Brief Report

Comparison of the Clinical Efficacy of Rituximab Infusion and Dexamethasone-Cyclophosphamide Pulse Therapy and Their Effect on Serum Th1, Th2, and Th17 Cytokines in Pemphigus Vulgaris–A Prospective, Nonrandomized, Comparative Pilot Study

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Abstract

Background: Rituximab infusion and dexamethasone-cyclophosphamide pulse (DCP) are the two most popular regimens used in pemphigus vulgaris (PV) in India. **Objective:** The present study compared the clinical efficacy of rituximab and DCP in Indian PV patients and their effects on serum Th1,2, and 17 cytokine levels. **Materials and Methods:** A total of 37 patients received DCP (Group A, *n* = 22) or rituximab (Group B, rheumatoid arthritis protocol (*n* = 15)) as per patients' preference. They were monitored for clinical response, adverse events (AEs), changes in serum anti-desmoglein-1,3 antibody titers and Th1,2 and 17 cytokine levels at baseline and weeks 20 and 52. **Results:** The proportion of patients attaining disease control, remission, and relapse in groups A and B were 82% and 93%; 73% and 93%; and 27% and 50%, respectively, after a median duration of 2 months each for disease control; 4 and 4.5 months for remission; and 5 and 7 months for relapse post remission. The musculoskeletal AEs were the highest in the two groups. Significant and comparable decreases in anti-dsg1 and 3 titers from baseline to weeks 20 and 52 were observed in both groups. Th1 and Th17 cytokine levels decreased, while Th2 cytokines increased post-treatment in both groups. However, no correlation was found between change in body surface area of involvement by PV and anti-dsg titers and cytokine levels before and after therapy in both groups. **Conclusion:** Comparable clinical efficacy between DCP and rituximab was observed.

Keywords: *Rituximab, dexamethasone-cyclophosphamide pulse, pemphigus vulgaris, cytokines*

Introduction

Pemphigus vulgaris (PV) is caused by pathogenic antibodies against desmoglein (Dsg) 1 and 3. Extensive PV is a dermatological emergency, mortality rate being 2.36-fold higher than the general population, necessitating prompt treatment.[1] Dexamethasone-cyclophosphamide pulse (DCP) and anti-CD20 chimeric monoclonal antibody rituximab are the two most popular treatment modalities in India. DCP is inexpensive, and produces rapid disease control and remission with milder side effects than daily oral steroids.[2] Rituximab has demonstrated good results in severe and recalcitrant PV, but its use is limited by moderately high cost.[3]

Both Dsg-specific B- and T-cells are necessary for the production of pathogenic autoantibodies, and the role of T-cell subsets and their cytokines is being increasingly recognized.[4] Cytokines can be categorized as Th1 (interleukin [IL]-2, interferon [IFN]-gamma), Th2 (IL4, IL10), and Th17 (IL-17, IL-23) type.

Herein, we report the results of a prospective nonrandomized, pilot study conducted to compare the efficacy of DCP with rituximab in PV, and their effect on serum Th1,2 and 17 cytokine levels.

Materials and Methods

Study design

An open-label, nonrandomized, prospective, comparative, pilot study was conducted in the dermatology department of a tertiary care hospital following institution ethics committee

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approval (NP/346/2012and RP-11/2012) and clinical trial registration (CTRI/2012/09/004047).

Participants

A total of 37 active mucocutaneous PV patients between 18 and 80 years, diagnosed on the basis of clinical features, histopathology, and ELISA test for anti-Dsg 1 and 3 antibodies, with completed family, were recruited over 2 years post written informed consent. A detailed history, general physical, dermatological, and systemic examination were undertaken, and extent of disease (cutaneous–based on body surface area involvement, mucosal–based on a number of mucosal erosions) and functional disability were recorded.

Intervention groups

The patients were allocated to two groups, i.e., group A (DCP, $n = 22$) and group B (rituximab, $n = 15$), based on patients' preference. Both groups concomitantly received oral prednisolone in a tapering schedule and oral cyclophosphamide 50 mg per day for a year. The details of treatment regimens are depicted in Figure 1.

