Original Article

Rituximab, Use and B Cell Depletion in Patients with Membranous Nephropathy– A Retrospective, Observational Study

Abstract

Introduction: Rituximab (Rtx), an anti-CD20 monoclonal antibody, results in selective B-cell depletion and has emerged as an important therapeutic option in idiopathic membranous nephropathy (MN). We conducted a retrospective observational study to evaluate the efficacy and tolerability of Rtx in MN with respect to the B-cell count depletion. **Methods:** Twenty patients with biopsy proven primary MN, both treatment naïve and treatment resistant, who received a fixed dose protocol of 500mg IV Rtx 1month apart were retrospectively observed with a minimum follow-up period of 12 months. The primary clinical outcome was complete (CR) or partial remission (PR) at 12 months in relation to B-cell depletion at 6 and 12 months. **Results:** All were patients (men, 90%) of PLA2R-Ab positive with MN with a mean age of 37.7 ± 12.5 years. The mean 24-h urinary protein was 7.5 ± 2.15 gm/day, serum albumin was 2.01 ± 0.6gm/dL, and eGFR was 86.5 ± 20 mL/min/1.73m^2. Primary composite outcome at 12 months was 66.7%, with 5.6% CR and 61.1% PR.The mean PLA2R-Ab at 12 months was low in those with remission compared to those who did not achieve (17.8 ± 21.2 RU/mL vs 311.7 ± 356.0; *P* = 0.01). Sustained B cell depletion at 6 months was seen in 84.3% (OR = 2.2, 95% CI = 0.11–42.7; *P* = 0.53) and 32% at 12 months (OR = 2.25, 95% CI = 0.18–27.7, and *P* = 0.66). **Conclusion:** Acceptable remission rates were seen with Rtx in both treatment naïve and treatment-resistant patients with MN. There was no significant association between B-cell depletion and remission.

Keywords: *CD-19 B-cell depletion, membranous nephropathy, rituximab*

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Background

Membranous nephropathy (MN) is the leading cause of nephrotic syndrome in adults.[1] Initial therapy for patients with MN is supportive; immunosuppressive therapy is recommended for patients with persistent nephrotic syndrome. A regimen of alternating glucocorticoids and cyclophosphamide is effective in 60–70% of patients but has been associated with clinically significant toxic effects.[2] Calcineurin inhibitors, including cyclosporine are effective, but associated with a high incidence of relapse after discontinuation and frequent side effects.[3]

B cell dysfunction plays a role in the pathogenesis of MN.[4] Rituximab, a selective B cell depleting agent, appears to be a promising approach. A number of studies using rituximab as first-line therapy as well as in patients resistant to other immunosuppressive regimens have shown a reduction in proteinuria of 60–80% in majority of patients. It is not clear, however, whether there is a direct link between B-cell depletion and efficacy of rituximab in inducing remission in membranous nephropathy. Hence, we aimed to the efficacy and tolerability of rituximab in primary MN with respect to the B cell depletion.

Methods

A single-center retrospective observational study was conducted at The Nizam's Institute of Medical Sciences, Hyderabad from January 2018 to December 2020. The study was approved by ethics committee (E NIMS/2582/2020).

All 18–65 years old patients with biopsy proven primary MN and 24-h urinary protein >4 gm/day with serum PLA2R positivity who received rituximab either as primary therapy or alternative therapy were reviewed. Clinical data was collected from patient's medical records.

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All the patients received a fixed dose protocol of rituximab 500mg IV, 1 month apart and supportive antiproteinuric therapy. The minimum follow-up period was 12 months. Laboratory evaluation included 24-h urinary protein, serum albumin, creatinine, eGFR, lipid profile. CD 19+ B lymphocyte depletion was defined as <5cells/mL and was evaluated after two doses, months 6 and 12 after rituximab infusion. Serum PLA2R was measured at baseline and after 12 months of receiving rituximab. Adverse events related to rituximab were evaluated during drug infusion and the entire follow-up period. Complete remission was defined as proteinuria <0.3g/24h, and partial remission was defined as proteinuria <3.5/24h or a reduction of >50% from baseline, with improvement or normalization of serum albumin concentration and stable or elevated <30% from baseline of serum creatinine. Patients who did not reach remission were considered non-responders. The recurrence of proteinuria >3.5g/24h after a period of remission was regarded as relapse.

