

The relationship between red cell distribution width and homozygous M694V mutation in familial Mediterranean fever patients

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BACKGROUND AND OBJECTIVE: Familial Mediterranean fever (FMF) is characterized by recurrent and self-limiting attacks with peritonitis, pleuritis, arthritis, and erysipelas-like erythema. We aimed to investigate the red cell distribution width (RDW) level as an inflammatory marker in FMF patients compared with normal subjects.

DESIGN AND SETTINGS: A retrospective study of FMF patients at the Department of Gastroenterology, Cumhuriyet University, between November 2011-February 2013.

METHODS: A total of 249 FMF patients and 131 age- and sex-matched control participants were included in the current study. RDW levels were also analyzed by standard methods. Each patient was given 2 mL of blood sample to obtain genomic DNA.

RESULTS: Statistically significant differences were observed in RDW values between the FMF patients and the control group. Also, RDW levels were higher in the FMF patients with the homozygous M94V mutation compared with those with other mutations. The receiver-operating characteristic curve analysis suggested that the optimum RDW cutoff point for the FMF patients was 13.95, with a sensitivity, specificity, negative predictive value, and positive predictive value of 70%, 64%, 68%, and 66%, respectively (area under the curve: 0.711, 95% confidence interval 0.627-0.795, $P < .0001$).

CONCLUSION: We suggest that RDW may show subclinical inflammation in FMF patients. RDW may be a promising marker in predicting the homozygous M694V mutation in FMF patients.

Familial Mediterranean fever (FMF) is well known as an autoinflammatory disease.¹ It has clinical features, which include recurrent and self-limiting attacks with peritonitis, pleuritis, arthritis, and erysipelas-like erythema. Subclinical inflammation in FMF patients is shown by high levels of acute-phase proteins, cytokines, and inflammation-induced proteins; overproduction of C-reactive protein (CRP) or serum amyloid-A (SAA); and persistently elevated fibrinogen levels and erythrocyte sedimentation rates (ESR). A number of genetic and environmental factors are effective in the severity of the disease. The M694V mutation was associated with clinical severity; it was expected to be associated with higher inflammation in these patients.²

A complete blood count is an easy examination

technique that gives us information about the patient's formed blood contents. Red cell distribution width (RDW) is a simple and inexpensive parameter, which reflects the degree of inflammation. RDW, a measurement of variability and size of erythrocytes, can be easily measured during routine complete blood counts.³ Increased RDW (independent of Hb values) has been demonstrated to be associated with negative clinical outcomes in autoimmune diseases.⁴⁻⁶ A strong correlation between RDW and widely used inflammatory markers including CRP and ESR have also been reported.⁷ Furthermore, some readily available markers in blood context such as neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), and mean platelet volume (MPV) were potential subclini-

cal inflammation markers in patients with FMF.⁸ NLR, PLR, MPV, and RDW values are higher in FMF patients during symptom-free periods.⁹

To the best of our knowledge, no study regarding the relationship between RDW and genetic factors in FMF patients are available in the published reports. From this point of view, the aim of our study was to evaluate RDW levels and its association with genetic factors in patients with FMF.

METHODS

We studied retrospectively 249 FMF patients according to Tel-Hashomer criteria¹⁰ and 131 control participants between November 2011-February 2013 at the Department of Gastroenterology, Cumhuriyet University. The clinical symptoms, laboratory values, and MEFV gene mutations were achieved from the archive records. Of the 249 patients, all were in attack-free period, and these patients had been followed up regularly. At least 2 weeks from the end of an FMF attack period was described as attack-free period according to the physical examination, clinical symptoms, and acute-phase proteins such as CRP, ESR, and leukocyte counts.

The patients with diabetes mellitus, coronary heart diseases, metabolic syndrome, anemia, history of transfusion, acute/chronic infection (within 3 weeks), autoimmune diseases, chronic obstructive pulmonary disease, amyloidosis with FMF, and history of smoking were excluded. We excluded amyloidosis according to clinical and laboratory parameters during the patient follow-up. Rectal biopsies and renal biopsies all of the patients were not performed. Also, the patients under medication except colchicine were not included in the study.

A written informed consent was obtained before enrollment from all participants. The Institutional Ethics Committee approved the study protocol, and the study was conducted in accordance with the Declaration of Helsinki.

