, CD8, CD19 and CD16 in FMF patients as compared to control group

	Patients n = 42	Control n = 20	p - value
CD3 %	51.91 ± 15.19	36.31 ± 10.41	0.001*
CD19 %	18.29 ± 9.07	19.78 ± 5.38	0.81
CD16 %	12.81 ± 5.38	19.06 ± 6.9	0.007*
CD4 %	33.74 ± 6.46	22.88 ± 6.81	0.002*
CD8 %	19.37 ± 5.98	12.2 ± 4.15	0.004*

*statistical significant p < 0.05.

Immunoglobulins IgA, IgM, IgG and also TLC and N/L ratio did not show the significant statistical difference between the patients and control groups (Table 3).

Table 3: Immunoglobulins IgA, IgM, IgG, TLC and N/L ratio in FMF patients and control group

parameters	Patients n = 42	Control n = 20	p - value
TLC (×10^6 /mm^3)	7.54 ± 2.39	9.16 ± 2.23	0.13
N/L ratio (NLR)	1.23 ± 0.62	0.8 ± 0.16	0.10
IgA (g/L)	1.35 ± 0.7	1.31 ± 1.06	0.89
IgM (g/L)	1.26 ± 0.45	1.41 ± 0.60	0.54
IgG (g/L)	10.67 ± 3.8	9.62 ± 3.45	0.49

*statistical significant p < 0.05

Discussion

Autoinflammatory diseases originate from inappropriate activation of antigen-independent inflammatory mechanisms [21]. FMF is characterised by fever and serositis which flare up as paroxysmal attacks, with intervening asymptomatic periods. The attacks of fever and diffuse abdominal pain in FMF are characterised by subclinical inflammation and associated endothelial dysfunction [11]. We aimed to

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investigate a possible immune regulation imbalance in familial Mediterranean fever (FMF) by measuring levels of peripheral blood lymphocyte subsets using flow - cytometry. In this study, we found the increased percentage of T cells CD3, CD4 and CD8 as compared to the control group. This was in agreement with Musabak et al., [22], who stated that CD3, CD4 and CD8 were increased in FMF patients than in the control group indicating that the T cell system is abnormally activated in patients with FMF. Rimar et al., [23] suggested that regulatory T cells play a role in cutting short FMF acute attacks. This may explain the fact that inflammation in FMF is self - limiting and also the absence of autoantibodies, antigen-specific T cells and tissue damage [24].

NK cells mediate cytotoxicity that is regulated by their inhibitory and activating surface receptors. An increase in the activation of NK cells may result in proliferation and change the immune response [25]. In this study, CD16+ NK cells were decreased in FMF patients group as compared to the control group in contrary to what was reported that NK cell numbers were significantly increased in FMF patients as compared to controls. In autoinflammatory diseases, dysregulation is believed to occur in the innate immune system without primary involvement of T and B lymphocytes. Nevertheless, autoinflammation involves crosstalk between the innate immune system – neutrophils, macrophages, and NK cells – with the adaptive immune system [26].

To investigate the B - cells, CD19 showed no significant difference between patients and control groups. As regards to serum immunoglobulins IgA, IgM and IgG estimated in this study, there was no statistically significant difference in patients when compared to the control group. In another genetic disease, results obtained showed a significant difference between patients and controls, in which the results of the control group is nearly the same [27]. However, Livneh et al., [28] compared the immunoglobulin levels in patients with FMF about patients with hyperimmunoglobulinemia D syndrome (HIDS) and not about healthy control subjects, and that the patients with HIDS showed the significantly higher prevalence of elevated immunoglobulins levels than patients with FMF.

The N/L ratio is a reliable marker for evaluating and monitoring the systemic inflammatory response, and prognosis of clinical outcome of inflammatory diseases [29].

No statistically significant difference was detected in TLC nor NLR in FMF patients as compared to the control group while Özer et al., [30] and Duksal et al., [29], found TLC and N/L ratio were significantly higher in children with FMF than in healthy individuals. Celikbilek et al., [31], reported that the N/L ratio was higher in active FMF than in FMF in remission as well as in control subjects.

In conclusion, dysfunction of the innate immune system is the central matter in FMF as a self - reactive autoinflammatory disease.

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