# A COMPARISON BETWEEN 9 MONTH VERSUS 6 MONTH CYCLOPHOSPHAMIDE INDUCTION CHEMOTHERAPY IN THE MANAGEMENT OF LUPUS NEPHRITIS IN A GOVERNMENT TERTIARY PEDIATRIC HOSPITAL IN THE PHILIPPINES

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## ABSTRACT

**BACKGROUND:** Lupus nephritis is very common complications in SLE, with clinical symptoms of renal involvement occurring in 30%–70% of patients. Outcomes in children with proliferative lupus nephritis (PLN) show 9–15% progress to end-stage renal disease (ESRD) at 5 years.

**OBJECTIVES:** This study compared the outcome of children and adolescent patients with lupus nephritis treated with 9 month versus 6 month induction of cyclophosphamide therapy. Renal frequency and adverse effects of IV cyclophosphamide during and after induction therapy were described and determined.

**DESIGN:** Retrospective Cohort Study

**SETTING:** Tertiary Hospital

**METHODS:** Retrospective cohort study comparing 6 and 9 month protocol of IV cyclophosphamide for lupus nephritis were conducted in a government tertiary pediatric hospital in the Philippines. A total of 39 patients with lupus nephritis were gathered, 23 patients underwent 6 months and 16 patients underwent 9 months protocol.

**RESULTS:** The comparison of two protocols in the administration of intravenous cyclophosphamide (IVCY) did not show significant difference between the two in terms of changes in GFR levels, but some evidence of a greater percent increase from baseline with the 6 months protocol post treatment were observed. Among 39 subjects, creatinine, albumin and urinalysis profile did not also differ between the two groups and levels within each group changed insignificantly over time up to 24 months. Proportion of subjects with renal flare ups, adverse effects and who expired during the study period were also essentially similar between the two groups.

**CONCLUSION:** IV Cyclophosphamide seems efficacious if given at the very beginning of the flare and at the start after patient was diagnosed with lupus nephritis. No statistically difference between the duration of the protocol. Renal flare ups and adverse effects of cyclophosphamide such as nausea, vomiting and headache were observed similarly between two protocols. Diligent follow up is needed for further studies and specificity of the results.

**KEYWORD:** systemic lupus erythematosus (SLE), IV cyclophosphamide (CYP), lupus nephritis (LN), pediatric

## INTRODUCTION

Systemic Lupus Erythematosus can occur in children as young as 4 years of age, with the majority of cases occurring after 12 years of age. Lupus nephritis is very common complications in SLE, with clinical symptoms of renal involvement occurring in 30%–70% of patients. Outcomes in children with proliferate lupusnephritis (PLN) show 9–15% progress to end-stage renal disease (ESRD) at 5 years. Thus early diagnosis and prompt treatment is required to prevent the progression of lupus nephritis to end stage renal disease. One of the recommended treatment is induction therapy with cyclophosphamide for 6 months. Unfortunately, there are no large, well-designed studies of induction therapy duration and its side effects and outcome for pediatric patient with lupus nephritis.

Monthly intravenous cyclophosphamide (IVCY) has been a recommended therapy for severe lupus nephritis or neurological flare-ups in lupus patients. But the optimal treatment regimen and duration remains unknown. DeBandt M, et.al report their experience in 1994, an open study of 37 patients treated with monthly IVCY, after six months of IVCY, a significant improvement was noticed, in terms of reduced serum creatinine and proteinuria. Serum creatinine was the best predictor of long-term renal outcome. Its usefulness was obvious at six months among clinical and biological data in patients with severe lupus nephritis or neurological flare.(1)

Based on the randomized control trials on adults published by the National Institutes of Health (NIH), most clinicians advocate the use of intravenous (i.v.) cyclophosphamide (CYP) for induction of lupus nephritis, as it has been shown to improve longterm renal outcomes (3,4). Long-term immunosuppression has been shown to improve renal survival and reduce the risk of renal flares (6,7). On another study by Niadet,P. three regimens were compared at the NIH to 65 patients with severe lupus nephritis, defined by an impairment of renal function and/or a high activity index on renal biopsy: (1) six monthly pulses of cyclophosphamide, (2) the same regimen followed cyclophosphamide pulses every 3 months for 2 additional years, and (3) six monthly pulses of methylprednisolone without cyclophosphamide. The probabilityof relapse of lupus nephritis was significantly higher in patients receiving cyclophosphamide pulses for 6 months compared with those receiving the long-course cyclophosphamide regimen (55% versus 10% after 5 years of observation).(10)

