

# Intima media thickness as an early predictor of atherosclerosis in Egyptian children with familial Mediterranean fever

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**Submitted:** 25 April 2018

**Accepted:** 2 July 2018

Arch Med Sci Atheroscler Dis 2018; 3, e106–e111

DOI: <https://doi.org/10.5114/amsad.2018.77545>

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## Abstract

**Introduction:** Familial Mediterranean fever (FMF) is an autosomal recessive disease. It is characterized by recurrent crises of fever and serosal inflammation. Although FMF patients are symptom free in between attacks, subclinical inflammation continues during the attack-free period. Such patients with inflammatory status have an increased risk of atherosclerotic cardiovascular complications. We attempted to elucidate the role of arterial wall thickening as a predictor of early atherosclerosis in children affected by FMF and to clarify the links between carotid intima media thickness and the markers of subclinical inflammation serum amyloid A (SAA), erythrocyte sedimentation rate (ESR), neutrophil-to-lymphocyte ratio (NLR) and platelet lymphocyte ratio (PLR).

**Material and methods:** It is a case control study. The study comprised 45 Egyptian children diagnosed with FMF and 45 healthy children of matched age and sex who served as controls, without family history or clinical manifestations suggestive of FMF. Laboratory investigations included complete blood count, NLR, PLR, ESR, C-reactive protein and lipid profile. Serum amyloid A levels were determined in both groups using enzyme linked immunosorbent assay. Assessment of the common carotid artery intima media thickness (CIMT) in the FMF patients was carried out.

**Results:** The level of SAA was significantly higher in patients than the control subjects with a mean value of 38.30 ng/ml and 23.43 ng/ml respectively ( $p < 0.001$ ). Our patients showed significantly higher PLR when compared to controls ( $p < 0.001$ ). The mean right and left carotid intima media thickness in patient and control groups showed a highly significant difference ( $p = 0.005$  and  $0.036$  respectively).

**Conclusions:** The mean carotid intima media thickness is higher in cases than the control group. Hence carotid intima media thickness may be used as a tool in the prediction of any atherosclerotic burden in those children.

**Key words:** familial Mediterranean fever, intima media thickness, atherosclerosis.

## Introduction

Familial Mediterranean fever (FMF) is an autoinflammatory disease that is characterized by recurrent attacks of fever and inflammation in serous

membranes, joints and skin [1]. Inflammation plays a key role in the pathogenesis of atherosclerosis, which starts by causing endothelial dysfunction, then progresses to cause a change in artery morphology [2]. Platelet activation is a common finding in inflammatory, thrombotic and atherogenic pathways. Activated platelets react with neutrophils and endothelial cells, through the production of P selectin and other pro-inflammatory compounds; this process enhances the role of immune cells in vascular inflammation and atherosclerosis [3].

Carotid intima media thickness (CIMT) is a non-invasive ultrasound screening test for detection of early atherosclerosis. Several studies have found a correlation between it and the risk of cardiovascular attacks [4].

White blood cell count is considered an important inflammatory marker especially in cardiovascular diseases. Platelet-to-lymphocyte ratio (PLR) is associated with chronic inflammation in cardiovascular and autoimmune diseases [5]. This study aims to evaluate arterial wall thickening as a potential marker for early atherosclerosis in FMF patients to correlate CIMT with markers of sub-clinical inflammation in FMF patients such as serum amyloid A (SAA), erythrocyte sedimentation rate (ESR) and PLR.

## Material and methods

### Study design and sample collection

This was a case control study carried out from January 2016 to May 2016 in which the patient group was represented by 45 Egyptian children diagnosed as FMF cases according to the new FMF criteria [6].

All patients were recruited from and followed up at the pediatric rheumatology clinic, Abou El Rish Pediatric Hospital. All participating patients were older than 7 years old and in an attack-free period.

Forty-five age- and sex-matched healthy children without family history or clinical manifestations suggestive of FMF served as a control group. Exclusion criteria for controls included any concomitant autoimmune disorder, associated congenital heart disease, renal affection, acute infection, or familial hypercholesterolemia.

