Evaluation of Oxidative Stress in Primary Glomerulonephritis with Serum Level of Ischemia Modified Albumin (IMA)

Primer Glomerülonefritlerde, Oksidatif Stresin Serum İskemi Modifiye Albümin (İMA) Düzeyi ile Değerlendirilmesi

ABSTRACT

OBJECTIVE: Oxidative stress (OS) is described as the imbalance of oxidative and anti-oxidative systems towards oxidants and plays a role in the pathogenesis of GN. In many studies, ischemia-modified albumin (IMA) is identified as a sign of OS. However, it has not yet been studied in patients with primary GN in the literature. In the present study, we aimed to determine the role of IMA in the pathogenesis of primary GN.

MATERIAL and METHODS: Forty-five primary GN patients were divided into two groups as proliferative GN (PGN) (n= 17, 37.8%) and non-proliferative GN (NPGN) (n= 28, 62.2%) according to the histopathological findings. IMA was studied by the cobalt binding method. Since serum albumin levels are commonly low in patients with GN, we calculated the adjusted IMA (aIMA) according to serum albumin.

RESULTS: There was no significant difference between the two groups regarding IMA compared with controls (n= 50). IMA was significantly higher in the PGN group compared with the control and NPGN groups (p= 0.009, 0.037; respectively). There was a negative correlation between serum albumin concentration and IMA.

CONCLUSION: These results support the role of OS in the pathogenesis of PGN in which inflammatory immune glomerular injury is predominant.

KEY WORDS: Glomerulonephritis, Oxidative stress, Ischemia-modified albumin (IMA)

ÖZ

AMAÇ: Oksidan ve antioksidan sistemler arasındaki dengenin oksidan maddeler lehine bozulması olarak tanımlanan oksidatif stresin (OS), glomerülonefrit (GN) patogenezinde rol aldığı gösterilmiştir. İskemi modifiye albümin (IMA) birçoğunda OS göstergesi olarak tanımlanmıştır. Ancak literatürde daha önce GN hastalarında çalışılmamıştır. Çalışmamızda, OS’ın GN patogenezindeki rolünü, IMA ile değerlendirilmesi amaçlanmıştır.

GEREC ve YÖNTEMLER: Kirkbeş primer GN hastası histopatolojik bulgulara göre proliferatif GN (PGN) (n= 17, %37,8) ve non-proliferatif GN (NPGN) (n= 28, %62,2) olarak iki gruba ayrıldı. IMA, albümin kobalt bağlama yöntemi ile ölçüldü. GN hastalarında serum albümin değerleri sıklıkla düşük olduğundan serum albümin konsantrasyonuna göre düzeltilmiş IMA (dIMA) hesaplanması yapıldı.

BULGULAR: Kontrol grubu (n= 50) ile gruplar arasında IMA değerleri açısından fark yoktu. PGN grubunda dIMA, kontrol ve NPGN gruplarına göre anlamlı (p= 0.009, 0.037) olarak yüksekti. Serum albümin konsantrasyonu ve IMA arasında negatif korelasyon tespit edildi.

SONUÇ: Elde ettiği bulgular OS’in, inflamatuvar immün yanıtın belirgin olduğu PGN patogenezinde rol oynadığı desteklemektedir.

ANAHTAR SÖZCÜKLER: Glomerülonefrit, Oksidatif stres, İskemi modifiye albümin (IMA)
INTRODUCTION

Primary GNs are an important heterogeneous pattern of chronic kidney diseases with various types, etiologies, and clinical presentations. Genetic defects, immunologic alterations, and inflammation are the main factors playing a role in the pathogenesis. Laboratory tests and renal biopsy are used to identify a specific etiology. Although etiology in primary GN is usually idiopathic, immune factors are supported as underlying mechanisms for most forms of primary GN (1, 2).

NPGN such as minimal change disease (MCD), focal segmental glomerulosclerosis (FSGS) and membranous GN (MGN) are typically associated with less glomerular injury that mainly affects the podocytes, which are away from the circulation. In contrast, immunologic injuries are localized to the mesangium or subendothelial space directly in contact with the circulation in PGN. In addition to urinary findings, a marked inflammatory response may cause an increased level of reactive oxygen species (ROS) in PGN compared to NPGN (3).

ROS are produced by the aerobic metabolism in small amounts and have important roles in normal cell physiology. Enzymatic and non-enzymatic systems efficiently eliminate ROS under normal circumstances. OS occurs when antioxidant defense systems are inadequate for the elimination of ROS and often results in tissue damage. ROS levels are increased during inflammation, oncogenesis and degenerative diseases and become relevant factors in the initiation and amplification of these deleterious processes (4-6).

