*Cardiology in the Young* (2015), **25**, 1276–1280
doi:10.1017/S1047951114002327


*Original Article*

# Plasma gelsolin as a biomarker of acute rheumatic carditis

Mustafa Argun,[1] Ali Baykan,[1] Figen Narin,[2] Abdullah Özyurt,[1] Özge Pamukçu,[1] Ferhan Elmalı,[3] Kazım Üzüm,[1] Nazmi Narin[1]

*Department of Pediatrics, Division of Pediatric Cardiology; [2]Department of Medical Biochemistry; [3]Department of Biostatistics and Medical Informatics, Erciyes University Medical Faculty, Kayseri, Turkey*

**Abstract** *Background:* Acute rheumatic fever is an autoimmune, inflammatory, and multi-systemic disease secondary to pharyngitis and is caused by group A streptococcus. In developing countries, acute rheumatic fever is the most common cause of acquired heart disease. Gelsolin is a calcium-dependent, multi-functional actin-regulatory protein circulating in the plasma of healthy human beings. The correlation between blood gelsolin levels and inflammatory conditions suggests the potential benefit of gelsolin as a prognostic marker. The aim of the present study was to appraise the association of gelsolin and acute rheumatic carditis in childhood. *Materials and Methods:* Plasma gelsolin levels were measured and echocardiographic examinations were performed in patients (n = 37) with acute rheumatic carditis and compared with those of age- and gender-matched healthy controls (n = 24). *Results:* The plasma gelsolin levels in children with acute rheumatic carditis were significantly lower compared with controls (197 ± 218 versus 322 ± 255 mg/L, p = 0.039). There was a significant correlation among gelsolin levels and the grade of mitral regurgitation (p = 0.030), left ventricular end-diastolic diameter (p = 0.017), and left ventricular end-systolic diameter (p = 0.028) at diagnosis. *Conclusions:* Levels of the gelsolin plasma isoform were decreased in patients with acute rheumatic carditis compared with healthy controls. Gelsolin may be used as a biochemical marker for acute rheumatic carditis.

Keywords: Acute rheumatic carditis; gelsolin; biomarker; children

Received: 29 June 2014; Accepted: 15 October 2014; First published online: 18 November 2014

Acute rheumatic fever is a non-suppurative, autoimmune, multi-systemic response to group A streptococcal pharyngitis. Its subsequent complication, rheumatic heart disease, is an important public health problem and the most common cause of acquired heart disease in developing countries.[1] Although acute rheumatic cardiac involvement presents in the form of pancarditis, valve insufficiency related to endocarditis is the most prominent clinical manifestation. Echocardiography is the gold standard method for demonstrating the grade of valve insufficiency and enlargements in the end-systolic/end-diastolic diameters of the left ventricle and, consequently, the severity of carditis. Echocardiography should be performed by experienced specialists; however, it is expensive, is not in common use, and, as a consequence, it cannot be performed in all patients diagnosed with acute rheumatic fever.[2]

As a calcium-dependent, actin-regulatory, 82–84 kDa protein, gelsolin is essential for cell locomotion and phagocytosis and was first identified in the cytoplasm.[3,4] Plasma gelsolin, the extracellular gelsolin isoform, is a circulating protein in the blood of healthy individuals and is a part of the "actin-scavenging" system.[5] The gelsolin-binding ligand, actin, is an extremely conserved cytoskeleton protein that is involved in cell motility and in maintenance of cell shape, and is ordinarily separated from the extracellular environment by intact plasma membranes. The deterioration of the cell membrane causes the release of intracellular proteins into the systemic circulation. Actin filaments in plasma increase the