Outcomes

Clinical Assessment: The clinical parameters assessed were proportion of patients achieving and time to achieve disease control (new lesions cease to form and established lesions begin to heal), remission (complete absence of new or established lesions on or off therapy), and relapse (appearance of three or more new lesions that do not heal within 1 week). The adverse events (AEs) were classified as per CTCAE, 5.0 Organ system classification, and assessed by Naranjo causality, modified Hartwig's severity, and Schedule Y, CDSCO seriousness scores. Clinical assessment was undertaken at weeks 2,4,8,12, and 16, until week 52 and further observed for atleast a year or until disease relapse, whichever was earlier.

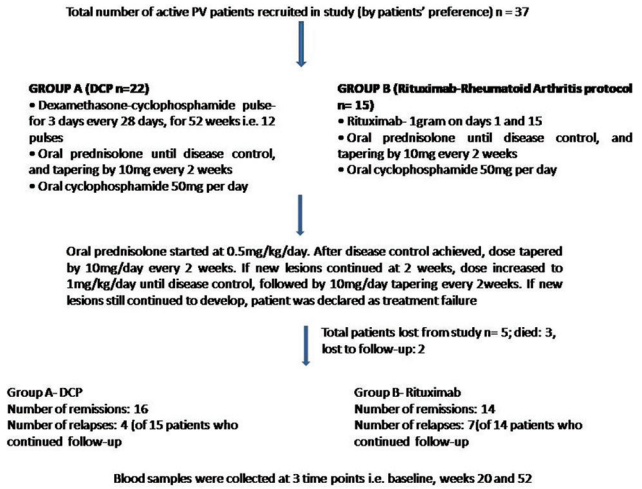

Figure 1: Flow of patients in the study

Immunological Assessment: Serum Dsg 1,3 antibody titers were assessed using commercially available ELISA kits (EUROIMMUN AG, Lübeck, Germany), and various cytokines; IFN-γ, IL-2, 4, 10, 17, and IL-23 were measured by highly sensitive ELISA method (G-Biosciences, MO, USA). They were measured at baseline and weeks 20 and 52.

Statistical methods

Statistical analysis was done using Stata version 14.1 software (Stata Corp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP.). Per protocol analysis was undertaken. The details of the statistical tests used are mentioned in respective footnotes of tables. A P value of < 0.05 was considered significant.

Results

Baseline demographic, clinical, and immunological characteristics of the two groups are depicted in Table 1. The two groups were comparable in terms of median time to achieve disease control, remission and relapse, and proportion of patients achieving them [Table 2]. The total cumulative dose (TCD) of oral prednisolone in the two groups was also comparable.

There was a significant reduction in median anti-dsg1 and dsg3 titers at weeks 20 and 52 from baseline within both groups A (DCP) and B (rituximab). On comparison between the groups, median percentage reduction in anti-dsg1 titers at weeks 20 and 52 and anti-dsg 3 at week 20 were comparable; however, significantly greater reduction in anti-dsg3 titer at week 52 was observed in the DCP group ($P = 0.03$) [Table 3]. On correlation analysis by Spearman rank correlation test between change in body surface area and change in dsg1 and 3 levels and cytokine levels at weeks 20 and 52 from baseline, it was found to be independent in both groups.

Musculoskeletal AEs were the commonest (DCP group–16.67%, rituximab group–25.3%), followed by dermatological events (13.78%, 10.84%), infections and infestations (13.76%, 15.66%), and general disorders (13.76%, 13.25%) [Figure 2]. On comparing the proportion of AEs with respect to causality in two groups, 89% and 11% had possible and probable causal association in the DCP group, whereas 98% had possible and 1% each had a probable and definite causal association with treatment in the rituximab group. The proportion of mild, moderate, and severe AEs were 26.3%, 36.2%, and 27.3% in the DCP group and 0, 5.07%, and 3.03% in the rituximab group.