The primary clinical outcome was the composite of complete or partial remission at 12 months. The secondary outcome included the association between sustained B-cell depletion at months 6 and 12 and clinical remission.

Statistics

Data was compiled in Microsoft Excel sheets; collected data was checked for its completenessand correction before analysis. Statistical software SPSS 19.0v (SPSS, Chicago, IL, USA) wasused for statistical analysis. Quantitative data were expressed as mean ± SD for normally distributed data with 95% confidence intervals. Categorical data were expressed as percentages. Comparison of various outcomes between the two groups with continuous data was done using two-sample unpaired *t*-test (parametric test). Statistical significance was considered as $P < 0.05$.

Results

There were 20 patients with MN who received rituximab treatment as an initial or alternative therapy. The clinical and pathological characteristics of the patients at baseline are presented in Table 1. The cohort included 18 (90%) males with a mean age of 37.7 ± 12.5 years. The baseline mean 24-h urinary protein was 7.5 ± 2.15 gm/day, serum albumin was 2.01 ± 0.6gm/dL, and eGFR was 86.5 ± 20 mL/min/1.73m^2. All the patients had serum PLA2R-Ab positivity with mean levels of 379.3 ± 300 RU/mL. All the patients underwent kidney biopsy with 100% tissue PLA2R positivity; IFTA of <30% was seen in 20% of patients. The electron microscopy revealed a mean GBM thickness of 690 ± 110 nm with 75 and 25% of patients having stage 2 and stage 3 membranous injury, respectively. Treatment naïve were 35 and 65% of patients had received immunosuppression previously [steroids along with mycophenolate mofetil (30%), tacrolimus (15%), and cyclophosphamide (10%)] but did not attain remission.

Table 1: Demographic and pathological characteristics of the patients

Characteristic	*n*=20
Age at onset (yrs)	37.7±12.5
Male: female	18:2
Weight (Kg)	66.4±12
Height (cm)	163.2±11.1
BMI (kg/m^2)	25.4±5.13
BSA (m^2)	1.71±0.18
Duration of illness (months)	23.5±12
Hypertension(%)	25
Hypothyroidism(%)	45
Previous immunosuppression (%)	65
Creatinine (mg/dL)	0.9±0.4
eGFR (mL/min/1.73m^2)	86.5±20
Hemoglobin (gm/dL)	11.4±1.8
Spot PCR (mg/gm)	7.19±1.5
Albumin (gm/dL)	2.01±0.6
Proteinuria (gm/day)	7.5±2.15
T. Cholesterol (mg/dLl)	258.9±90.4
HDL (mg/dL)	58.16±12.7
LDL (mg/dL)	164.4±74.38
TG (mg/dL)	160±83.5
TSH (mIU/mL)	6.17±8.46
Serum PLA2R-Ab (RU/mL)	379.3±300
Light microscopy	
Membrane thickening(%)	100
Glomerular sclerosis(%)	10
IFTA <30%(%)	20
Vessels thickened(%)	10
Tissue PLA2R positivity(%)	100
Electron microscopy	
GBM thickness (nm)	690±110
Stages	
1	0
2	75
3	25
4	0
Diffuse foot process effacement	100