Correspondence to: M. Argun, MD, Department of Pediatrics, Erciyes University Medical Faculty, 38039 Kayseri, Turkey. Tel: +90 352 207 6666; Fax: +90 352 437 5825; E-mail: dr.margun@hotmail

blood viscosity, obstruct small blood vessels, enhance the severity of bacterial infection, and has also been shown to be toxic to endothelial cells in culture. The integrative explanation for low plasma gelsolin levels under an acute inflammatory condition is the exposure of actin to plasma because of injury. Plasma gelsolin is primarily related to the fast severing and removal of actin filaments released from dead cells into the blood stream.[6,7] The main function of plasma gelsolin is to protect seriously ill patients from their own inflammatory response. Reduced plasma gelsolin levels have been observed in severe trauma, critical illness, sepsis, chronic haemodialysis, myocardial infarction, and in many inflammatory diseases, such as systemic lupus erythematosus, rheumatoid arthritis, and multiple sclerosis.[3–7]

The aim of this study was to determine whether plasma gelsolin depletion was associated with acute rheumatic carditis. In addition, we investigated whether there were correlations among the reduction in plasma gelsolin level and serum inflammatory markers, the grade of valvular insufficiency detected on echocardiography, and enlargement in the left ventricular end-diastolic and end-systolic diameters – namely, the severity of carditis.

## Materials and methods

This prospective study was carried out at Erciyes University Medical Faculty between January, 2012 and December, 2012 with the approval of the Ethics Committee. A total of 37 patients diagnosed with acute rheumatic carditis were included in the patient group and 24 healthy children were included in the control group.

All patients were examined by a paediatric cardiologist. The diagnosis of acute rheumatic fever was made according to modified Jones criteria, revised in 1992.[8] The major criteria include carditis, polyarthritis, Sydenham's chorea, erythema marginatum, and subcutaneous nodules. The minor criteria include arthralgia, fever, elevated acute-phase reactants such as erythrocyte sedimentation rate, C-reactive protein, and a prolonged PR interval on the electrocardiogram. The diagnosis of acute rheumatic fever was based on the presence of two major or one major and two minor criteria in the patient. Echocardiography was performed by the same paediatric cardiologist. The World Health Organization's echocardiographic criteria for carditis include length of colour jet >1 cm, colour jet identified in at least two planes, mosaic colour jet with a peak rate >2.5 m/s, and presence of a signal – holodiastolic for aortic regurgitation or holosystolic for mitral regurgitation.[9]

Patients with any chronic disease, using medications, with associated infections, and renal and hepatic dysfunction were excluded from the study. In the patient group, antistreptolysin O, erythrocyte sedimentation rate, C-reactive protein, white blood cell count, haemoglobin level, platelet counts, mean platelet volume, platelet distribution width, creatine kinase-MB, troponin I, pro-brain natriuretic peptide levels, and plasma gelsolin levels were measured and echocardiography was performed at the time of initial diagnosis in the patient group. In the control group, the plasma gelsolin level was determined and echocardiography was performed.

Venous blood samples of the healthy individuals and patients were drawn on admission. The blood samples were immediately placed in sterile etilen diamin tetra asetik asit test tubes and centrifuged at 2000–3000 rpm for 20 minutes at 4°C to collect plasma. Plasma was stored at – 70°C until assayed. The concentration of gelsolin in plasma was analysed by enzyme-linked immunosorbent assay using commercial kits (Hangzhou Eastbiopharm Co., Ltd, Hangzhou, Zhejiang, China) in accordance with the manufacturer's instructions.

Secondary prophylaxis with benzathine penicillin G with a 3-week interval was started in all patients after establishing the diagnosis of acute rheumatic carditis. Oral steroids were given to the patients with moderate-to-severe carditis at a dose of 2 mg/kg daily (maximum 60 mg daily). Although the dosage of the steroid was gradually decreased 2 to 4 weeks later, oral aspirin was included in the therapy regimen at a dose of 80–100 mg/kg daily. Oral aspirin was given alone at a dose of 80–100 mg/kg daily to patients with mild carditis. Anti-congestive therapy was started in patients revealing signs of heart failure.