A total of seven AEs were classified as serious. These included three deaths (two in DCP and one in rituximab group; one each in the two groups due to sepsis, and another in the DCP group due to gastric carcinoma post remission). The Naranjo causality assessment showed a "possible" causal association

Table 1: Comparison of demographic, baseline clinical and immunological profile of pemphigus vulgaris patients in two groups

Parameter	Group A (DCP) (*n*=22)	Group B (Rituximab- RA protocol) (*n*=15)	P
Age in years Median (range) Mean±SD	41 (25-67) 42.27±11.52	47 (30-68) 47.47±9.33	0.16
Sex			
Male *n* (%)	8 (36.36)	4 (26.67)	0.72
Female *n* (%)	14 (63.64)	11 (73.33)	
Duration oral lesions (months) Median (range) Mean±SD	6 (0.3-180) 12.736±16.892	13 (1-30) 34.506±62.277	0.16
Duration cutaneous lesions (months) Median (range) Mean±SD	6 (0.2-180) 16.340±37.973	9 (0.2-240) 31.746±63.287	0.59
Other sites of involvement *n* (%)			
Genitalia	6 (27.3)	2 (13.3)	-
Nasal	9 (40.9)	4 (26.7)	
Perianal	2 (9.1)	1 (6.7)	
Ocular	6 (27.3)	1 (6.7)	
Co-morbidities *n* (%)			
HTN	1 (4.5)	2 (13.3)	-
DM	1 (4.5)	3 (20)	
TB	1 (4.5)	0	
Liver disease	1 (4.5)	0	
Neurological	0	1(migraine) (6.7)	
Previous treatment *n* (%)			
Treatment naïve	0	0	-
Non-specific	6 (27.3)	2 (13.3)	
Daily oral steroids	17 (77.3)	13 (86.7)	
Pulse steroids	4 (18.2)	8 (53.3)	
Immuno-suppressants	8 (36.4)	11 (73.3)	
Topical steroids	4 (18.2)	4 (26.7)	
Tzanck showing acantholytic cells *n* (%)			
Positive	22 (100)	15 (100)	-
Negative	0	0	
Histopathology *n* (%)			
Positive	22 (100)	14 (93.3)	-
Negative	0	1 (6.7)	
DIF *n* (%)			
Positive	13 (59.1)	8 (53.3)	-
Negative	4 (18.2)	1 (6.7)	
Not available	5 (22.7)	6 (40)	
% Body Surface Area (BSA) Median (Range) Mean±SD	6 (2-20) 6.545±3.826	4 (1-30) 7.467±8.149	0.28
anti- Dsg1 titre (RU/ml) Median (Range) Mean±SD	220.99 (3.71-353.28) 206.497±80.395	211.5 (21.5-336.5) 203.091±94.411	0.97
anti- Dsg3 titre (RU/ml) Median (Range)Mean±SD	276.50 (58.33-466.57) 286.982±105.556	291 (32-395.17) 260.029±105.527	0.59
IFN γ Median (Range) Mean±SD	37.75 (11.8-142) 45.328±33.674	56.5 (23.5-325.11) 97.980±92.718	0.02
IL-2 Median (Range) Mean±SD	20.65 (0-110.56) 31.070±34.035	20.06 (1.39-117.42) 36.765±38.376	0.69
IL-4 Median (Range) Mean±SD	41.5 (7.23-236) 58.259±56.976	65 (18-145) 63.12±37.913	0.27
IL-10 Median (Range) Mean±SD	20.28 (1.5-100.8) 25.193±22.988	21.5 (3-105) 26.460±24.872	0.001
IL-17 Median (Range) Mean±SD	81.5 (37.67-380) 103.848±68.490	96 (14.28-548.57) 153.373±144.818	0.42
IL-23 Median (Range) Mean±SD	655 (236-1945) 795.309±481.775	735 (216-1689) 754.733±406.488	0.89

Desmoglein 1 and 3 titers are measured in RU/ml (relative units per milliliters) and cytokines in pg/ml (picogram per milliliters).
DCP - dexamethasone–cyclophosphamide pulse, RA - rheumatoid arthritis, *n* - number, HT - hypertension, DM - diabetes mellitus, TB - tuberculosis, DIF - direct immunofluorescence, IL - interleukin, IFN - interferon, Dsg - desmoglein. The test used to compare two groups: Two-sample Wilcoxon rank-sum (Mann–Whitney) test