### Methodology

Blood samples from all participants were withdrawn under complete aseptic conditions. Laboratory investigations for all participants at the time of study including complete blood picture by CELL-DYN, neutrophil to lymphocyte ratio (NLR) and PLR were performed, ESR and C-reactive protein were measured by Nephstar, lipid profile including low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides and cholesterol was done by Erba XL-300 and urine analysis for

proteinuria quantitative determination of serum amyloid A (SAA) levels was assayed using EIAab ELISA Kit, Co., Ltd, Cat E08885h.

Measurement of the CIMT in the FMF patients was performed by grey scale ultrasonography using GELOGIQ P6 ultrasound system with a 7.5–10 MHz linear-array transducer.

### CIMT measurement

A single experienced vascular sonographer, who was blind to the clinical and laboratory data of the study subjects, performed all imaging studies. The images were obtained using a General Electric medical ultrasonographic machine (model: GE LOGIQ P6) equipped with a 7.5–10 MHz linear-array transducer. Imaging of the carotid arteries was performed at the radiology department at Abo el Rish Cairo University by a specialized pediatrics hospital, the subject resting in the supine position with his/her neck extended, and the head turned 45° toward the contralateral side. A transverse section of the common carotid artery was imaged first to screen for any atheromatous plaques and then a longitudinal section at the middle third of the common carotid artery was imaged to achieve a consistent site of measurement. Generally, images are recorded in the plane where the maximal CIMT can be visualized. Magnification of the vessel wall allows easy identification of the intima-medial complex, defined by the border between the echolucent vessel lumen and the echogenic intima and the border between the echolucent media and echogenic adventitia.

### Statistical analysis

Data were coded and entered using the statistical package SPSS (Statistical Package for the Social Sciences) version 23 for Windows (SPSS 23, Inc., Chicago, IL, USA). Data were summarized using mean, standard deviation, median, minimum and maximum for quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. Comparisons between quantitative variables were done using the non-parametric Kruskal-Wallis and Mann-Whitney tests. For comparing categorical data, chi square ( $\chi^2$ ) test was performed. The exact test was used instead when the expected frequency was less than 5. Correlations between quantitative variables were done using Spearman correlation coefficient. *P*-values less than 0.05 were considered as statistically significant.

## Results

### Demographic data

All demographic data of patients are summarized in the Table I.

### Laboratory data

Platelets to lymphocytic ratio (PLR) mean was found higher in patients group than control group with a highly significant difference ( $p < 0.001$ ) (Table II).

Neutrophils to lymphocytic ratio (NLR) mean was found roughly the same between the cases and the control with no significant difference ( $p = 0.765$ ) (Table II).

Erythrocyte sedimentation rate (ESR) was found to be elevated in patients ( $p < 0.001$ ).

C-reactive protein (CRP) was elevated in patients with a significant difference ( $p < 0.001$ ). Also the mean of both ESR and CRP were higher in patients than controls with a highly significant difference ( $p < 0.001$ ) (Table II).

Serum amyloid A level was found higher in the patients group than the control group with a highly significant difference ( $p < 0.001$ ) (Table II).

The lipid profile was done for both groups showing higher levels of cholesterol and LDL in patients ( $p < 0.001$ ) (Table III).

### Radiological data

Regarding the carotid intima media thickness, the mean of the right and left carotid intima media thickness higher in patient than the control group with a highly significant difference  $p$ -value are 0.005 and 0.036 respectively (Table IV).

Carotid intima media thickness correlation with inflammatory markers as ESR, CRP and SAA but there was no statistical significance ( $p > 0.005$ ) (Table V).

Correlation between mean CIMT, Lipid profile, NLR and PLR showed no statistical significance ( $p > 0.05$ ) (Table VI).

### Discussion

Atherosclerosis and cardiovascular diseases are important causes of morbidity and mortality in FMF patients [7]. Systemic inflammation leads to endothelial dysfunction, which causes oxidative stress, vascular damage and finally atherosclerosis [8, 9].