## Results

### *Study population characteristics*

The study included 37 children with rheumatic carditis, comprising 20 girls and 17 boys, and 24 healthy control children, comprising 16 girls and 8 boys. The ages of children ranged from 5 to 15 years (mean $11 \pm 2.37$ years) in the study group and from 3.5 to 18 years (mean $11.8 \pm 4.36$ years) in the control group. There were no statistically significant differences between the study and control groups regarding age ($p = 0.40$) and gender ($p = 0.42$) distribution. At diagnosis, 29 patients had carditis and arthritis, and eight patients had carditis and chorea.

Although mitral regurgitation was detected in 36 out of 37 patients (97%), three patients had grade 3 mitral insufficiency, 19 patients had grade 2 mitral insufficiency, 12 patients had grade 1 mitral insufficiency, and two patients had very mild mitral regurgitation. Although aortic insufficiency was detected in 17 out of 37 patients (45%), one patient had grade 2 aortic insufficiency, 10 patients had

https://doi.org/10.1017/S1047951114002327 Published online by Cambridge University Press

Table 1. Comparison of echocardiographic values in patients and control patients (z scores).

| Variables | Patient (n = 37) | Control (n = 24) | p value |
|---|---|---|---|
| Left ventricular end-diastolic diameter | 0.71 ± 1.24 | – 0.21 ± 1.09 | 0.010 |
| Left ventricular end-systolic diameter | 0.30 ± 1.03 | – 0.19 ± 1.00 | 0.100 |

grade 1 aortic insufficiency, and six patients had very mild aortic insufficiency. Of 37 patients, 16 had both aortic insufficiency and mitral insufficiency. Among the echocardiographic parameters, although a statistically significant difference was detected between the left ventricular end-diastolic diameters of the patient and control group, no statistically significant difference was detected between the left ventricular end-systolic diameters of the patient and control group (Table 1).

### *Change of plasma gelsolin level in acute rheumatic carditis at diagnosis*

The plasma gelsolin levels of the patients with rheumatic carditis were significantly lower than those of control patients ($p < 0.05$). The mean plasma gelsolin levels were found to be 197.27 ± 218.51 mg/l for the patient group and 322.0 ± 255.46 mg/l for the control group. There was no statistically significant difference between the patients who had carditis and arthritis (201.93 ± 220.49 mg/l) and patients who had carditis and chorea (180.37 ± 225.09 mg/l) in terms of gelsolin level at diagnosis ($p = 0.10$).

### *Correlation between plasma gelsolin level and echocardiographic parameters*

Negative correlations were detected among the plasma gelsolin level and the severity of mitral insufficiency ($\rho = -0.290$; $p = 0.030$), left ventricular end-diastolic diameter z score ($\rho = -0.333$; $p = 0.017$), and left ventricular end-systolic diameter z score ($\rho = -0.307$; $p = 0.028$) (Fig 1). No correlation was detected, however, between the plasma gelsolin level and severity of aortic insufficiency ($p > 0.05$).

### *Changes in inflammatory markers and haematologic parameters in acute rheumatic carditis at diagnosis*

Antistreptolysin O, erythrocyte sedimentation rate, C-reactive protein, white blood cell count, haemoglobin count, platelet count, mean platelet volume, platelet distribution width, creatine kinase-MB, Troponin I, and pro-brain natriuretic peptide tests were measured in the patient group (Table 2). As evidence of prior group A streptococcus infection, the elevated antistreptolysin O titer was 100%.

