The Relationship Between Plasma Gelsolin Levels and Myeloperoxidase in Patients Undergoing Hemodialysis: A Prospective, Observational, Controlled Study

Hacı Huseyin Tural, Kader Kose, Inayet Gunturk, Ismail Kocyigit, Cevat Yazıcı

1Department of Biochemistry, Erciyes University Faculty of Medicine, Kayseri, Türkiye
2Department of Medical Biochemistry, Erciyes University Institute of Health Sciences, Kayseri, Türkiye
3Department of Midwifery, Nigde Omer Halisdemir University, Faculty of Health Sciences, Nigde, Türkiye
4Division of Nephrology, Department of Internal Medicine, Erciyes University Faculty of Medicine, Kayseri, Türkiye

Objective: Given the association of inflammatory conditions with the development of comorbidities, particularly cardiovascular diseases, it is crucial to monitor inflammation in hemodialysis patients. This study aimed to evaluate plasma gelsolin and myeloperoxidase (MPO) levels before and after dialysis sessions and to assess their relationship with inflammation.

Materials and Methods: The study included 16 healthy volunteers and 30 patients receiving regular hemodialysis treatment. Along with routine biochemical analyses, plasma gelsolin and MPO levels were measured in blood samples taken from the study group before and after the sessions.

Results: Plasma gelsolin levels were found to be statistically higher both before and after dialysis compared to the control group (p=0.000); however, there was no significant change during the session (p=0.094). Conversely, plasma MPO activity, which was significantly higher before dialysis, increased at the end of the session (p=0.000).

Conclusion: It can be concluded that elevated levels of gelsolin are associated with a chronic inflammatory response, as indicated by high-sensitivity C-reactive protein (hs-CRP) and MPO levels. Consequently, gelsolin could be considered a supportive treatment strategy for these patients.

Keywords: Hemodialysis, plasma gelsolin, myeloperoxidase, inflammation, kidney failure.

INTRODUCTION

Chronic kidney disease (CKD) is widely recognized as one of the leading causes of death, affecting more than 10% of the general population, which amounts to over 800 million individuals.¹ The role of inflammation and oxidative stress in the progression of CKD has been particularly demonstrated. Chronic inflammation and the weakening of the antioxidant system increase in direct proportion to the degree of kidney failure in patients with CKD.²
Hemodialysis (HD) therapy, a lifesaving treatment for patients with kidney failure, also induces inflammation. The biocompatibility of dialysis may vary depending on the filter used, blood flow rate, and dialysis fluid. However, even under optimal conditions, providing blood flow outside the body mechanically activates different systems and cells, such as leukocytes, triggering pro-inflammatory and pro-oxidant pathways. Therefore, monitoring the oxidative and inflammatory status in these patients is important.

Myeloperoxidase (MPO), an enzyme mainly stored in neutrophils and responsible for the formation of reactive oxygen species, has been demonstrated to be an independent marker in determining the progression of CKD. In the context of kidney disease, MPO is directly related to oxidative stress and allows the evaluation of inflammation, oxidative stress, and endothelial dysfunction together.

Gelsolin (GSN), a protein secreted from myocytes, plays a role in reshaping the cytoskeletal structure by cutting, closing, and binding actin filaments. While cytoplasmic GSN (cGSN) is involved in the dynamics of actin monomers in the intracellular environment, plasma GSN (pGSN) is actively secreted into the extracellular environment. Studies focusing on the role of pGSN have revealed that it is primarily responsible for the rapid elimination of actin filaments released from circulating dead cells. The release of actin, which constitutes almost 10% of the total cellular proteins in eukaryotic cells, into the systemic circulation in response to necrosis associated with injury or disease, may result in pathological conditions such as platelet aggregation, microvascular thrombosis, release of pro-inflammatory mediators, and fibrinolysis disorder. Additionally, studies on pGSN can be extended to include apoptosis, signal transduction, epigenetic processes, transcriptional regulation, regulation of the inflammatory response, and their involvement in the pathogenesis of diseases. It has also been shown that pGSN can bind to bioactive components such as platelet activating factor and lipopolysaccharides, thus exhibiting protective properties against inflammation.

A growing body of research suggests that pGSN depletion may predict secondary inflammation and tissue damage, and hypogelsolinemia may be an indicator of poor prognosis or complications.

However, studies on HD patients are limited and appear to be worth exploring. Therefore, the current study aimed to evaluate pGSN levels before and after dialysis sessions in HD patients and to investigate the relationship between pGSN and MPO levels.

MATERIALS AND METHODS
This study was supported by the University Scientific Research Projects Commission (Project no: TYL-2019-8691) and approved by the Erciyes University Clinical Research Ethics Committee (Date: 18.07.2018; Decision no: 2018/392).

The study groups consisted of 30 patients who have been followed up and treated at the Erciyes University Faculty of Medicine Organ Transplant and Dialysis Hospital, receiving regular HD treatment for four hours three times a week for at least six months (HD group), and 16 healthy volunteers (Control group). All patients and healthy volunteers who were planned to be included in the study were informed about the study, and informed consent forms were obtained.