Carotid intima media thickness is a safe, simple, and inexpensive method for evaluating car-

**Table I.** Demographic data of the studied FMF cases

Parameter	Cases				
	Mean	Standard deviation	Median	Minimum	Maximum
Age of first symptoms	5.82	2.52	6.00	1.00	10.00
Delay in diagnosis [years]	3.21	1.62	3.00	0.00	7.00
Disease duration [years]	5.97	1.50	5.00	5.00	10.00
	Count		%		
First degree consanguinity	14		31.1		
Family history of FMF	13		28.9		
History of appendectomy	4		8.9		

**Table II.** Analysis of the inflammatory markers in the studied FMF patients and the controls

Variable	Cases					Control					P-value
	Mean	SD	Median	Minimum	Maximum	Mean	SD	Median	Minimum	Maximum	
Neutrophil to lymphocyte ratio (NLR)	1.12	0.57	1.00	0.30	2.70	1.18	0.85	1.00	0.20	4.30	0.765
Platelet to lymphocyte ratio (PLR)	118.89	39.41	115.00	58.80	208.90	84.33	34.00	76.70	28.50	195.00	< 0.001*
ESR value	15.51	17.84	11.00	0.00	108.00	7.40	2.66	7.00	4.00	15.00	< 0.001*
CRP value	9.48	34.43	0.00	0.00	170.00	0.00	0.00	0.00	0.00	0.00	< 0.001*
Serum amyloid A [ng/ml]	38.30	21.51	34.70	11.60	114.80	23.43	8.02	23.40	6.50	38.10	< 0.001*

**Table III.** Lipid profile of patients and controls

Parameter	Cases					Control					P-value
	Mean	SD	Median	Minimum	Maximum	Mean	SD	Median	Minimum	Maximum	
LDL level [mg/dl]	107.49	32.30	107.00	54.00	219.00	82.96	22.43	83.00	42.00	141.00	< 0.001*
HDL level [mg/dl]	38.16	13.93	38.00	18.00	79.00	42.44	9.26	41.00	22.00	63.00	0.049*
Triglycerides [mg/dl]	80.04	31.25	73.00	43.00	162.00	75.44	15.02	75.00	55.00	116.00	0.599
Cholesterol level [mg/dl]	162.04	31.21	162.00	100.00	271.00	140.71	21.23	140.00	101.00	190.00	< 0.001*

**Table IV.** Comparison between FMF patients and controls as regards right and left CIMT

Variable	Cases					Control					P-value
	Mean	SD	Median	Minimum	Maximum	Mean	SD	Median	Minimum	Maximum	
Right carotid intima media thickness	0.05	0.01	0.05	0.03	0.06	0.04	0.01	0.04	0.03	0.06	0.005*
Left carotid intima media thickness	0.05	0.01	0.05	0.02	0.07	0.04	0.01	0.04	0.03	0.06	0.036*

**Table V.** Correlation between mean CIMT, ESR, CRP and SAA

Variable	Mean CIMT					P-value
	Mean	Standard deviation	Median	Minimum	Maximum	
ESR	0.05	0.00	0.05	0.04	0.06	0.934
CRP [mg/dl]	0.05	0.01	0.05	0.04	0.06	0.207
<b>Serum amyloid A [ng/ml]</b>						
Mean CIMT	<i>R</i>				0.021	
	<i>P</i> -value				0.890	

**Table VI.** Correlation between mean CIMT, lipid profile, NLR and PLR

Parameter	Mean CIMT
LDL level [mg/dl]	Correlation coefficient
	0.223
	<i>P</i> -value
	0.141
HDL level [mg/dl]	Correlation coefficient
	-0.272
	<i>P</i> -value
	0.071
Triglycerides [mg/dl]	Correlation coefficient
	-0.029
	<i>P</i> -value
	0.848
Cholesterol level [mg/dl]	Correlation coefficient
	0.109
	<i>P</i> -value
	0.474
Neutrophil to lymphocyte ratio (NLR)	Correlation coefficient
	0.180
	<i>P</i> -value
	0.236
Platelet to lymphocyte ratio (PLR)	Correlation coefficient
	0.228
	<i>P</i> -value
	0.133

diovascular risk by measuring the combined thickness of the intima and medial layers of the arterial wall [10].

A study was done by Duşunsel *et al.* [11], who reported that 30.4% of their patients had consanguineous parents and 26.5% of patients had a positive family history of FMF. Another Turkish study by Kilic *et al.* [12] displayed a positive family history in 44.6% of the studied patients. An Iranian study presented by Salehzadeh *et al.* [13] reported that 20% of their patients had positive family history of FMF, while parental consanguinity was present in 50% of the patients. We revealed positive family history of FMF in 28.9% of our studied cases and 14 out of the 45 cases showed first degree consanguinity.