The protein creatinine ratio (mg/gm) showed a significant decline from a baseline mean value of 7.34 ± 1.4 to 2.95 ± 2.26 at 12 months ($P = 0.03$). The serum albumin (gm/dL) showed a significant rise from 2.09 ± 0.5 to 3.31 ± 0.96 at 12 months ($P = 0.04$). There was a mild increase in S. creatinine (mg/dL) from 0.9 ± 0.4 at baseline to 1.17 ± 0.6 at 12 months, which was not statistically significant ($P = 0.09$) [Table 2]. The treatment naïve group had higher remission rates compared to alternative therapy group (85% vs 54.5%), although statistical significance could not be found. The mean serum PLA2R-Ab levels at baseline was 249 ± 165.5 RU/mL in patients who attained remission and 521.17 ± 497.15 RU/mL in patients who did not attain

remission (*P* = 0.099). The mean PLA2R-Ab at 12 months was 17.77 ± 21.23 RU/mL in patients who attained remission and 311.67 ± 356.05 RU/ml in patients who did not attain remission (*P* = 0.01) Primary composite outcome of complete/partial remission at 12 months was 66.7%, with complete remission rates of 5.6% and partial remission rates of 61.1% [Table 3]. At 6 months, 47.4% had attained partial remission; none of them attained complete remission.

The mean B-cell count at the start of therapy was 225.2 ± 96 cell/μL (12.75%). All the patients in the study population had B cell depletion after two doses of rituximab with a mean B cell count of 8 ± 2 cells/μL (0.91%). At 6 months, the mean B cell count was 50.3 ± 8 cells/μL (1%), with a median value of 5 cells/μL. Sustained B cell depletion was seen in 84.3% of the study populationat at 6 months, whereas in 15.7% of the study population, B cells had repopulated (OR = 2.2, 95% CI = 0.11–42.7, and *P* = 0.53). At 12 months, the mean B cell count was 125 ± 20 cells/μL (7%). At 12 months, 32% of the study population had sustained B cell depletion and in 68% of the study population B cells had repopulated. (OR = 2.25, 95% CI = 0.18–27.7, and *P* = 0.66). There was no statistical significance between B-cell depletion and remission rates. The mean body surface area of the study population who attained B cell depletion of <1% was 1.62 ± 0.117 kg/m^2 and those who did not attain B cell depletion was higher at 1.74 ± 0.194 kg/m^2 (*P* = 0.305).

There were nine (45%) adverse events in the study population. Six (30%) were mild not requiring hospitalization, all the 3 (15%) patients with cellulites required hospitalization of which 2 (10%) died due to sepsis [Table 4]. Two patients died after the second dose of rituximab, one at 1month and another at 8 moths. One patient died after 18 months of the second dose of rituximab due to COVID-19 infection.

Table 2: Efficacy end points at 6 and 12 months of treatment

Parameter	*n*=20	*P*
Protein creatinine ratio (mg/g)	7.34±1.4	<0.001
Baseline	4.22±2.4	
6 months	2.95±2.2	
12 months		
Serum Albumin (gm/dL)		
Baseline	2.09±0.5	<0.001
6 months	2.95±0.8	
12 months	3.31±0.96	
Serum creatinine (mg/dL)		
Baseline	0.9±0.4	0.67
6 months	1.05±0.5	
12 months	1.17±0.6	

Table 3: Remission rates with rituximab at 6 and 12 months follow-up

Remission (Complete/Partial)	No attained/Total (%)
At 6 months	
Partial remission	9/19 (47.4)
Complete remission	0/19
Total remission	9/19 (47.4)
No remission	10/19 (52.6)
At 12 months	
Partial remission	11/18 (61.1)
Complete remission	1/18 (5.6)
Total remission	12/18 (66.7)
No remission	6/18 (33.3)

Table 4: Adverse effects of rituximab

Event	*n* (%)
Any adverse events	9 (45%)
Grade ≥3	3 (15%)
Grade <3	6 (30%)
Serious adverse events	2 (10%)
Fatal	2 (10%)
Nonfatal	0
Upper respiratory tract infection	1 (5%)
Lower respiratory tract infection	1 (5%)
Acute gastroeneteritis	2 (10%)
Infusion reaction (chills)	2 (10%)
Cellulitus	3 (15%)

