Research Article (Araştırma Makalesi)

Pre-Treatment Serum Complement 3 Levels And Rituximab Response In Idiopathic Membranous Nephropathy

İdiopatik Membranöz Nefropatide Tedavi Öncesi Serum Kompleman 3 Seviyesi ve Rituksimab Yanıtı

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Özet

Amaç: İdiyopatik membranöz nefropati (MN) hastalarında rituksimabın etkinliği rapor edilmiştir. Merkezimizde diğer tedavilere yanıt vermeyen idiyopatik MN hastalarında tanıda kullanılan biyokimyasal testler, immünhistokimyasal profil ve rituksimab yanıtı arasındaki ilişkiyi değerlendirmeyi amaçladık.

Gereç ve Yöntemler: Bu çalışmada 2017-2022 yılları arasında diğer immünsüpresif tedavileri alan ve merkezimizde böbrek biyopsisi yapılan idiyopatik MN'li dokuz hasta değerlendirildi. Tedavi öncesi fosfolipaz A2 antikor düzeyi 6 hastada pozitifti, 3 hastada ise antikor analizi yapılamadı. Tüm hastalara renin-anjiyotensin-aldosteron sistemi (RAAS) blokeri, siklofosfamid, steroid ve kalsinörin inhibitörlerinin ardından ≥2 gram rituksimab verildi.

Bulgular: Çalışmaya dahil edilen dokuz hastanın 7'si (%78,2) erkek olup yaş ortalaması 39,7±13,2 yıl idi. Dört hastada rituksimab tedavisi ile tam remisyon (KR), beş hastada ise kısmi remisyon (PR) görüldü. Renal biyopside sklerotik glomerül sayısı, IgG, A, M, C1q, C3, C4d, fibrinojen, kappa ve lambda boyanması, tübüler atrofi ve interstisyel fibrozis bulguları benzerdi. Ancak serum kompleman 3 (C3) düzeyi normal sınırlar içinde anlamlı derecede düşüktü (1,22 ± 0,26 vs 1,560 ± 0,56 p=0,016). Ortalama arter basıncı kısmi remisyonda olan hastalarda tam remisyonda olanlara göre anlamlı derecede yüksekti (96.2 ± 2.5 mmHg vs 84.75 ± 4.27 mmHg, p: 0.018).

Sonuç: İdiyopatik membranöz nefropatili hastalarda tedavi öncesinde normal sınırlar içerisinde düşük bir bazal serum C3 düzeyi, diğer immünsüpresif tedavilere yanıtsızlığın ve rituksimab tedavisine kısmi yanıtın öngörülmesinde yardımcı olabilir.

Anahtar kelimeler: Membranöz Nefropati, Serum Kompleman 3, Rituksimab

Abstract

Objective: The efficacy of rituximab has been reported in patients with idiopathic membranous nephropathy (MN). We aimed to evaluate the relationship between biochemical tests at diagnosis, immunohistochemical profile, and rituximab response in patients with idiopathic MN unresponsive to other therapies in our center.

Materials and Methods: In this study, nine patients with idiopathic MN who received other immunosuppressive therapies between 2017-2022 and who underwent renal biopsy in our center were evaluated. Pre-treatment phospholipase A2 antibody levels were positive in 6 patients, and antibodies could not be analyzed in 3 patients. All patients received rituximab ≥2 grams after renin-angiotensin-aldosterone system (RAAS) blocker, cyclophosphamide, steroid, and calcineurin inhibitors.

Results: Of the nine patients included in the study, 7 (78.2%) were male, and the mean age was 39.7 ± 13.2 years. Four patients had complete remission (CR) with rituximab treatment, and five had partial remission (PR). Sclerotic glomeruli count, IgG, A, M, C1q, C3, C4d, fibrinogen, kappa and lambda staining, tubular atrophy, and interstitial fibrosis findings on renal biopsy were similar. However, the serum complement 3 (C3) level was significantly lower within normal limits (1.22 ± 0.26 vs 1.560 ± 0.56 p=0.016). The mean arterial pressure was significantly higher (96.2 ± 2.5 mmHg vs 84.75 ± 4.27 mmHg, p=0.018) in patients with partial remission compared to those with complete remission.

Conclusion: A low baseline serum C3 level within normal limits before treatment in patients with idiopathic membranous nephropathy may help predicting unresponsiveness to other immunosuppressive therapies and partial response to rituximab treatment.

Key words: Membranous Nephropathy, Serum Complement 3, Rituximab

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INTRODUCTION

Membranous nephropathy (MN) is one of the most common causes of nephrotic syndrome in non-diabetic adults worldwide. Its histology is characterized by glomerular basal membrane thickening with little cellular proliferation or infiltration on light microscopy. Adult MN is primary mainly (about 75 to 80% of cases) and is caused by circulating autoantibodies against podocyte antigens. In about 20-25% of cases, MN may also develop secondary to infections (such as hepatitis B and syphilis), autoimmune diseases, malignancies, allogeneic hematopoietic stem cell transplantation, non-steroidal anti-inflammatory drugs (NSAIDs), alpha-lipoic acid, and some conventional drugs.