Biochemical measurements

Blood samples were withdrawn without stasis in the morning of the day following a fasting period of 12 hours. The blood samples of all groups were analyzed after 2 hours of venipuncture using an automatic blood counter (Mindray BC-6800, Genova, Italy). In all groups, RDW levels were measured in a blood sample collected in tripotassium EDTA (7.2 mg) tubes. Hematological parameters, including hemoglobin (Hb), white blood cell (WBC) count, and platelet count were also analyzed by standard methods. The reproducibility of the RDW test (intra- and interassay variability) are under 1% and 3%, respectively.

Mutation analysis

All data of genetic analyses of patients were obtained from hospital records in the Medical Genetic Department of our university hospital. All molecular examinations of patients who had FMF or possible FMF were performed in the laboratory of the Medical Genetics Department. Each patient was given 2 mL of blood sample to obtain genomic DNA; for this process, a Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, USA) was used. The spectrophotometric analysis of DNA molecules (Nanodrop ND-1000) was done to detect the amount and purity of the molecules. The MEFV mutations (M694V, M694I, M680I and V726 located in the tenth exon, and E148Q located in the second exon) in patients were determined with the polymerase chain reaction-enzyme-linked immunosorbent assay using a PRONTO FMF Kit (Pronto Diagnostics, Rehovot, Israel), while P369S, K695R, A744S, R202Q, and R761H mutations were determined with an FV-PTH-MTHFR Strip Assay Kit (Vienna, Austria). The patients were divided into 2 groups according to the presence or absence of the M694V mutation to determine the relationship between genetic structure and RDW, and 2 groups were compared in terms of RDW values.

Statistical analysis

SPSS version 15.0 (Statistical Package for the Social Sciences, Chicago, Illinois, USA) was used for statistical analyses. Continuous variables were given as mean (standard deviation), and categorical variables were defined as percentage. Data were tested for normal distribution using the Kolmogorov–Smirnov test. To compare continuous variables, one-way analysis of variance test or Kruskal-Wallis test was used as appropriate. When a significant difference was observed between the 2 groups, the post hoc Tukey test or Man-whitney U was used for determining the difference between the groups. To compare categorical variables, the chi-square test was used. The receiver-operating characteristic (ROC) area under the curve (AUC) is a popular measure for the accuracy of a diagnostic test. A diagnostic test that has a greater AUC is a better predictor of the presence of a disease. To determine the accuracy and respective best cutoff values of RDW for predicting disease activity in patients with FMF, ROC curves and their corresponding AUC were used. A 2-sided $P < .05$ was considered significant.

RESULTS

The attack and baseline clinical characteristics are given in **Table 1**. The distribution of MEFV gene mutations

is given in **Table 2**. The heterozygous M694V mutation was the most common gene mutation in our study. In addition, the comparisons between the 2 groups about demographic features and laboratory findings are shown in **Table 3**. Hematological parameters including Hb, WBC, and platelet count did not have statistically significant differences in the 2 groups (**Table 3**). Statistically significant differences were observed in RDW values between the FMF patients and the control group ($P < .0001$) (**Table 3**). Also, RDW levels were higher in FMF patients with the homozygous M94V mutation compared with those with other mutations (**Table 4**) (**Figure 1**). RDW levels were higher in FMF patients with other mutations compare with those of controls (**Table 5**) (**Figure 1**).

The ROC curve analysis suggested that the optimum RDW cutoff point for FMF patients was 13.95, with a sensitivity, specificity, negative predictive value, and positive predictive value of 70%, 64%, 68%, and 66%, respectively (AUC: 0.711, 95% confidence interval 0.627-0.795, $P < .0001$) (**Figure 2**).

DISCUSSION

The present study investigated the relationship between *MEFV* gene mutation and RDW levels in patients with FMF. RDW values were demonstrated to be significantly higher in FMF groups compared with those of control subjects. Also, RDW levels were shown to increase in the FMF patients with the homozygous M94V mutation compared with those with other mutations. These results may indicate that elevated serum RDW levels may be associated with the ongoing inflammation in the pathophysiology of FMF.

RDW is known as the variation of red blood cell volume and can easily be measured by the routine analysis of whole-blood count.¹¹ Furthermore, systemic inflammation can affect erythrocyte heterogeneity, and it can result in increased RDW levels. High RDW has been associated with inflammatory diseases. Vayá et al¹² investigated the association of RDW with inflammatory markers in systemic lupus erythematosus (SLE). In SLE without anemia, RDW correlated directly with fibrinogen and CRP. These results indicated that RDW was influenced by inflammation in SLE patients. Chronic inflammation is one of the major components of many autoimmune diseases, and RDW may reflect the severity of these autoimmune diseases as well. Hu et al¹³ investigated the correlation between RDW and disease activity of SLE. An increased RDW level was observed in SLE patients. RDW was positively correlated with serum immunoglobulin M, CRP, ESR, and SLE disease activity index.