HD Group
When selecting patients for the study, those with systemic diseases such as chronic respiratory failure, acute infection, fever, malignant tumors, hepatitis, and those using lipid-lowering drugs were excluded from the study.

During the patients’ dialysis sessions, Althin TINA and Dialog B Braun dialysis machines were used. Dialyzers made of cellulose membrane (Dicea 150 G), dialysis fluids containing bicarbonate (Baxter, Eczacibaşı; Bikardi, Eczacibaşı; Ren-Acet SFY/Ren-Bikar SFY, Ren-Med Medical Products), and heparin as an anticoagulant were employed. The dialysate flow rate was set at 500 mL/minute, and the blood flow rate was adjusted to 250–300 mL/minute. Kt/V and urea reduction rate (URR) values were calculated using the standard formula.

Additionally, all HD patients were taking folic acid, vitamin B complex, and iron supplements.

Control Group
Sixteen healthy volunteers were selected among healthy individuals with routine laboratory results within the reference range, no systemic disease, no medication use in the last month (including vitamins and/or mineral preparations), and non-smokers.

Study Plan
Blood samples were collected twice from the HD group, before and after dialysis, and only once from the healthy volunteers. These samples were taken in both anticoagulant (Ethylenediaminetetraacetic Acid [EDTA]) and non-anticoagulant tubes. A complete blood count [CBC; (Siemens Advia 2120i)] was performed on the same day using whole blood samples from the volunteers. Tubes with and without anticoagulant were centrifuged for 10 minutes at 2000 g at 4 °C within 30 minutes. Some of the serum samples were used for routine measurements on the same day. The residual serum/plasma was stored at -80 °C until the working days.
CBC in whole blood samples obtained from study groups was analyzed, in addition to routine biochemistry analyses [such as blood urea nitrogen (BUN) and creatinine] in serum samples. High sensitivity C-reactive protein (hs-CRP, Dade Behring Nephelometer) measurements were also made.

MPO activity was determined in EDTA plasma samples, and pGSN levels were measured in serum samples.

**Determination of MPO Activity**
Plasma MPO activity was determined using the method described by Bradley et al.\textsuperscript{18} This method is based on the oxidation of o-dianisidine dihydrochloride, known as peroxidase substrate, by MPO in the presence of H\textsubscript{2}O\textsubscript{2}. The intensity of the yellow-orange colored oxidation product, which correlates directly with the enzyme activity in the environment, is monitored as an increase in optical density at a wavelength of 460 nm.

MPO activity measured in plasma samples was reported per liter of plasma (U/L). The coefficient of variation (CV) for this method was found to be 5.0%.

**Determination of Serum pGSN Levels**
Serum pGSN levels were measured using a commercial Enzyme-Linked Immunosorbent Assay (ELISA) kit (Aviscera Bioscience, INC. USA, Catalog No: SK00384-01).

**Statistical Analysis**
The data were evaluated using the “IBM Statistical Package for the Social Sciences (SPSS) Statistics 23.0” software package. The Shapiro-Wilk test was used to determine whether the data were normally distributed. Continuous variables with normal and abnormal distributions were expressed as mean with standard deviation (SD) and median (first-third quartiles), respectively. In the comparison of the study groups according to age and gender distribution, the Mann-Whitney U and Chi-square tests were used, respectively. For comparing quantitative data between groups, Student’s t-test was used for data conforming to normal distribution, and the Mann-Whitney U test was used for non-normally distributed data. In comparing HD session input and output values of patients, paired samples t-test was used for data conforming to normal distribution, and the Wilcoxon test was used for non-normally distributed data. Relationships between numerical variables within the same study group were evaluated using Pearson or Spearman correlation analysis, depending on their suitability for normal distribution. The level of statistical significance was defined as p<0.05. Estimations of effect size were also performed by calculating Cohen’s d. Post-hoc power analysis was performed using the G*Power (3.1.9.7) software with a 5% alpha margin of error (on both sides).

**RESULTS**
When comparing the working groups according to gender distribution, there was no significant difference between the groups (p=0.611). Similarly, in the patient group, there was no statistical difference between males (n=14/46.7%) and females (n=16/53.3%), and in the control group between males (n=8/50%) and females (n=8/50%) in terms of age (p=0.421).

Compared with the control group, serum BUN, creatinine, and hs-CRP levels were found to be significantly elevated in patients prior to HD (p=0.000). At the end of dialysis, there was no statistically significant difference in BUN values between the control and patient groups (p=0.936). However, creatinine and hs-CRP levels were found to be significantly higher in patients compared to the control group (p=0.000) (Table 1).

When comparing values before and after HD, it was observed that BUN and creatinine levels decreased statistically (p=0.000). All patients had Kt/V ≥ 1.2 and URR ≥ 65%. However, it was found that hs-CRP levels remained very high despite dialysis (Table 1).