The complement system has a fundamental role in the pathogenesis of many renal diseases. In studies on Heymann Nephritis, it has been suggested that subepithelial immune complex-induced complement activation plays an essential role in the pathogenesis of MN, resulting in massive proteinuria (1). Glomerular deposition of C3 deposits in MN was first recognized in 1984 in 8 of 16 patients with primary MN (2). In the study, patients with glomerular complement 3 (C3) deposition were found to have more proteinuria than those without glomerular C3 deposition. Today, C3c deposition can be detected in almost all patients with MN using more sensitive immunohistologic staining (3), and approximately 70% of patients with MN elevated levels of C3d, a stable degradation product of C3 (4).

Rituximab (RTX) is a chimeric monoclonal antibody that kills B cells by binding to the CD20 surface molecule, which is present at all stages of B cell maturation, except early B cell progenitors and plasma cells. In membranous nephropathy, rituximab is the first-line immunosuppressive treatment of choice for high or very high-risk patients with normal or near-normal renal function and intermediate-risk patients. No correlation has been demonstrated between rituximab treatment response and serum C3 level. We aimed to evaluate the response to rituximab treatment combined with immunosuppressive therapy through biochemical and immunologic tests at diagnosis.

MATERIAL AND METHODS

This study evaluated nine patients with idiopathic MN who underwent renal biopsy in our center between 2017 and 2022, could not achieve remission with other immunosuppressive therapies at least for one year, and received rituximab. The pre-treatment phospholipase A2 antibody was positive in 6 patients, and the antibody could not be evaluated in 3 patients. All patients received rituximab treatment of ≥2 grams for at least two cycles after renin-angiotensin-aldosterone system (RAAS) blocker, cyclophosphamide, steroid, and calcineurin inhibitors. For MN, partial remission (PR) was defined as proteinuria of ≥0.3 and <3.5 g/24 h, 50% reduction in proteinuria from the baseline level, normal serum albumin (serum albumin of ≥3.5 g/dl), and stable renal function; complete remission (CR) was defined as proteinuria of <0.3 g/24 h, normal serum albumin, and stable renal function in at least two successive visits. Relapse was defined as recurrence of a proteinuria level of ≥3.5 g/24 hours (5).

The study was designed retrospectively. Informed consent was waived by the Institutional Review Board of Baskent University Medical and Health Sciences because this study involved a retrospective review of existing data. This study was approved by Baskent University Institutional Review Board (Date: 09/2023; Project No: KA23/344). This study complied with the ethical principles of the Declaration of Helsinki.

Statistical Evaluation

The study data were analyzed in Jamovi Version 2.3.18, an open-source R programming language user interface. Continuous numerical data were given as mean and standard deviation (mean±sd); nominal and categorical data were given as number-percentage (n%). The Mann-Whitney U test was used to analyze numerical data, and the Chi-square test was used to analyze categorical data. The level of statistical significance was taken as $p<0.05$ in the evaluation of the findings.

RESULTS

Seven (78.2%) of the patients were male; the mean age was 39.7 ± 13.2. With rituximab treatment, complete remission was achieved in 4 patients and partial remission in 5 patients. Sclerotic glomeruli count, IgG, A, M, C1q, C3, C4d, fibrinogen, kappa and lambda staining, tubular atrophy, and interstitial fibrosis findings in renal biopsy of patients with complete and partial remission were similar. Also, no difference was found in patients' BUN, creatinine, and body mass index values in partial and complete remission before and after the treatment (**Table 1**).

The mean blood pressure was significantly higher in patients in partial remission than in patients in complete remission (96.2 ± 2.5 mmHg vs 84.75 ± 4.27 mmHg, p: 0.018). Serum C3 level was significantly lower within normal limits in patients in partial remission than in patients in complete remission (1.22 ± 0.26 vs 1.560 ± 0.56 p: 0.016). No significant difference was found in serum complement 4 (C4) levels and C3/C4 ratios between the two groups (**Table 1**).

DISCUSSION

Although rituximab treatment is considered a first-line treatment option in MN, other conventional immunosuppressive therapies are often preferred as the first-line treatment due to the costs of rituximab treatment. Our study evaluated nine patients with idiopathic MN who could not respond to ACEI/ARB, protein-restricted diet, cyclophosphamide, and calcineurin inhibitor treatments.

Table 1. Comparison of laboratory, renal biopsy, and immunohistochemical profile findings of patients with complete and partial remission.