Table 2: Comparison of clinical results in the two treatment groups

Parameter	Group A (DCP) (*n*=22)	Group B (Rituximab- RA protocol) (*n*=15)	*P value*
Number of patients achieving disease control *n* (%)	18 (81.8) (*n*=2 died; 2 LTFU)	14 (93.3) (*n*=1 died)	0.26
Time to disease control (months)			
Median (Range)	2 (0.5–11)	2 (0.5–8)	0.77
Mean±SD	2.778±2.787	2.607±2.194	
Number of patients achieving disease remission *n* (%)	16 (72.7)	14 (93.3)	0.23
Time to disease remission (months)			
Median (Range)	4 (2–11)	4.5 (0.5–8)	0.73
Mean±SD	5.5±3.265	4.25±2.007	
Duration of follow-up (months)			
Median (Range)	13 (0.5–37)	20 (1–33)	0.05
Mean±SD	15.704±11.627	21.8±9.344	
Time to relapse (post remission) in months			
Median (range)	5 (2–10.6)	7 (0.5–17)	0.63
Mean±SD	5.65±3.681	7.357±5.977	
Relapse *n* (%)	(*n*=15 continued follow-up) 4 (26.6)	(*n*=14 continued follow-up) 7 (50)	0.06
Occurrence of relapse *n* (%)	Within treatment period (*n*=2) / Post-treatment period (*n*=2)	Within treatment period (*n*=5) / Post-treatment period (*n*=2)	
Total cumulative dose of oral prednisolone (mg)			
Median (Range)	2222.5 (0–5750)	2325 (0–10095)	0.69
Mean±SD	2612.5±1926.118	3141.071±2592.509	

LTFU=Lost to follow up, mg = (milligrams) SD- Standard deviation, DCP- dexamethasone-cyclophosphamide pulse, RA- rheumatoid arthritis. The comparison has been done by two-sample Wilcoxon rank-sum (Mann–Whitney) test

with the respective group. Four additional patients required hospitalization/prolongation of hospitalization (one patient each in the two groups had disease exacerbation, one on rituximab had facial swelling and breathlessness post infusion and the other developed breast carcinoma (Miller Payne Grade III)).

Immunological results

Among serum Th1 cytokines, median IFN-γtiters significantly reduced at weeks 20 and 52 from baseline in the two groups. The median IL-2 level slightly decreased in the DCP group at weeks 20 and 52, while in the rituximab group, the level increased slightly at week 20 and then decreased and came at par with baseline at week 52. The median titers of IL-4 and IL-10 significantly increased at weeks 20 and 52 from baseline in the rituximab group, while with DCP there was a decrease at week 20 and then an increase (higher than baseline) at week 52 (significant change in IL-4 level). The Th17 cytokine IL-17 reduced at weeks 20 and 52 from baseline in both groups, but the decrease was significant only in the DCP group. Serum IL-23 levels significantly decreased at weeks 20 and 52 in the two groups. On comparison between the two groups, a comparable median percentage change at weeks 20 and 52 from baseline was observed in Th1 and 17 cytokine levels. However, among TH2 cytokines, IL-4 significantly increased from baseline (P = 0.03) at week 52 in DCP group, while IL-10 was significantly higher at week 20 from baseline ($P < 0.001$) in the rituximab group [Table 4].

Discussion

Considering high morbidity and mortality in PV, treatment strategies need to be well defined. DCP revolutionized PV treatment in India; however, frequent hospital visits and a few distressing symptoms are its limitations. Rituximab is effective in severe and refractory PV with limited hospital visits. It has been found to significantly reduce the time to attain disease remission with a significantly greater proportion of patients achieving remission and also a decrease in total cumulative steroid dose when compared with oral corticosteroids or nonsteroidal conventional immunosuppressants.[5-8] However, its high cost may be a limiting factor in resource-limited settings, necessitating comparison between the two agents.