The oxidative process is an integral part of inflammation, and phagocytic cells as neutrophils and macrophages produce ROS (7). Because inflammation plays an important role in the pathogenesis of GN, there has been an interest in OS in glomerular diseases (3, 8-10). The contribution of oxidative processes to the pathogenesis and disease progression of GN has been documented by in vivo and in vitro experimental studies (3, 10-16).

In ischemia, an N-terminus form of albumin changes and metal binding capacity decreases. This new variant form of albumin is called IMA (17). Increased ROS levels affect the N-terminus of albumin and contribute to IMA formation (18). IMA has been identified as a sign of OS in a variety of diseases or conditions including acute coronary syndrome and diabetic nephropathy in which ischemia is a part of the pathogenesis (19-26).

This novel marker has not yet been studied in patients with primary GN in the literature. We therefore aimed to investigate the role of OS in the pathogenesis of primary GN with IMA in this study.

MATERIALS and METHODS

Patients

Out of 54 patients included in the study, nine were excluded due to various reasons (amyloidosis n=4, tubulointerstitial nephritis n=1, hypertensive and diabetic glomerulosclerosis n=3, inadequate histopathology n=1). Finally, a total of 45 patients (mean age 41.1±14.7 years, range 19-71 years) and 50 healthy controls (mean age 34.9±10.2 years, range 21-73 years) were enrolled in the study. The study cohort included 28 (62.2%) males, 17 females (37.8%) in the patient group, and 24 (48%) males, 26 (52%) females in the control group. The diagnosis of primary GN was based on renal histopathology. Renal biopsy was performed in patients with asymptomatic proteinuria (> 1 g/day), nephrotic syndrome, nephritic syndrome, and rapidly progressive GN. Patients with secondary GN (lupus nephritis, diabetic nephropathy, and hypertensive nephropathy), those on vitamin supplementation, and patients who had cardiovascular disease within the last month were excluded.

Patients with primary GN were divided into two groups according to histopathologic findings as PGN (n= 17, 37.8%) and NPGN (n= 28, 62.2%). The patients with MCD (n= 3, 6.7%), FSGS (n= 2, 4.4%), MGN (n=23, 51.1%) were grouped as NPGN and patients with membranoproliferative GN (MPGN) (n= 4, 8.9%), Ig A nephritis (n= 5, 11.1%) and crescentic GN (n= 8, 17.8%) as PGN.

The study was approved by the local ethics committee of Uludag University, Medical Faculty. Written informed consents were obtained from all patients and controls before inclusion in the study.

Study Protocol and Sample Collection

Following an overnight fast, blood samples were collected, and sera were separated by centrifugation for 10 minutes at 3000 rpm. Sera were stored at -80°C until analysis (maximum 6 months).

Proteinuria was negative among controls with the dipstick test. The MDRD formulation was used to calculate eGFR. Proteinuria was studied with 24-hour urine samples. Other laboratory results and medical history records of the patients and controls were obtained from the electronic file system.

IMA was determined by a manual spectrophotometric assay called Albumin Cobalt Binding (ACB) test described by Bar-Or et al. (27). The amount of albumin-bound cobalt was measured at 470 nm (Shumadzu U.V. Visible 1601) in comparison with a serum cobalt blank without DTT. IMA results were given in absorbance units (ABSU). Albumin levels were measured spectrophotometrically with the Brom cresol Green (BCG) method using the ARCHITECT c16000 (Abbott) autoanalyzer. Albumin results were given in mg/dl.

It is reported that evaluation of IMA result in cases with low or high serum albumin levels may be misleading (28-30). Determination of IMA with the ACB method is based on defining of the amount of cobalt unbound to albumin. A negative correlation has been found between IMA and serum albumin values. Accordingly, since the binding rate of cobalt will be decreased in persons with low serum albumin levels, the IMA
level will be high. Therefore, formulas of adjusted IMA (aIMA) according to serum albumin levels have been developed (29,30).

Serum albumin concentrations were low also in our patient group. Consistently with the literature, there was a significant negative correlation between IMA and serum albumin concentrations. Because previous studies have reported misleading results in the event of low serum albumin levels, we used an adjusted IMA formula in our study as described by Lippi et al. (30) as follows:

\[
aIMA = \text{case IMA} \times \left( \frac{\text{case albumin concentration}}{\text{median albumin concentration of group}} \right)
\]

Statistical Analysis

Statistical analysis of the obtained data was performed using the SPSS 13.0 statistical software. Normality of the data was examined with the Shapiro-Wilk test. For the normally distributed variables, comparison of two groups was carried out using the t-test, while comparison of more than 2 groups was with the one-way analysis of variance. For the non-normally distributed data, comparison of two groups was performed using the Mann-Whitney U test and more than 2 groups with the Kruskal-Wallis test. Correlations between the variables were studied with the Pearson correlation coefficient. Evaluation of the categorical data was with the Pearson Chi-square test. The significance level was set at \( p = 0.05 \).