In our study, we tried to focus on carotid intima media thickness (CIMT) as a marker of early atherosclerosis in Egyptian children with FMF. CIMT is considered a reflection of early arterial wall morphological changes caused by many risk factors over a period of time and it is considered a noninvasive marker of premature atherosclerosis [14].

The mean of the right and left CIMT in our FMF patients was higher than controls with a significant difference ( $p$  values are 0.005 and 0.036 respectively).

Our results matched the study offered by Peru *et al.*, [14] where the mean of the CIMT in patients versus controls was  $0.038 \pm 0.007$  in patients vs.  $0.032 \pm 0.004$  in controls, as well as another study done by Bilginer *et al.* [15], where the mean in patients was higher than controls (0.03 and 0.02) respectively.

Other studies that were done in adults with FMF matched our results, such as the study done by Gürbüz *et al.* [16]. Another study that was conducted by Akdogan *et al.* [17] showed that the mean CIMT in patients was higher than the mean in the controls  $0.06 \pm 0.07$  and  $0.05 \pm 0.07$  respectively. Another Turkish study by Ugurlu *et al.* [18] showed similar results where the mean CIMT was  $0.05 \pm 0.01$  in patients and  $0.04 \pm 0.01$  in the controls. This agreement among different studies suggests the increased risk of atherosclerotic burden in FMF patients, which needs to be monitored closely.

On the other hand, a study carried out by Sari *et al.* [19] showed that there was no difference between the mean CIMT in both FMF patients and the control group. Regarding the correlation between CIMT and the lipid profile, our results showed no correlation between CIMT and lipid profile. Our results matched the study done by Peru *et al.* [14] and Sari *et al.* [19]. This finding might reflect the ability of CIMT to be an early predictor of atherosclerotic risk.

Our results go hand in hand with other studies that tried to link CIMT with lipid profile in diseases other than FMF, like the Egyptian study done by Kandil *et al.* [20] on children with obesity that also failed to find a correlation between CIMT and lipid profile. On the other hand, a study done by Ugurlu *et al.* [18] encompassing adult FMF patients revealed positive correlation between CIMT and total cholesterol level and LDL. Again, the difference may be due to different numbers of patients and different study methodologies. The study done by Abd el dayem *et al.* [10], which measured CIMT in type 1 DM in adolescents, found a positive correlation between CIMT and cholesterol level and triglycerides.

In our study there was no significant correlation between CIMT and SAA. This result matched the studies done by Peru *et al.* [14] and Ugurlu *et al.* [18]. In contrast a study by Bilginer *et al.* [15] revealed a significant correlation between CIMT and SAA. This may be because of different study methodologies.

No significant correlation was found between CIMT and CRP. Our results matched the studies done by Peru *et al.* [14], Ugurlu *et al.* [18], and Bilginer *et al.* [15]. This might be due to the fact that CIMT needs more time to be affected by subclinical inflammation than the subclinical markers.

No significant correlation was found between CIMT and ESR. Our results matched the study done by Ugurlu *et al.* [18]. On the other hand the Turkish study reported by Bilginer *et al.* [15] showed a significant correlation between CIMT and ESR. This may be due to different ethnic groups and the degree of the disease severity.

Recent studies implicated the role of platelets in triggering inflammation of arterial wall [21]. Hence PLR for both patient and control groups were calculated showing a mean of 118.89 and 84.33 respectively with a statistically significant  $p < 0.001$ ; these results matched the study by Ozer *et al.* [22].

Our study displayed a higher risk of atherosclerosis in Egyptian children with FMF than their normal counterparts. CIMT can be considered a useful noninvasive tool to detect early arterial morphological changes in FMF patients for appropriate cardiovascular care settings and follow-up.

## Acknowledgments

We would like to extend our gratitude to all the contributing authors for their sincere efforts.

The study has been approved by the Ethical Committee of the National Research Centre, which is in accordance with the ethical standards of the Declaration of Helsinki and its later amendments. In addition, written informed consent was obtained from parents of each participant prior to their inclusion in the study.

## Conflict of interest

The authors declare no conflict of interest.

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