In our study, since treatment allocation was based on patient preference, the number of patients prospectively recruited over a fixed time period, i.e. 2 years in the two groups were dissimilar. A slight variation from the conventional guidelines was that we continued oral prednisolone at the initial dose until disease control rather than remission before tapering, since oral cyclophosphamide was also being concomitantly administered. We administered daily cyclophosphamide in the rituximab arm too, to balance

Table 3: Comparison of serum anti-desmoglein 1 and 3 antibody titers in the two treatment groups

Serum anti-Dsg1, 3 antibody titers			Baseline	Week 20	Week 52	% change at Week 20	% change at Week 52
Group A (DCP) (*n*=22)	Anti-Dsg1	*n*	22	15	14		
		Median (range)	220.985 (3.71–353.28)	121.85 (0–245.52)	0.225 (0–295.85)	37.768 (−36.726–100)	99.912 (−730.458–100)
		Mean±SD	206.497±80.395	118.468±94.551	31.173±77.524	47.023±43.01	34.692±213.202
		P within the group[#]	-	0.003	0.002		
	Anti-Dsg3	*n*	22	15	14		
		Median (range)	276.495 (58.33–466.57)	232.5 (0–350.76)	114.02 (0–329.8)	30.803 (−2.636–100)	64.007 (15.146–100)
		Mean±SD	286.982±105.556	183.375±117.456	115.161±105.359	39.961±37.229	63.180±32.135
		P within the group*	-	0.001	0.001		
Group B (Rituximab-RA protocol) (*n*=15)	Anti-Dsg1	*n*	15	14	13		
		Median (range)	211.5 (21.5–336.5)	19.5 (0–211.5)	0.24 (0–201)	92.979 (9.424–100)	99.865 (27.749–100)
		Mean±SD	203.091±94.411	55.297±68.531	31.621±71.115	76.425±30.561	89.374±23.583
		P within the group[#]	-	0.001	0.001		
	Anti-Dsg3	*n*	15	14	13		
		Median (range)	291 (32–395.17)	218.595 (0–330.71)	208.28 (7.86–410.14)	32.776 (−38.268–100)	33.471 (−82.755–96.6)
		Mean±SD	260.029±105.527	186.73±111.026	189.730±134.901	30.708±38.158	30.056±49.419
		P within the group*	-	0.016	0.039		
P	Anti-Dsg1[a]		0.97	0.16	0.74	0.15	0.71
	Anti-Dsg3[b]		0.59	0.91	0.09	0.81	0.03

[#]and * represents *P* values for change in median titers of desmoglein 1 and 3 at weeks 20 and 52 within the groups A and B, while [a,b]represents *P* values for median percentage change in desmoglein1 and 3 titers at weeks 20 and 52 between the groups. The comparison has been done by two-sample Wilcoxon rank-sum (Mann–Whitney) test. Dsg -desmoglein, DCP - dexamethasone-cyclophosphamide pulse, RA - rheumatoid arthritis, SD – Standard deviation

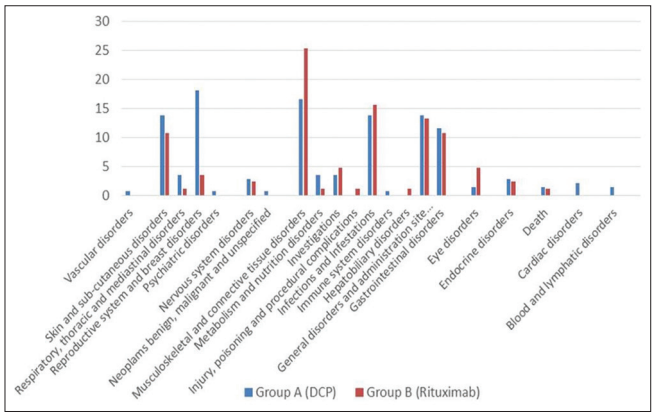

Figure 2: Graph depicting proportion of adverse events in the two groups classified as per organ system criteria. The *P* values were calculated by Chi-square test

the daily immunosuppressive being received in both arms. Some PV guidelines do not recommend the addition of daily immunosuppressive to the rituximab protocol for fear of a higher incidence of infection. However, so far there are no head-to-head studies in this regard. Moreover, methotrexate and cyclophosphamide in combination with rituximab in DMARD-resistant rheumatoid arthritis (RA) patients have been successfully used, without increased risk of infection.[9] Since cyclophosphamide was administered to both groups, only patients with completed families were included.