RESULTS

Demographic Findings

Demographic findings are presented in Table I. No significant differences were found between the primary GN and control groups, and between the PGN and NPGN groups in terms of gender distribution. The mean age of the control group was lower than the patient group.

At presentation, nephrotic syndrome was detected in 22 (48.9%), asymptomatic proteinuria in 15 (33.3%), and nephritic syndrome in 7 (15.6%) patients, and rapidly progressive GN in 1 (2.2%) patient.

Laboratory Findings

When the patient and control groups were compared regarding laboratory outcomes; serum urea (51.9±45.1 vs. 26.7±7.6, respectively, \( p < 0.001 \)) and creatinine (1.7±2.6 vs. 0.7±0.1, respectively, \( p < 0.002 \)) levels were significantly high, and eGFR (90.2±48.6 vs. 118.2±17.3, respectively, \( p = 0.002 \)) and albumin levels (2.9±0.8 vs. 4.4±0.2, respectively, \( p < 0.001 \)) were significantly low in the patient group (Table I). Also, laboratory outcomes were compared between the PGN and NPGN groups. Serum urea (76.2±63.1 vs. 37.2±19, respectively, \( p = 0.016 \)), and creatinine (2.9±3.9 vs. 0.9±0.4, respectively, \( p = 0.006 \)) were significantly higher and eGFR was significantly lower (63.4±52.4 vs. 106.5±38.7, respectively, \( p = 0.005 \)) in the PGN group.

Although serum IMA (\( p = 0.612 \)) and aIMA (\( p = 0.304 \)) levels were higher in the patient group compared with the control group, the difference did not reach statistical significance. IMA values adjusted according to serum albumin levels, aIMA, were significantly higher in the PGN group compared with the NPGN (\( p = 0.037 \)) and control groups (\( p = 0.009 \)). Whereas, no significant difference was observed between the control and NPGN groups in term of aIMA values (\( p = 0.662 \)) (Table II).

Correlation Analysis

In correlation analysis; a negative correlation was found between IMA values and serum albumin values (\( r = -0.349; p = 0.019 \)). There was a positive correlation between serum aIMA values and serum albumin levels (\( r = 0.72; p = 0.001 \)) and a negative correlation between serum aIMA values and daily proteinuria value (\( r = -0.404; p = 0.007 \)). Also, there was also a negative correlation between serum albumin concentrations and daily proteinuria value (\( r = -0.556; p <0.001 \)). There was no correlation between IMA levels and urea, creatinine or eGFR that reflect renal functions.

<table>
<thead>
<tr>
<th>Table I: The comparison of demographic characteristics and laboratory results in controls and patients</th>
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</thead>
<tbody>
<tr>
<td><strong>Control (n= 50)</strong></td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Gender (m/f)</td>
</tr>
<tr>
<td>Serum Urea (mg/dL)</td>
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<tr>
<td>Serum creatinine (mg/dL)</td>
</tr>
<tr>
<td>eGFR (MDRD) (ml/min/1.73 m$^2$)</td>
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<tr>
<td>Albumin (g/dl)</td>
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<tr>
<td>IMA (ABSU)</td>
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<td>a-IMA (ABSU)</td>
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eGFR: Estimated glomerular filtration ratio, IMA: Ischemia modified albumin, aIMA: Adjusted ischemia modified albumin, ABSU: Absorbance unit
DISCUSSION

OS and increased production of ROS have been demonstrated to play an important role in the pathophysiology of renal diseases and GN (31). Increased OS is accused of the development of proteinuria with GBM alterations, morphological changes and glomerular hemodynamic changes (12,32). In the present study, we showed that aIMA levels which are defined as a marker of OS, are high in patients with PGN.

Numerous in vitro and in vivo studies have shown effects of OS on the pathogenesis of GN with increased levels of ROS and decreased antioxidant enzyme activity (3,10-16). Markan et al. (3) showed that pro-oxidant markers (malondialdehyde, 8-isoprostane, total homocysteine) were significantly higher and antioxidant markers (superoxide dismutase) were significantly lower in primary GN patients compared to the control subjects. When patients were grouped as PGN and NPGN; OS was shown to be significantly profound in the PGN group compared with NPGN group (3).

In the present study, there was no significant elevation in IMA/ aIMA levels in NPGN group, and aIMA levels were found higher only in the PGN group as a marker of OS.

Immunologic mechanisms involved in GN pathogenesis contribute to glomerular injury either through inflammatory or non-inflammatory processes. The inflammatory injury is characterized by glomerular hypercellularity that results from infiltrating cells such as neutrophils and macrophages and proliferating glomerular cells. In GN classified as PGN, the inflammatory injury is predominant. Non-inflammatory lesions resulting from immune injury in NPGN usually involve glomerular podocytes (33).