Azkenazi et.al had a retrospective review of charts of 25 patients between 1990 and 2004 who had received 9 monthly doses of cyclophosphamide induction treatment. Clinical and biopsy results greatly improved after 9 monthly intravenously administered cyclophosphamide pulses in most children with class IV Proliferative Lupus Nephritis. Those who did not improve were at risk for flares and progression of disease. The tailoring of therapies based on findings from a biopsy after induction may improve outcomes.(2)

From the study of Tangnararatchakit K, thirty one children with severe lupus nephritis who received intravenous pulse cyclophosphamide for six months have been followed-up for at least 6 months. After 3 months of treatment, most patients were clinically improved as evidenced by significant improvements in 24-hour urine protein, creatinine clearance, serum creatinine, BUN, serum albumin and C3 level.

These improvements were sustained up to 18 months and renal outcome at the last follow-up (range = 6-76 months) demonstrated that twelve patients (38.7%) had complete remission, 18 patients (58.0%) still had significant proteinuria and only one had serum creatinine of 1.6 mg/dl at 42 months.(8)

This study compared the outcome of children and adolescent patients with lupus nephritis treated with 9 month induction of cyclophosphamide therapy versus 6 month induction of cyclophosphamide therapy.The frequency of occurrence of renal flare of patients with lupus nephritis treated with 6 months induction of cyclophosphamide therapy compared to 9 months therapy at the end of induction and 24 months after induction were determined.The adverse effects in induction of cyclophosphamide therapy for patients with lupus nephritis were described and determined.

**METHODOLOGY**

This was a retrospective cohort study conducted in a government tertiary pediatric hospital in the Philippines. Included in the study were patients less than 18 years old diagnosed with proliferative lupus nephritis based on the World Health Organization Criteria and confirmed by Renal Biopsy and treated with either 6 months or 9 months induction of cyclophosphamide therapy from January 2002 up to December 2004 for 6 months therapy and January 2006 to Dec 2008 for 9 months therapy.

A list of patients with a diagnosis of Lupus Nephritis were generated. Group sample sizes of at least 24 and 40 achieve 80% power to detect a difference of 20 in GFR between the 9 mos and 6 months group with the estimated group standard deviation of $26.3_2$ at the 5% level of significance using a one sided two sample t test.

Data present in PhililippineChildrens Medical Center medical records of patients with diagnosis of lupus nephritis from 2000-2008was reviewed. Data gathered were divided into two groups, those patients who underwent treatment of cyclophosphamide for 6 month alone and patients who continued the treatment for 9 months. Baseline characteristic of included patient such as age, gender, age of onset of lupus nephritis and glomerular filtration rate prior to induction therapy were included in data analysis.

The only difference for both protocol was only the duration of treatment. Patients at first were closely follow up every two weeks with CBC and urinalysis. All patients were advised to record in their notebook symptoms experienced after every therapy and followed up every month. After 1 month of treatment a repeat creatinine, CBC and urinalysis was requested. In case during treatment patient will experienced persistent adverse effects of cyclophosphamide such as nausea and vomiting, bone marrow suppression, stomach ache, diarrhea, darkening of the skin/nails, alopecia (hair loss) or thinning of hair, changes in color and texture of the hair, and lethargy. Patient will not be included in the study. After completion of 6 months or 9 months regimen, patient will have a series of laboratory workup such as estimated glomerular filtration rate using the schwartz formula, quantitated 24 hour proteinuria, C3, serum bun and creatinine, serum albumin and urinalysis.

For those with complete remission IV cyclophosphamide was discontinued and prednisone was taper. For those with partial remission,

continue IV cyclophosphamide for 3 months and include prednisone (2mg/kg/day) for 4 weeks.

Records of subjects in each group were reviewed up to 2 years after induction.

At the beginning of induction therapy,following parameters were determined and compared at the end of induction therapy thus identify response rate of pediatric patients with lupus nephritis.The same parameter at 24 month follow up after induction therapy was reviewed to determine remission or relapse rate of patients. Death of patients within the observation period of the study was included as an outcome.

Data were described using means, standard deviations and frequency counts. T-test for both paired and independent samples were used to analyse the data. For comparison of correlated continuous data of more than two groups, ANOVA one way for correlated samples was used. McNemar's test for frequency data before and after treatment was also employed. For comparison of categorical variables, chi-square and Fischer's exact test, whichever was appropriate was used. For continuous variables of paired samples of less than or = 10, we used Wilcoxon Rank Signed test .