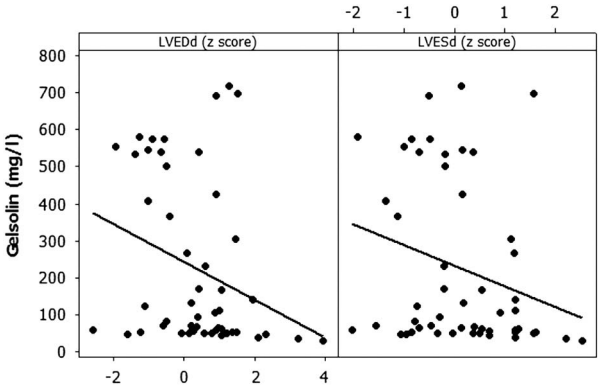


**Figure 1.**
*Gelsolin and left ventricular end-diastolic diameter z score (LVEDd) ($\rho = -0.333$; $p = 0.017$) and gelsolin and left ventricular end-systolic diameter z score (LVEDs) ($\rho = -0,307$; $p = 0.028$); negative correlation in acute rheumatic carditis.*

### *Correlation among inflammatory markers, haematologic parameters, and echocardiographic parameters*

A significant positive correlation was found between the pro-brain natriuretic peptide level and grade of mitral insufficiency ($\rho = 0.456$; $p = 0.019$). No correlations were detected, however, among the pro-brain natriuretic peptide level and severity of aortic insufficiency, left ventricular end-diastolic diameter z score, and left ventricular end-systolic diameter z score ($p > 0.05$). On the other hand, although there was a negative correlation between the plasma gelsolin and pro-brain natriuretic peptide level, it was not statistically significant ($\rho = -0.062$; $p = 0.763$). A significant negative correlation was found between white blood cell count and the left ventricular end-diastolic diameter z score ($\rho = -0.479$; $p = 0.006$). No significant correlation was found among the other parameters in question ($p > 0.05$).

### *Statistical analysis*

Data were analysed using the IBM SPPS Statistics 21 SigmaStat 3.5 package program. Normal distribution of the data was analysed by the Shapiro–Wilk test. The independent samples t-test was used for comparing normally distributed variables between two groups and the Mann–Whitney U test was used for analysing non-normally distributed variables. The Kruskal–Wallis test was used in the comparisons

https://doi.org/10.1017/S1047951114002327 Published online by Cambridge University Press

Table 2. Laboratory characteristics of patients with rheumatic carditis at diagnosis.

| Variables | Patients (n = 37) |
|---|---|
| White blood cell (count/mm$^3$) | 9011 ± 2686 |
| Haemoglobin (g/dl) | 12.0 ± 1.1 |
| Platelet (count/mm$^3$) | 371,916 ± 109,623 |
| Mean platelet volume (fl) | 8.45 ± 0.88 |
| Platelet distribution width (%) | 40.95 ± 6.83 |
| Erythrocyte sedimentation rate (mm/hour) | 58.0 ± 29.8 |
| C-reactive protein (mg/dl) | 35.35 ± 35.3 |
| Antistreptolysin O (IU/ml) | 1194 ± 1055 |
| Creatine kinase-MB (U/L) | 17.95 ± 14.41 |
| Troponin I (ng/ml) | 0.038 ± 0.083 |
| Pro-brain natriuretic peptide | 398.6 ± 557.6 |

Data are presented as mean and standard deviation

among three groups and the Dunn test was used as a multiple comparison test (post-hoc test). The $\chi^2$ test and Fisher's exact test were used for the comparison between categorical variables. Spearman's correlation analysis was used for analysing the correlation among the numerical variables. A p value $<0.05$ was accepted as statistically significant.

## Discussion

As far as we know, this is the first study on plasma gelsolin levels in patients with acute rheumatic carditis. In this study, the plasma gelsolin levels in patients with acute rheumatic carditis were found to be low at diagnosis. In addition, strong negative correlations were found among the low plasma gelsolin levels and the grade of mitral insufficiency, left ventricular end-diastolic, and end-systolic diameters.