Table II: The comparison of laboratory results in controls, and the PGN and NPGN groups

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=50)</th>
<th>PGN (n=17)</th>
<th>NPGN (n=28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mg/dL)</td>
<td>26.7±7.6</td>
<td>76.2±63.1†‡</td>
<td>37.2±19.0</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.7±0.1</td>
<td>2.9±3.9†‡</td>
<td>0.9±0.4</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²)</td>
<td>118.2±17.3</td>
<td>63.4±52.4†‡</td>
<td>106.5±38.7</td>
</tr>
<tr>
<td>IMA (ABSU)</td>
<td>0.473 (0.229-0.840)</td>
<td>0.549(0.175-0.937)</td>
<td>0.442(0.252-0.746)</td>
</tr>
<tr>
<td>aIMA (ABSU)</td>
<td>0.473 (0.229-0.840)</td>
<td>0.562 (0.244-1.305)†‡</td>
<td>0.470(0.242-0.944)</td>
</tr>
<tr>
<td>Albumin (mg/dL)</td>
<td>4.4±0.2D</td>
<td>3.1±0.8</td>
<td>2.8±0.8</td>
</tr>
<tr>
<td>Proteinuria (g/day)</td>
<td>4.1±4.5</td>
<td>6.1±4.9</td>
<td></td>
</tr>
</tbody>
</table>

eGFR: Estimated glomerular filtration ratio. IMA: Ischemia modified albumin, aIMA: Adjusted ischemia modified albumin, ABSU: Absorbance unit.

PGN vs. control and NPGN groups (†p< 0.05) by one-way ANOVA test.
PGN vs. NPGN group (‡p< 0.05) by student t-test, (†p< 0.05) by Mann-Whitney U test.
PGN vs. control group (¥p< 0.05) by Mann-Whitney U test.
PGN vs. control and NPGN groups (*p< 0.05) by Kruskal Wallis test.
Control vs. PGN and NPGN groups (∆p< 0.05) by one-way ANOVA test.

Different cell groups may be sources of ROS. Neutrophils, monocytes/macrophages, mesangial and epithelial cells and complement components are responsible for the increased production of ROS with the influence of many immune stimuli such as immune complexes and immunoglobulins (34). Neutrophils and monocytes/macrophages have been reported in PGN, and mesangial and epithelial cells in NPGN as the sources of the production of ROS (8,34-36). We thought that high aIMA levels were a marker of severe OS caused by the activated neutrophils, monocytes/macrophages and mesangial cells in patients having PGN with prominent inflammation.

Data about effect of elevated serum creatinine on IMA levels is limited. Several studies demonstrated that IMA levels were higher among hemodialysis (HD) patients (37,38). Conversely, Carrega et al. concluded that the IMA and albumin ratio was not significantly different in HD patients and controls (39). In another study IMA showed correlation with creatinine levels (40) whereas there was no correlation between IMA/aIMA levels with serum creatinine levels in our findings. In the present study, increased aIMA levels were higher and deterioration in renal function was significant among PGN compared with NPGN patients whereas there was no correlation between aIMA and serum creatinine levels or eGFR. These findings suggested that prominent inflammation, which was thought to be source of OS, might be responsible for the high aIMA levels and decrease in renal function in PGN patients. Although we did not study any inflammation marker in the serum, histopathologic findings support prominent inflammation in PGN patients.

In the correlation analysis; a significant negative correlation was found only between IMA and serum albumin concentrations,
consistent with the literature. We think that the use of the aIMA value in the mostly hypoalbuminemic GN patient group would be more appropriate.

IMA has been studied as a marker of OS especially in coronary artery disease and other ischemic conditions. There are only a few studies showing its association with inflammation other than ischemic conditions (41,42). Although we did not study any inflammatory marker, high levels of aIMA in patients with PGN where inflammation is prominent suggests that this marker could be used as a marker of OS also in conditions other than ischemia-like inflammation.

The most important limitation of our study is low serum albumin levels in the patients. Data on this issue are limited as patients with altered serum albumin values have been excluded in many studies (43). We think that the use of aIMA value in the mostly hypoalbuminemic GN patient group would be more appropriate. Elevation of aIMA levels should be supported with other OS markers, and this might be the second limitation of the study. The control group was younger; however, there was no data about age and IMA association and IMA/aIMA levels were not significantly different between controls and whole patients. Studies with IMA in patients with kidney diseases are limited. IMA has been studied in patients with diabetic nephropathy. Given that diabetes mellitus might affect IMA levels due to increased OS (44), our study is the first in the literature to investigate IMA levels in primary glomerular diseases.

In conclusion; our findings would seem to suggest that high levels of aIMA are associated with OS among PGN patients in whom glomerular inflammation is prominent in the pathogenesis. We also believe that IMA/aIMA could be used as a marker of OS in conditions other than ischemia, such as inflammation. Furthermore, comprehensive studies are needed to support these findings.

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