Table 1. During the attack and baseline clinical characteristics of patients with FMF.

| | Patients (n=249) |
|--|------------------|
| Age at diagnosis (y) ^a | 20.0 (10.0-52.0) |
| Disease duration (y) ^a | 4.0 (1.0-45.0) |
| Dose of colchicine (mg/d) ^b | 1.3 (0.6) |
| Family history of FMF, (%) | 137 (55) |
| Fever, (%) | 231.0 (92.8) |
| Peritonitis, (%) | 228.0 (91.6) |
| Pleuritis, (%) | 138.0 (55.4) |
| Arthritis, (%) | 57.0 (22.9) |
| Erysipelas-like erythema, (%) | 17.0 (6.8) |
| Pericarditis, (%) | 15 (6) |

^aMedian (min-max value); ^bMean (SD); FMF, Familial Mediterranean fever.

Table 2. *MEFV* gene mutations.

| | |
|--------------------------|-------------|
| Homozygous M694V | 27.0 (10.8) |
| Homozygous M680I | 6.0 (2.4) |
| Homozygous V726A | 5 (2) |
| Heterozygous M694V | 50.0 (20.0) |
| Heterozygous M680I | 19.0 (7.6) |
| Heterozygous E148Q | 18.0 (7.2) |
| Heterozygous V726A | 4.0 (1.6) |
| Heterozygous M694V/M680I | 19.0 (7.6) |
| Heterozygous M694V/E148Q | 12.0 (4.8) |
| Heterozygous M694V/V726A | 9.0 (3.6) |
| Heterozygous M680I/V726A | 7.0 (2.8) |
| Others | 73.0 (29.3) |

All datas: n (%).

Table 3. Comparison of clinical and laboratory values of patients and controls.

| | Patients (n=249) | Controls (n=131) | P value |
|----------------------------|----------------------|-----------------------|---------|
| Age, (y) | 32.0 (11.8) | 29.9 (8.8) | .057 |
| Gender, M/F, n (%) | 162 (65.1)/87 (34.9) | 76 (58.0) / 55 (42.0) | .178 |
| CRP, mg/L | 2.0 (1.0-8.0) | 2.0 (1.0-7.0) | .011 |
| ESR, mm/h | 6.0 (1.0-23.0) | 7.0 (1.0-14.0) | .122 |
| Leukocyte, $\times 10^9/L$ | 7.4 (2.0) | 7.1 (1.6) | .162 |
| Platelet, $\times 10^9/L$ | 275.4 (72.1) | 275.9 (58.5) | .940 |
| Hemoglobin, g/dL | 14.1 (1.3) | 14.2 (1.3) | .229 |
| RDW, % | 13.8 (1.1) | 13.1 (0.9) | <.0001 |

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; RDW, red blood cell distribution width.

Table 4. Comparison of clinical and laboratory values of FMF with homozygous M694V mutations and other mutations.

| | FMF with homozygous M694V mutations (n=27) | FMF with other mutations (n=222) | P value |
|----------------------------|--|----------------------------------|---------|
| Age (y) | 30.2 (9.3) | 32.2 (12.1) | .655 |
| Gender, M/F, n (%) | 18 (66.7)/9 (33.3) | 144 (64.9)/78 (35.2) | .853 |
| Leukocyte, $\times 10^9/L$ | 7.9 (1.9) | 7.35 (2.0) | .356 |
| Platelet, $\times 10^9/L$ | 278.0 (75.6) | 275.1 (71.8) | .976 |
| Hemoglobin, g/dL | 13.6 (1.1) | 14.1 (1.3) | .089 |
| RDW, % | 14.5 (1.1) | 13.8 (1.1) | .001 |

FMF, Familial Mediterranean fever; RDW, red cell distribution width.