For all tests, a 95% confidence level was considered significant.

## RESULTS

The mean age of patients diagnosed lupus nephritis on the averagewas 13yrs old with gender distribution predominantly female. Age at onset of lupus and lupus nephritis wasabout 13 yrs old also, and this is true for both groups.

**Table 1. Profile of Subjects**

| | Protocol for Cyclophosphamide Induction Chemotherapy | | |
|---|---|---|---|
| | 6 months | 9 months | P value |
| Age in yrs, mean + sd | 13.2 + 3.3 | 12.8 + 3.3 | 0.750 |
| Sex | | | |
| Male | 2 | 2 | 0.952 |
| Female | 21 | 14 | |
| Age at onset of Lupus, in yrs | 13.4 + 3.3 | 12.9 + 3.2 | 0.952 |
| Age at onset of lupus nephritis, in yrs | 13.4 + 3.3 | 13.4 + 3.4 | 0.968 |
| Interval between onset of lupus and lupus nephritis, in yrs | 0.39 years (4.7 months) | 0.5 years ( 6 months) | 0.968 |

Table 2 shows that although the baselineGFR was lower in the 6 months Protocol group compared to the 9 months protocol group, the difference was not statistically significant. Hence, baseline level was not considered as confounding, and therefore comparison of GFR levels at 6 and 24 months was done without adjusting for baseline levels.

GFR levels of both groups did not significantly differ from each other at 6 and 24 months. Within the 6 months protocol group, GFR levels increased steadily over time, but the increases at 6 and 24 months were small and were not statistically different from baseline. For the 9 months protocol group, there was noted a decrease after 9 months, and an increase at 24 months,

but this increase did not exceed baseline levels. Changes in GFR level within this group over time was not statistically significant.

With regards to percentage increase from baseline, we found significant difference between the two groups, with the 6 months protocol group showing a higher percentage increase than the 9 months protocol group ( 15% vs 0.2%) post treatment. At 24 months , however, no significant difference was found.

In terms of proportion of subjects who showed > 25% increase in GFR from baseline, we found no significant difference between the two groups at 6, 9 months and at 24 months.

**Table 2. GFR Levels After Treatment With Cyclophosphamide Induction Chemotherapy Protocols**

| | Protocol | | |
|---|---|---|---|
| | 6 months | 9 months | P value |
| GFR | | | |
| Baseline | 108.9 + 43.9 | 129.8 + 38.5 | 0.389 |
| At 6 months post treatment | 111.0 + 40.5 | 121.3 + 42.1 | 0.446 |
| At 24 months post treatment | 114.9 + 44.2 | 126.9 + 38.1 | 0.379 |
| P value | 0.592 | 0.446 | |
| % increase at 6/9 months | 15% | 0.2% | <0.05 |
| % increase at 24 months | 23% | 8% | >0.05 |
| No. and proportion of subjects who showed > 25% increase from baseline levels | | | |
| Post treatment( 6 and 9 months) | 4/23 | 2/14 | 1.00 |
| At 24 months | 6/21 | 3/14 | 0.711 |

Table 3 shows that serum creatinine levels did not significantly differ between the two groups post treatment. Within each group, the absolute average amount of decrease was also not significant. In terms of percent decrease from baseline, again we did not find significant difference between the two groups also.

For serum albumin, we found that both protocols resulted in significant increase post treatment. The percent increase from baseline, was nearly similar and statistically, there was no significant difference between the two groups. Likewise, comparing the average serum levels of albumin posttreatment to baseline levels, no significant difference was found.

**Table 3. Serum Creatinine and Albumin Levels**

| | Protocol | | |
|---|---|---|---|
| | 6 months | 9 months | P value |
| Creatinine | | | |
| Pre treatment | 63.5 + 30.3 | 68.2 + 33.4 | 0. 648 |
| Post treatment ( at 6 and 9 months) | 59 + 22.9 | 63.2 + 31.2 | 0.627 |
| P value | 0.119 | 0.226 | |
| Percent decrease | 6% | 7% | >0.05 |
| Albumin | N=10 | N=9 | |
| Pretreatment | 17.1 + 6.6 | 21.8 + 9.1 | 0.253 |
| Posttreatment | 27.2 + 6 | 29.2 + 3.0 | 0.386 |
| P value (Wilcoxon rank sign test) | <0.005 | 0.01 (one tailed) | |
| Percent increase | 58% | 33% | >0.05 |
| | | | |

- P value vertical= from t-test for matched pair
- P value horizontal = from t-test for independent samples

There was marked improvement in the urinalysis results before and after treatment for both groups. Proteinuria and hematuria were markedly improved posttreatment in both groups.