Discussion

We found that nearly two-thirds of our patients treated with rituximab had remission (partial/complete) at 12 months, with improvement in serum albumin. This is consistent with few prospective studies done earlier.[5-9] Despite the fact that these studies differ in several features like eligibility criteria, treatment protocols, and criteria to define remission, the overall remission rates (complete and partial) of rituximab at 12 months are approximately 60– 70%, ranging from 44 to 85%. Important to note that about 30– 40% of all treated patients do not respond to initial treatment with rituximab and might need additional treatments.

In the present study, the mean age of the study population was lower 37 ± 12.5 than that in GEMRITUX[5] and MENTOR[6] trials and higher number of males (90%) and more number of patients who previously were treated with immunosuppression (65%) as compared to GEMRITUX (2.7%) and MENTOR (29%). The baseline protein to creatinine ratio of 7.19 ± 1.5 was comparable with that in GEMRITUX trial 7.68 ± 2.2. The present study had lower baseline serum albumin levels of 2.01 ± 0.6 and higher PLA2R-Ab at baseline in comparison with the

other two studies. All these parameters indicate that the present study population had patients with more severe disease and also, many of them being resistant to other immunosuppressive agents used in the past. The baseline characteristics of patients in different studies are presented in Table 5.

Several studies showed that male sex, old age (>50 years), hypertension, massive proteinuria (>10gm/24h,) and elevated serum creatinine concentration at the time of renal biopsy are poor prognostic factors of MN.[7-12] However, none of these factors were found to have statistical significance in the present study, probably due to small sample size. In the present study, 85% of the patients who received rituximab as primary therapy attained remission. On the other hand, among those who had a history of usage of previous immunosuppression, only 54% attained remission. A meta-analysis done by Bomabak *et al.*[13] suggested that the drug is equally efficacious as both primary and secondary immunosuppression, with patients having a 15–20% chance of complete remission whether they have failed previous immunomodulatory treatment.

At 12 months, the PLA2R values in those who attained remission were significantly lower than those who did not attain remission. Hofstra *et al.*[14] found that in patients with low antibody titers spontaneous remissions occurred in 38% of patients whereas only occasionally observed in patients with high titers. De Vriese *et al.*[15] suggested immunosuppressive therapy withdrawal if PLA2R-Ab is reduced by 90% from the baseline. The role of PLA2R-Ab has widened in the recent times from diagnostic and prognostic marker to a therapeutic target.

The optimal dose of rituximab and whether dosing should be targeted at B-cell response has largely been inconclusive. Different regimens used have been highlighted in Table 6. Several studies done before differ in the dose of rituximab as well as duration of therapy. Most of the studies used 375 mg/m^2, either 2 or 4 weeks apart and few studies also studied a rituximab dose of 1 gm, 1month apart. A single dose of rituximab 1 gm was also used in studies with either 2 or 4 weeks apart. In the present study, we studied the efficacy of low-dose regimen of 500 mg on day 1 and day 30 to decrease the risk of infection and for cost effectiveness. The rationale for this regimen being that the conventional four weekly high doses of rituximab was originally used to treat lymphoma.[16] The estimated median terminal elimination half-life was 22 days (range 6.1–52 days);[17] hence, the doses were given 1month apart. Also, the clearance of rituximab might be increased in patients with nephrotic range proteinuria.[18]

All the patients in the study group achieved CD-19 B-cell depletion (<5cells/µLor ≤1%). In total, 84.3 and 32% of the population had sustained B cell depletion at 6 and 12 months respectively. A four weekly regimen of 375mg/m^2 achieves a persistent B-cell depletion for 6–9 months in >80% of patients.[19] Cravedi *et al.*[20] found that rituximab treatment titrated to circulating B-cells was effective as the standard four-dose protocol in inducing remission but showed a better risk/benefit profile, fewer hospitalizations, and was 4-fold less expensive. They noted that circulating CD-20 cells were fully depleted already after the first rituximab administration and remained below normal throughout observation period.