Acute rheumatic fever is an autoimmune disease that develops secondary to abnormal immune response against group A streptococcal infections as a result of genetic predisposition.[1] Acute rheumatic fever is a major public health problem in developing countries. Carditis is the most serious manifestation of acute rheumatic fever and may result in rheumatic heart disease. The incidence of carditis in acute rheumatic fever has been reported in the range of 30–45% in previous studies.[10]

Despite the fact that the development of the disease has been largely explained in studies on the aetiopathogenesis of acute rheumatic fever, it is obvious that numerous future studies are required when we consider the complexity of immune mechanisms and the point at which science stands today. These studies will provide new information about the aetiopathogenesis of the disease while mediating the development of more powerful and specific therapies against the immune response, which is difficult to prevent with the current therapies, and, consequently, against the process leading to rheumatic heart disease.

As far as is known, the abnormal immune response in rheumatic carditis includes cellular and humoral mechanisms. Inflammation, cellular inflammation, and lesions specific to rheumatic heart disease develop as a result of a process involving numerous cytokines.[11–13]

Plasma gelsolin is an important member of the extracellular actin-scavenger system, which is capable of severing and scavenging circulating actin microfilaments. Actin is a cytoskeleton protein responsible for maintaining the shape and motility of the cell, and it is released into circulation as a result of cell death. Circulating actin microfilaments change blood flow characteristics by increasing blood viscosity, and they may even cause the obstruction of small blood vessels.[14] Actin may give rise to an increase in certain major components of proinflammatory cytokine products.[4] The continual presence of actin microfilaments in the circulatory system may result in a condition similar to multiple organ dysfunction syndrome.[7] Its potential toxicity to endothelial cells has also been demonstrated in cell cultures.[15]

Until now, reduced gelsolin levels have been reported in acute respiratory distress syndrome, acute lung damage, acute liver damage, surgery, sepsis, trauma, and septic shock.[7] Lee et al[5] reported that plasma gelsolin was a potential prognostic biomarker for critically ill surgical patients. Cohen et al[16] obtained evidence indicating that exogenous gelsolin might reduce morbidity from sepsis in a rat model. Furthermore, a reduction in the circulating gelsolin level was reported in rheumatoid arthritis, which is a chronic inflammatory disease.[17] Hu et al[18] reported reduced plasma gelsolin levels and a negative correlation between disease activity and plasma gelsolin level in patients with rheumatoid arthritis or systemic lupus erythematosus. The main function of plasma gelsolin is to protect the host from the severe disease related to the inappropriate inflammatory response of the host against itself. The reduced gelsolin level in acute rheumatic carditis may be explained as the use of gelsolin by the

https://doi.org/10.1017/S1047951114002327 Published online by Cambridge University Press

body as a protective mechanism against the inflammatory process. Although rheumatic carditis presents in the form of pancarditis, endocarditis is the essential lesion that causes most of the clinical manifestations and is mainly responsible for morbidity.

Previous studies have revealed that brain natriuretic peptide levels are increased in rheumatic heart diseases because of atrial dilatation. Esen et al[2] pro-brain natriuretic peptide levels were shown to be positively correlated with left atrium diameters. Similarly in the present study, significant positive correlation was found between the pro-brain natriuretic peptide level and the grade of mitral insufficiency.

The limitations of the study are the measuring of plasma gelsolin level only at diagnosis and the relatively small sample size.

## Conclusion

This study revealed evidence that the plasma gelsolin level might be used as a biomarker for demonstrating the severity of disease in patients with acute rheumatic carditis. Data obtained from the serial measurements of plasma gelsolin levels in the course of the disease may provide a better understanding of the relationship between acute rheumatic carditis and gelsolin. In addition, further studies involving acute rheumatic fever patients without carditis would provide a better understanding of the pathogenesis and allow the development of new treatment modalities.

## Acknowledgements

All authors contributed extensively and equally to this work.

## Financial Support

This research received no specific grant from any funding agency, commercial or not-for-profit sectors.

## Conflicts of Interest

None.

## Ethical Standards

The ethical aspects were respected and the research was approved by the Committee of Ethics and Research of Erciyes University.