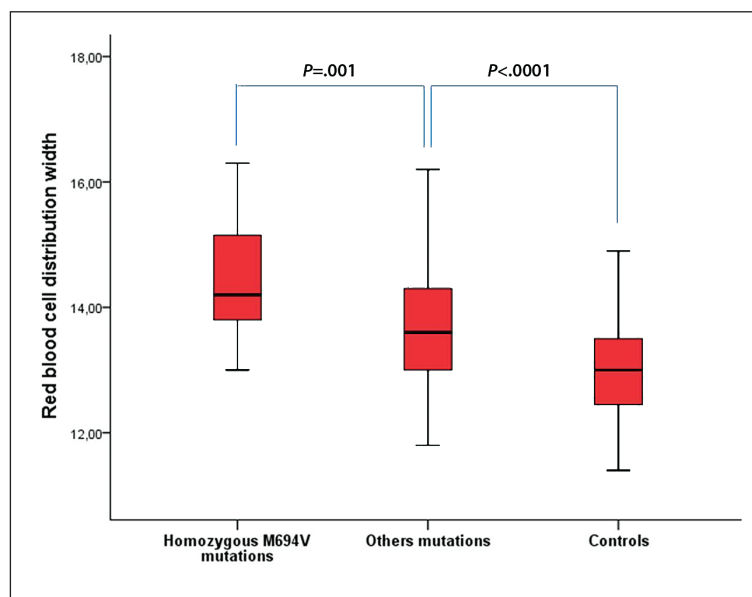


Figure 1. Red blood cell distribution width significantly increased in FMF patients compared with that in the control group ($P<.01$).

Table 5. Comparison of clinical and laboratory values of FMF with other mutations and controls.

| | FMF with other mutations (n=222) | Controls (n=131) | P value |
|----------------------------|----------------------------------|-------------------------|---------|
| Age (y) | 32.2 (12.1) | 29.9 (8.8) | .139 |
| Gender, M/F, n (%) | 144.0 (64.9)/78.0 (35.2) | 76.0 (58.0)/55.0 (42.0) | .200 |
| Leukocyte, $\times 10^9/L$ | 7.4 (2.0) | 7.1 (1.6) | .577 |
| Platelet, $\times 10^9/L$ | 275.1 (71.8) | 275.9 (58.5) | .993 |
| Hemoglobin, g/dL | 14.1 (1.3) | 14.2 (1.3) | .731 |
| RDW, % | 13.8 (1.1) | 13.1 (0.9) | <.0001 |

FMF, Familial Mediterranean fever; RDW, red blood cell distribution width.

Behçet Disease (BD) is a chronic, multisystemic, inflammatory disorder, characterized by recurrent oral aphthous ulcers, genital ulcers, uveitis, and skin lesions.¹⁴ It is a systemic immuno-inflammatory vasculitis and is characterized by endothelial dysfunction.^{15,16} Vayá et al¹⁷ reported that RDW was statistically higher in patients with BD than in controls. RDW has been shown to be associated with disease activity in several inflammatory disorders. RDW in systemic sclerosis may represent an integrative measure of multiple pathological processes including extensive vasculopathy, fibrosis, or ongoing inflammation. An increase in RDW may indicate an impairment of cardiorespiratory function. RDW showed a positive correlation with inflammatory markers, including ESR and CRP in patients with systemic sclerosis. Rodríguez-Carrio et al¹⁸ investigated the relationship between disease parameters and RDW levels in rheumatoid arthritis. RDW is associated with disease activity and acute-phase reactants in patients with rheumatoid arthritis.

Inflammation has been shown to be associated with endothelial dysfunction in patients with FMF.^{19,20} Subclinical inflammation is shown by high levels of acute-phase proteins, cytokines, and inflammation-induced proteins. Inflammatory cytokines may cause increased heterogeneity of erythrocyte maturation and impairment.²¹ Interleukin (IL-6) and IL-8 were the first 2 interleukins identified as being elevated in the sera of FMF patients during attack-free periods. Subsequent studies reported high serum concentrations of cytokines in attack-free patients with FMF.²² In experimental studies, inflammatory cytokines have been found to suppress the maturation of erythrocytes, so immature erythrocytes enter into the circulation and RDW levels increase.²³ Accordingly, cytokines inhibit the growth of erythroid precursor cells and erythropoietin production, and reduce iron availability for erythropoiesis by increasing hepcidin synthesis, which could explain the link between RDW and adverse outcome.²⁴ Cetin et al²⁵ have investigated the relationship between RDW levels (as indicators of subclinical inflammation and tools) and FMF patients for treatment decision and inflammatory condition. The authors concluded that high RDW levels may provide additional information about persistent subclinical inflammation in FMF patients during attack-free periods. The positive correlation was found between RDW and ESR.