Although 84.3% of the study population attained B-cell depletion, it was not found to be significantly associated with remission rates. Similar results were found in GEMRITUX trial where B-cell counts did not predict proteinuria remission. It also confirmed that PLA2R-Ab depletion rather than CD20 depletion achieved in all patients matters for prediction of rituximab response. This was thought to be due to the persistence of B cells in secondary lymphoid organs.[21]

The strength of study lies in the fact that use of a low-dose regimen with CD-19 B cell monitoring helped us understand the pharmacokinetics and pharmacodynamics of rituximab. This study also corroborates with the observations by Fervenza

Table 5: Baseline characteristics in different studies

Parameter	Gemritux (*n*=37)	Mentor (*n*=65)	Present study (*n*=20)
Age (yrs)	53.0±11.5	51.9±12.6	37.7±12.5
Males; *n*(%)	28 (75.7)	47 (72)	18 (90)
Weight (kg)	76±20	96±23	66.4±12
History of immunosuppressive therapy, *n*(%)	1 (2.7)	19 (29)	13 (65)
S.creatinine (mg/dL)	1.11±0.5	1.3±0.4	0.9±0.4
eGFR (mL/min/1.73m^2)	66.5±14	84.9±29.8	86.5±20
Protein to creatinine ratio (mg/g)	7.68±2.2	6.2±2.6	7.19±1.5
Albumin (gm/L)	2.2±0.5	2.5±0.4	2.01±0.6
Serum PLA2R-Ab positive patients; *n*(%)	27 (73)	50 (77)	20 (100)
Median Serum PLA2R-Ab (Ru/mL)	40.5 (0–-275.5)	409 (163–-834)	435 (0.6–1500)

Table 6: Rtuximab protocol and remission rates in different studies

Study	Rituximab dose	Follow-up period	Remission rates
Remuzzi *et al*.[8]	375mg/m^2 once weekly for 4 weeks	20wks	62%
Ruggeneti *et al*.[9]	375mg/m^2 once weekly for 4 weeks	12 months	62%
Cravedi *et al*.[10]	375mg/m^2 single dose, repeated if B-cell count <5cells/µLafter 1 week of treatment	12 months	67%
Fervenza *et al*.[11]	1gm on day 1 and 15; repeated at 6 months if proteinuria >3gm/24h and CD19 B cell >15/µL	12 months	53%
GEMRITUX[6]	375mg/m^2 at day 1 and 8 and repeat dose based on clinician's discretion	6 months	35%
MENTOR[7]	1gm on day 1 and 15; repeated at 6 months if >25% decrease in proteinuria but failed to attain complete remission irrespective of B cell count	24 months	60%
Raja *et al*.[12]	100 mg with repeat dosing based on CD19 depletion.	6 months	50%
Present study	500mg on day 1 and 30.	12 months	66.7%

et al.[6] that CD19 depletion does not predict response to therapy, and hence, we suggest that rituximab dosing in MN should be tailored to PLA2R levels rather than CD-19 depletion. The limitations of the study include the small sample size and observational nature of the study. The effect of other antiproteinuric measures like ACE-I/ARBs could not be avoided, and the impact of prolonging the follow-up period to 24 months on the remission rates needs further study.

Conclusion

Therapy with rituximab was effective in inducing remission in both treatment naïve and previously treated patients of primary MN. Low-dose rituximab was effective in achieving sustained B cell depletion. However, there was no significant association between CD-19 B cell depletion and attaining remission. A lower dose of rituximab but with additional repeat doses monitoring PLA2R may provide even more promising results with lower adverse events.

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Conflicts of interest

There are no conflicts of interest.

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Visual Abstract