In the present study, the median time to disease remission in the DCP group was 4 months, which is comparable to other studies with remissions attained at 3–4.2 months.[10,11] Rituximab showed a median time of 4.5 months to attain remission, comparable to 4.36–8.8 months in previous studies.[12,13] These variations in remission rates may be attributed to heterogeneity in the treatment regimens used.

The majority of Indian studies have reported remission rates of 40–100% with DCP, ours being 73%.[11] Few Western studies have shown inferior results. Zivanovic *et al*. showed only 60% remission with DCP, while Shaik *et al.* reported it in 50% of patients receiving either methylprednisolone or DCP along with oral cyclophosphamide.[14,15] Rituximab in our study showed a higher, i.e. 93.3% remission rate, with previous studies reporting it in 74–87% of patients after a single cycle.[13,16,17]

In our study, the proportion of patients achieving disease control with DCP was lower (82%), though not statistically significant, compared to 93% with rituximab after a median of 2 months in both groups. This was much shorter than that reported by Roga and Augustine (6.7months) with pulse therapy since both PV and PF patients were included, and the latter took more time to achieve control.[18] Sethy *et al*. reported disease control after a mean of 1.5 months (range 0.5–7months)

Table 4: Comparison of serum Th1 (IFN gamma, IL-2), Th2 (IL-4, IL-10) and Th17 (IL-17, IL-23) cytokine levels at weeks 20 and 52 in the two treatment groups

Serum cytokine levels	Group		Baseline	Week 20	Week 52	% change at Week 20	% change at Week 52
IFN gamma	Group A (DCP) (*n*=22)	*n*	22	15	14		
		Median (range)	37.75 (11.8-142)	20 (0-63)	13.5 (1.22-45.11)	32.692 (-207.317, 100)	67.842 (-112.891, 97.719)
		Mean±SD	45.328±33.674	22.429±16.873	16.381±13.001	11.528±91.553	44.123±57.779
		P within the group[#]	-	0.036	0.005		
	Group B (Rituximab) (*n*=15)	*n*	15	14	13		
		Median (range)	56.5 (23.5-325.11)	22.945 (15-125.39)	19.285 (0-58)	51.547 (0.221-93.574)	78.614 (-2.654,100)
		Mean±SD	97.980±92.718	47.417±42.056	19.750±16.106	44.835±29.081	62.744±40.347
		P within the group*	-	0.001	0.004		
	P[a]		0.02	0.09	0.58	0.57	0.25
IL-2	Group A (DCP) (*n*=22)	*n*	22	15	14		
		Median (Range)	20.645 (0-110.56)	14.1 (0-41.78)	12.44 (3.03-45.36)	-16.501 (-1328.571, 89.093)	12.414 (-336.227, 88.075)
		Mean±SD	31.070±34.035	17.061±14.257	16.7130±13.912	-127.930±377.125	-33.832±125.895
		P within the group[#]	-	0.507	1		
	Group B (Rituximab) (*n*=15)	*n*	15	14	13		
		Median (range)	20.06 (1.39-117.42)	23.86 (9.75-73.37)	20.475 (4-96.22)	15.496(-386.842,76.222)	19.994 (-1714.39, 81.922)
		Mean±SD	36.765±38.376	26.849±17.767	30.240±27.377	-47.409±164.198	-282.901±588.868
		P within the group*		0.136	0.721		
	P[b]		0.69	0.15	0.15	0.51	0.59
IL- 4	Group A (DCP) (*n*=22)	*n*	22	15	14		
		Median (Range)	41.5 (7.23-236)	40 (7.16-147)	88.5 (28.41-342)	-12.121 (-854.356-28.571)	-169.128 (-1075.657-26.315)
		Mean±SD	58.259±56.976	51.564±37.876	110.605±82.526	-166.815±300.166	-382.787±414.723
		P within the group[#]	-	0.02	0.001		
	Group B (Rituximab) (*n*=15)	*n*	15	14	13		
		Median (Range)	65 (18-145)	81.5 (6.24-187)	94 (2.36-266)	-18.878 (-195-71.828)	-46.153 (-735.214-93.444)
		Mean±SD	63.12±37.913	77.542±50.996	102.783±66.102	-32.070±74.035	-126.239±216.661
		P within the group*	-	0.035	0.023		
	P[c]		0.27	0.13	0.88	0.4	0.03
IL-10	Group A (DCP) (*n*=22)	*n*	22	15	14		
		Median (Range)	20.28 (1.5-100.8)	17 (7-61.17)	26.92 (16.85-58.24)	-21.428 (-900, 64.420)	-98.485 (-2732,72.474)
		Mean±SD	25.193±22.988	22.390±13.379	31.448±13.934	-88.966±229.853	-368.782±734.232
		P within the group[#]		0.088	0.177		
	Group B (Rituximab) (*n*=15)	*n*	15	14	13		
		Median (Range)	21.5 (3-105)	26.855 (13-78.28)	32.7 (17.57-99.71)	-87.356 (-539.25-77.828)	-84.697 (-2392.75-80.276)
		Mean±SD	26.460±24.872	36.169±21.533	41.253±28.968	-131.218±177.905	-292.669±659.317
		P within the group*	-	0.001	0.001		
	P[d]		0.001	0.06	0.64	<0.001	0.06