FMF is an inherited autosomal recessive disorder, which is ethnically restricted, commonly found among individuals of Mediterranean descent, and is caused by *MEFV* gene mutations on chromosome 16. However, approximately 10% to 20% of FMF patients have not

any *MEFV* mutations.²⁶ *MEFV* gene, and these mutations have been identified mostly in exon 2 (E148Q) and exon 10 (M694V, M694I, V726A, M680I). The *MEFV* gene encodes pyrin/marenostrin. Pyrin inhibits the proinflammatory cytokines and/or increases the secretion of anti-inflammatory mediators.¹ Homozygous for the *MEFV* mutation resulting in the pyrin variant met 694 val seems to correlate with the degree of subclinical inflammation. In a group of attack-free FMF patients who were homozygous for this specific mutation, CRP and SAA levels considerably elevated compared with those of patients who harbored 2 other *MEFV* mutations. Other indirect findings suggesting the association between M694V and amyloidosis are as follows: homozygous M694V patients had higher disease severity scores, the resistance to colchicine treatment was more common among homozygous M694V patients, and the M694V/M694V genotype was the most common cause of phenotype 2 disease.²

We presented higher RDW levels in FMF patients compared with those of controls in this study. Also, FMF patients with the homozygous M694V mutation have higher RDW levels compared with those with other *MEFV* mutations. In this context, since above studies showed that the homozygous M694V mutation was associated with higher inflammatory conditions, RDW, which is present in higher levels in this mutation according to the findings of the present study may be used as an inflammatory marker in FMF patients.

Study limitations

The major limitation of our study was the comparatively small size of the study population. Importantly, in our study, the number of homozygous M694V mutation patients was comparatively small. So, future studies should investigate a novel inflammatory markers like RDW in patients with the homozygous M694V mutation. In patients with amyloidosis, there is a need to

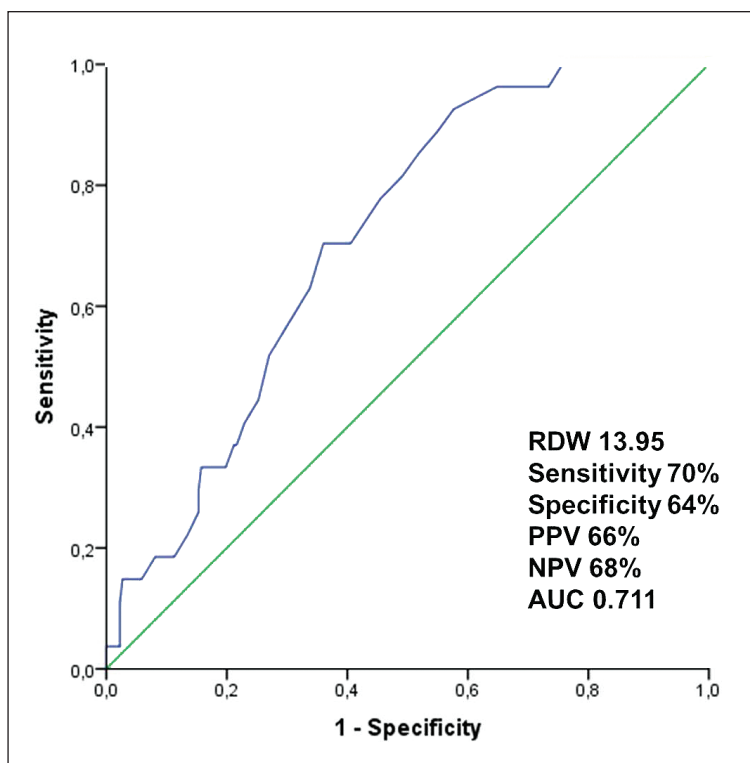


Figure 2. Receiver-operating characteristic curve of red cell distribution width (RDW) for predicting familial Mediterranean fever. PPV, positive predictive value; NPV, negative predictive value; AUC, Area under the curve.

study the involvement.

In conclusion, we have shown higher RDW levels in the FMF patients compare with the control group. Also, we first reported that the FMF patients with the homozygous M694V mutation had higher RDW values compared with those the control subjects. We suggest that RDW may show subclinical inflammation in FMF patients, and hence RDW may be a promising marker in predicting the homozygous M694V mutation in FMF patients.

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