## References

1. Marijon E, Mirabel M, Celermajer DS, Jouven X. Rheumatic heart disease. Lancet 2012; 379: 953–964.
2. Esen F, Argun M, Pamukçu O, et al. Importance of N-terminal pro-brain natriuretic peptide in monitoring acute rheumatic carditis. Cardiol Young 2013; 28: 1–5.
3. Bucki R, Byfield FJ, Kulakowska A, et al. Extracellular gelsolin binds lipoteichoic acid and modulates cellular response to proinflammatory bacterial wall component. J Immunol 2008; 181: 4936–4944.
4. Wang H, Cheng B, Chen Q, et al. Time course of plasma gelsolin concentrations during severe sepsis in critically ill surgical patients. Crit Care 2008; 12: 1–6.
5. Lee PS, Drager LR, Stossel TP, Moore FD, Rogers SO. Relationship of plasma gelsolin levels to outcomes in critically ill surgical patients. Ann Surg 2006; 243: 399–403.
6. DiNubile MJ. Plasma gelsolin as a biomarker of inflammation. Arthritis Res Ther 2008; 10: 124.
7. Peddada N, Sagar A, Ashish GR, et al. Plasma gelsolin: a general prognostic marker of health. Med Hypotheses 2012; 78: 203–210.
8. Dajani AS, Ayoub E, Bierman FZ, et al. Guidelines for the diagnosis of rheumatic fever: Jones criteria, 1992 update. Special writing group of the committee on rheumatic fever, endocarditis, and Kawasaki disease of the council on cardiovascular disease in the young of the American Heart Association. JAMA 1992; 268: 2069–2073.
9. WHO. Rheumatic fever and rheumatic heart disease. World Health Organ Tech Rep Ser 2004; 923: 1–122.
10. Lee JF, Naguwa SM, Cheema GS, Gershwin ME. Acute rheumatic fever and its consequences: a persistent threat to developing nations in the 21st century. Autoimmun Rev 2009; 9: 117–123.
11. Guilherma L, Kalil J. Rheumatic fever and rheumatic heart disease: cellular mechanisms leading autoimmune reactivity and disease. J Clin Immunol 2010; 30: 17–23.
12. Narin N, Kütükçüler N, Ozyürek R, Bakiler AR, Parlar A, Arcasoy M. Lymphocyte subsets and plasma IL-1 alpha, IL-2, and TNF-alpha concentrations in acute rheumatic fever and chronic rheumatic heart disease. Clin Immunol Immunopathol 1995; 77: 172–176.
13. Kütükçüler N, Narin N. Plasma interleukin-7 (IL-7) and IL-8 concentrations in acute rheumatic fever and chronic rheumatic heart disease. Scand J Rheumatol 1995; 24: 383–385.
14. Haddad JG, Harper KD, Guoth M, Pietra GG, Sanger JW. Angiopathic consequences of saturating the plasma scavenger system for actin. Proc Natl Acad Sci USA 1990; 87: 1381–1385.
15. Erukhimov JA, Tang ZL, Johnson BA, et al. Actin-containing sera from patients with adult respiratory distress syndrome are toxic to sheep pulmonary endothelial cells. Am J Respir Crit Care Med 2000; 162: 288–294.
16. Cohen TS, Bucki R, Byfield FJ, et al. Therapeutic potential of plasma gelsolin administration in a rat model of sepsis. Cytokine 2011; 54: 235–238.
17. Osborn TM, Verdrengh M, Stossel TP, Tarkowski A, Bokarewa M. Decreased levels of the gelsolin plasma isoform in patients with rheumatoid arthritis. Arthritis Res Ther 2008; 10: R117.
18. Hu YL, Li H, Li WH, et al. The value of decreased plasma gelsolin levels in patients with systemic lupus erythematosus and rheumatoid arthritis in diagnosis and disease activity evaluation. Lupus 2013; 22: 1455–1461.