Compared with the control group, plasma MPO activity before HD was found to be significantly higher in patients (p=0.001). At the end of the dialysis, it was determined that the MPO activity increased beyond the control values (p=0.000). When comparing MPO activities before and after HD, a statistically significant increase was observed in MPO activity after dialysis (p=0.000) (Table 2).
pGSN levels were higher in patients both before and at the end of dialysis compared to the control group (p=0.000). Comparing pGSN levels before and after dialysis in HD patients revealed that although there was a numerical decrease in pGSN levels following dialysis, this decrease was not statistically significant (p=0.094) (Table 2).

When conducting correlation analyses between measured parameters in HD patients, there was no significant correlation between MPO activity before (p=0.393) and after (p=0.263) HD and pGSN values. Apart from the pGSN-MPO relationship, significant positive or negative correlations are presented in Table 3.

Examining the values before HD, a significant positive correlation was observed between MPO and hs-CRP (p=0.009). After HD, a significant negative correlation was observed only between pGSN and hs-CRP values (p=0.004) (Table 3).

**DISCUSSION**

In this study conducted with long-term HD patients, pGSN levels, which did not show a significant difference before and after HD sessions, were found to be higher in patients than in healthy volunteers. Additionally, no significant correlation was found between MPO and pGSN levels in these patients.

MPO is a biomarker of oxidative stress and is associated with the development of cardiovascular disease and estimated Glomerular Filtration Rate (eGFR) decline in CKD. An observational study by the Chronic Renal Insufficiency Cohort (CRIC) showed that serum MPO levels were associated with the risk of disease progression in CKD. In the current study, MPO levels increased in the patient group compared to healthy volunteers and further increased after the HD session. These findings are consistent with those of Himmelfarb et al., who showed that MPO levels were increased in CKD patients receiving HD treatment compared to controls, and increased directly with HD treatment. More recently, Bieber et al. demonstrated that HD-related neutrophil activation, a well-known source of MPO, contributes to endothelial inflammation and damage. In the HD patients of this study, hs-CRP levels, which were already high before treatment, further increased after the HD session, along with MPO. These results support the presence of chronic inflammation in these patients, as well as the pro-inflammatory effect of HD. However, in this study, the fact that the positive correlation between MPO and hs-CRP levels observed before HD was not seen after HD suggests that the acute increase in MPO levels occurs more rapidly. Also, Kitabayashi et al. found that MPO levels increased significantly at the start and end of HD sessions, but there was no significant change in CRP levels. Considering these data, the relatively low synthesis rate of CRP compared to MPO, which is directly secreted by neutrophil degranulation, may be explanatory.

To the best of our knowledge, this is the first study to investigate the correlations between MPO and pGSN in HD patients. However, although both molecules are often shown to be associated with poor disease prognosis and
inflammation, no significant relationship was found between these two parameters in this study. This may be due to the inadequacy of the sample size or because pGSN changes occur more prominently in the acute stages of the disease. A previous study has shown that pGSN levels are lower in patients receiving HD treatment. However, in this study, the patient group consisted of individuals who had just started HD treatment. Therefore, it can be posited that pGSN levels decreased because the patients were in the acute phase of the disease and undergoing HD treatment. On the other hand, in another study with patient population characteristics similar to ours, where patients received HD treatment for an average of 2.4 years, no difference in pGSN levels was found between healthy volunteers and HD patients.

Considering that the accumulation of pGSN at sites of injury and/or clearance with circulating actin are the principal causes of pGSN decrease, especially after acute insults, it is possible that the expected reduction in pGSN could not be observed in a relatively stable group of patients receiving long-term HD therapy.

Furthermore, studies showing that gelsolin levels differ in patients with different degrees of aortic arch calcification and lower values in IgA nephropathy compared to other glomerular nephritis suggest that in different etiologies, the change in pGSN levels could differ. Therefore, the differing etiological characteristics of the patient groups and the methods used may explain the varied results.

In the present study, the presence of a chronic inflammatory condition, supported by high hs-CRP levels, may be the major cause of the elevated pGSN levels observed before and after the HD session. Li et al. explained the paradoxical increase in pGSN levels in cases like Finnish type familial amyloidosis and pediatric multiple sclerosis, suggesting that gelsolin may exhibit either protective or damaging properties depending on the condition. Additionally, they hypothesized that this phenomenon might be related to oxidation/reduction reactions due to the thiol groups in cGSN. In other words, they suggested that increased expression of gelsolin could reduce cell death in response to oxidative stress through its antioxidiant properties.

CONCLUSION

In conclusion, CKD patients receiving regular HD treatment have higher pGSN levels compared to healthy volunteers, a response to chronic inflammation that is also evidenced by high hs-CRP levels before the HD session. Considering this study’s results and previous research showing that pGSN levels are associated with morbidity and mortality, it may be advisable to use gelsolin as a supportive treatment in these patients.

Ethics Committee Approval: The Erciyes University Clinical Research Ethics Committee granted approval for this study (date: 18.07.2018, number: 2018/392).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

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