Contd...

Table 4: Contd...

Serum cytokine levels		Group	Baseline	Week 20	Week 52	% change at Week 20	% change at Week 52
IL-17	Group A (DCP) (*n*=22)	*n*	22	15	14		
		Median (Range)	81.5 (37.67-380)	48 (4.75-117.215)	67 (0-171)	51.7006 (12.709, 87.390)	72.631 (-175.806-100)
		Mean±SD	103.848±68.490	52.939±35.645	59.384±55.442	50.242±25.516	42.776±74.879
		P within the group[#]	-	0.001	0.023		
	Group B (Rituximab) (*n*=15)	*n*	15	14	13		
		Median (Range)	96 (14.28-548.57)	66.44 (0-298.57)	83 (0-206)	44.661 (-151.388-100)	83.776 (-1335.574-100)
		Mean±SD	153.373±144.818	91.765±91.782	76.846±83.218	21.261±77.489	-63.244±389.006
		P within the group*	-	0.064	0.173		
	P[e]		0.42	0.54	0.95	0.63	0.93
IL-23	Group A (DCP) (*n*=22)	*n*	22	15	14		
		Median (Range)	655 (236-1945)	450 (155-1605)	207.5 (92-910)	40.154(-0.536-62.323)	65.419 (25.632-85.714)
		Mean±SD	795.309±481.775	524.857±363.885	294.714±244.828	34.516±20.184	62.039±19.175
		P within the group[#]	-	0.002	0.001		
	Group B (Rituximab) (*n*=15)	*n*	15	14	13		
		Median (Range)	735 (216-1689)	473.5 (126-995)	223 (123-1011)	35.097(-2.721-72.302)	59.314 (-37.551-86.796)
		Mean±SD	754.733±406.488	489.928±305.113	313.846±257.690	33.600±24.589	56.114±33.2
		P within the group*		0.002	0.004		
	P[f]		0.89	0.98	0.62	0.84	0.89

[#],*represents *P* values of change in median cytokine levels at weeks 20 and 52 within the two groups, while [a–f]represents *P* values of the median percentage change in cytokine levels at weeks 20 and 52 between the two groups. DCP - dexamethasone-cyclophosphamide pulse, RA - rheumatoid arthritis, IL - interleukin, IFN - interferon, Th - T-helper

with DCP.[11] Disease control at 2 months (range 1.33–3months) with rituximab as seen in our study was also achieved by Kanwar *et al.*[19]

In our study, 27% of DCP patients who had undergone remission relapsed after a median of 5 months of achieving remission, while relapse rate with rituximab was 50%, observed after a median of 7 months post remission. The higher relapse rate recorded in the rituximab group was probably due to a relatively longer duration of follow-up post treatment. Shaik *et al*. reported 26% relapse rate after DCP, while relapse rates of 36% and 65% with rituximab RA protocol have been observed in other studies.[15-17]

The median TCD of oral steroids (used as a surrogate marker of treatment efficacy) in the two groups was comparable. The addition of azathioprine, mycophenolate mofetil, or intravenous cyclophosphamide pulse to daily prednisolone reduced the TCD to 7712, 9798, and 8276 mg, respectively, compared to only prednisolone that required a TCD of 11631 mg to produce remission in PV.[20] Our median TCD of 2325 mg with the RA protocol was lower than that reported in previous studies, which was 2432, 3535, and 3496 TCD respectively[13,17,19] This could probably be the effect of concomitant use of daily cyclophosphamide in our patients.