Variables	Complete remission (n:4)	Partial remission (n:5)	p
Age	35.2±15.9	43.211	0.523
Sex (Male)	**n: 3. (75%)**	**n: 4. (80%)**	0.233
Biopsy Findings			
Glomeruli count in the biopsy	17.5±9.57	±27±1.87	0.215
Sclerotic glomeruli count	0.75±0.95	4.4±3.36	0.105
Ig G	1 (25%)	3 (75%)	0.204
C3	4 (57.1%)	3 (42.9%)	0.347
C1q	-	1 (100%)	0.343
Fibrinogen	1 (100%)	-	0.236
Kappa	-	1 (100%)	0.343
Lambda	1 (100%)	-	0.236
C4d	1 (50%)	1 (50%)	1.00
Tubular Atrophy	3 (60%)	2 (40%)	0.294
Interstitial Fibrosis	3 (75%)	1 (25%)	0.099
Laboratory Findings			
Baseline Phospholipase A2 antibody	70.11±113.82	521.38±848.01	0.372
Serum Ig G	3.29±0.59	4.68±1.69	0.055
Serum Ig A	1.34±0.44	1.80±1.15	0.905
Serum Ig M	1.50±1.13	0.46±0.25	0.063
C3	1.56±0.56	1.22±0.26	0.016*
C4	0.67±0.52	0.38±0.22	0.140
C3/C4 ratio	3.16±1.43	3.76±1.21	0.556
Mean blood pressure (mmHg)	84.75±4.27	96.2±2.5	0.018*
BMI (Body mass index)	26.12±1.28	26.52±2.29	1.00
Pre-treatment BUN (mg/dl)	12.75±5.05	14.60±5.22	0.902
Post-treatment BUN (mg/dl)	12.50±3.87	16.40±7.30	0.387
Pre-treatment creatinine (mg/dl)	0.67±0.07	0.93±0.036	0.413
Post-treatment creatinine (mg/dl)	0.76±0.06	0.93±0.29	0.459
Pre-treatment e-GFR (ml/min)	82.1±28.7	122.6±12.9	0.383
Pre-treatment proteinuria (gram/day)	4587.5±3807.6	8360±4218.17	0.219
Post-treatment proteinuria (gram/day)	187±103.96	3140±960.72	0.019*
Amount of decrease in proteinuria	4400.5±3795.75	5220±3773.19	0.73

Abbreviations: e-GFR: estimated glomerular filtration rate; *p<0.05, statistically difference.

The activation of the complement system is effective in the pathogenesis of MN, as in other glomerulonephritis cases. In renal biopsy, immunohistochemical demonstration of C3 accumulation is among the supportive findings for MN. Our study found no significant difference between CR and PR groups regarding C3 accumulation in tissue. The qualitative determination of C3 accumulation in tissue rather than quantitative determination may be the reason for the lack of difference in comparing C3 levels in tissue. However, our study found that serum C3 level was within normal limits but significantly lower in patients with partial remission. Low serum C3 levels observed during diagnosis or remission in lupus nephritis have been associated with renal flare-ups (6). The rate of progression to end-stage renal failure has also been found to be high in patients with low serum C3 levels (7). It has been shown that higher serum C4 levels are associated with poorer renal survival in patients with MN. In this study, serum C3 levels were found to be correlated with serum C4 levels (8). Although the number of patients was small in this study, low serum complement three levels were associated with lower treatment

response. A study conducted with patients with IgA nephropathy reported that patients with a low C3/C4 ratio had more severe clinical symptoms and showed faster progression to chronic renal disease compared to patients with a higher ratio (9). Our study determined that serum C3/C4 ratio was not associated with treatment responses. No study in the literature demonstrates the effect of serum C3/C4 ratio on the renal process in patients with MN.

In an in vivo animal study with rituximab in which non-Hodgkin's lymphoma was modeled, it was shown that the response to rituximab was eliminated in animals lacking the C1q gene and that complement activation determined the therapeutic activity of rituximab in vivo (10). These data are consistent with previous in vitro findings, evidencing that rituximab lyses human lymphoma cells more effectively via CDC (complement-dependent cytotoxicity) compared to ADCC (antibody-dependent cellular cytotoxicity) in vitro (11). It has also been shown that the IgG4 form of the molecule, which does not activate CDC or ADCC, does not consume peripheral blood B cells in non-human primates (12). Moreover, complement is rapidly activated in vivo after rituximab infusion (13). These explanations may be consistent with the finding that patients in partial remission had significantly lower serum C3 levels within normal limits after rituximab treatment than patients in complete remission.

In our study, although a standard dose of RAAS (renin-angiotensin-aldosterone system) blocker was used, the mean blood pressure was statistically significantly higher in patients in partial remission than in patients in complete remission.

The limitations of the study are that the study was conducted at a single center and included a small number of patients, to have a retrospective design, and the fact that the C3 value was only evaluated at the diagnostic stage.

As a result of our study, pre-treatment baseline serum C3 levels within normal limits in patients with idiopathic membranous nephropathy may help predicting unresponsiveness to other immunosuppressive therapies and partial response to rituximab treatment.

Ethical approval: This study was approved by Baskent University Medical and Health Sciences Research Board. Informed consent was waived by the Institutional Review Board of Baskent University Medical and Health Sciences because this study involved a retrospective review of existing data. This study was approved by Baskent University Institutional Review Board. (Date: 09/2023; Project No: KA23/344). This study complied with the ethical principles of the Declaration of Helsinki.

Conflict of Interest and Financial Status: Our study has not been financed by an institution and institution. In this study, there is no conflict of interest among the authors on any subject.

Authors' contribution: The authors declare that they have contributed equally to the study.

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