Deaths occurred in two (9%) patients in the DCP group, due to sepsis or gastric carcinoma post remission (off pulse). One death (3.4%) occurred in the rituximab group due to sepsis. Previous studies have reported mortality rates of 3.8–11% in patients receiving DCP, compared to 1.09% with the RA protocol of rituximab.[18,21-24]

Both therapies produced a significant and comparable reduction in serum anti-dsg1 and 3 titers at weeks 20 and 52, with, however, greater reduction in anti-dsg3 titer at 52 weeks with DCP. Kanwar *et al*. observed a 98% reduction in mean anti-Dsg1 and 67% in anti-Dsg3 titer with rituximab after a mean of 33.4 weeks.[19]

Several studies have reported the role of cytokines in PV pathogenesis. With the aim of studying the effect of rituximab and DCP on Th1 and Th2 cytokines, this objective was included. This was a good opportunity to recognize treatment targets and improve our understanding of PV pathogenesis. As far as can be ascertained, there have been no studies undertaken in a prospective and comparative manner to study the effect of DCP and rituximab on serum cytokine levels.

We observed a significant but comparable decrease in IFN-γ at weeks 20 and 52 in both our study groups. IL-2 also decreased though not significantly in both groups. Verhoef *et al.* observed a transient but significant decrease in IFN-γ by inhibiting mRNA levels in normal human lymphocytes after dexamethasone treatment in RA patients.[25] Treatment with 10μg/ml of rituximab in an *in vitro* setting in activated peripheral blood mononuclear cells (PBMCs) co-cultured with RA synoviocytes, led to a 62.8% decrease in serum IFN-γ levels.[26]

Dexamethasone-mediated transcriptional inhibition of IL-2 in normal human lymphocytes with a reduction in T cell proliferation, resulting in a decrease in B cell clone expansion and autoantibody titers, has been observed.[25,27] A significant fall in mean IL-2 titers post rituximab has also been observed in RA, which correlated with disease activity.[28]

IL-4 exerts its anti-inflammatory effect by suppressing Th1 responses. We observed a significant increase in median IL-4 level in both the groups post treatment. Verhoef *et al*. observed falling titers of IL-4 on days 7 and 42 post high-dose dexamethasone in RA patients.[25] A study on the effect of rituximab on functional activities of PBMC, isolated monocytes of Type 1 diabetes patients showed significant upregulation in IL-4 and IL-10.[29] Our study did not show a significant rise in serum IL-10 post DCP; however, a significant increase was seen following rituximab, similar to that reported by Hamouda *et al.* in Type 1 diabetes patients.[29]

The role of amalgamation of Th1/Th17 immune response has been validated in our study. Following treatment with both DCP and rituximab, IL-17 and IL-23 titers were reduced. A decrease in IL-17 was observed in 12 RA patients with a combination treatment of rituximab and 125 mg IV methylprednisolone after 12 weeks, and it corroborated with improvement in severity scores.[30]

In our study, it is possible that changes in cytokine levels may have also been influenced by the concomitant administration of oral prednisolone (in tapering doses) and cyclophosphamide. However, their use in both groups in the same schedule would certainly make a reliable comparison possible on the effect of rituximab and DCP on serum cytokine profile.

Limitations

They included a small sample size (can be responsible for Type 2 error), nonrandomized design, and a shorter follow-up period of 1 year post remission.

Conclusions

The present study suggests that both DCP and rituximab in combination with oral steroid (in tapering schedule) and oral cyclophosphamide are similar in their ability to influence disease progression and serum levels of pro- and anti-inflammatory cytokines in PV.

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Conflicts of interest

There are no conflicts of interest.

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