**Table 4. Results of Urinalysis Pre and Post treatment**

| | Protocol | | | |
|---|---|---|---|---|
| | 6 months | | 9 months | |
| CHON | Pre | Post | Pre | Post |
| 0-trace | 0 | 16 | 1 | 13 |
| +1-2 | 10 | 1 | 9 | 1 |
| +3-4 | 7 | 0 | 4 | 0 |
| P value = <0.0001 | | | P value= <0.0001 | |
| RBC | | | | |
| 0-1 | 1 | 10 | 2 | 13 |
| 2-6 | 3 | 6 | 3 | 1 |
| 7-11 | 7 | 1 | 5 | 0 |
| 12-16 | 2 | 0 | 1 | 0 |
| 16+ | 4 | 0 | 3 | 0 |
| P value=<0.0001 | | | P value=<0.0001 | |

*McNemar's Test

The proportion of flare-ups during the study period was higher in the 6 months protocol, but the difference was not significant. Two mortality cases were recorded, one in each group. The proportion of mortality and was not significantly different between the two groups.

**Table 4. Flare-ups and Mortality**

| | Protocol | | |
|---|---|---|---|
| | 6 months N=23 | 9 months N=16 | P value |
| + Flare | 5 (21%) | 2 (13%) | 0.677 |
| Mortality | 1 | 1 | 1.00 |

*Fischer's exact test

## DISCUSSION

This is a preliminary study done in tertiary government hospital in comparison between 9 month versus 6 month Cyclophosphamide induction chemotherapy in the management of Lupus Nephritis. The comparison of two protocols in the administration of intravenous cyclophosphamide (IVCY) did not show significant difference between the two in terms of changes in GFR levels, but we did find some evidence of a greater percent increase from baseline with the 6 months protocol post treatment. In this study a very low rate of chronic renal failure was observed. In one patient that progressed to end stage renal disease was lost to follow up for 5 months the had poor compliance to treatment due to lack of funds. According to study by Gunnarssonet. Al, prospective studies have shown that delaying the start of induction therapy more than three months after diagnosis is associated with a progression towards end stage renal disease. On the other hand, the initial response to treatment also influences the long-term evolution of the disease: complete and partial remission are accompanied by greater renal survival than in those cases with no response to treatment. Poverty may account for some of this explanation. In a population based ecological study, Ward reported that lower socio-economic areas had higher incidence of endstage renal disease due to SLE suggesting that limited access to care results in poorer SLE renal outcomes.

Creatinine, albumin and urinalysis profile among 39 subjects did not also differ between the two groups. Creatinine levels within each group changed insignificantly over time up to 24 months, but albumin levels improved significantly post treatment, for both groups.

Proteinuria and hematuria were also markedly improved posttreatment in both groups. The proportion of subjects with renal flare ups and who expired during the study period were also essentially similar between the two groups. Limitation of this study was only few numbers had quantitated 24 hour urine protein and C3 at the end of the induction therapy, some of the patients had a missed follow up schedules of protocol. In study of S. K. Annavarajula et.al, they demonstrated that a number of previously neglected or rarely studied predictors were important prognostic markers. It confirms the predictive importance of serum creatinine, 24-h urinary excretion of protein, C3, and of the activity and chronicity indices on biopsy(16).

Nausea and vomiting were nearly universal with infusion of cyclophosphamide. Hemorrhagic cystitis was not seen. No occurrence of malignancy was reported.
The proportion of flare-ups during the study period was essentially similar in the two groups. 5 of the patients had a doubling of serum creatinine and one of them was dialysis dependent. The improved outcome may have been definitely influenced by the use of cyclophosphamide. The persistence of nephritic syndrome for more than 6 months is a strong risk factor for ESRD.(17). Limitation of this study is long term follow up with

patients is not done. On the other hand 2 of the patients had renal flare after 1 month post 9months induction therapy which described as proteinuria. No literature published regarding nine months protocol.

IVCY seems efficacious if given at the very beginning of the flare and at the start after patient was diagnosed with lupus nephritis. One can gain much information by performing a protocol biopsy prior to induction and after induction therapy with mean interval of at least 2 years. Diligent follow up is needed for further studies. Its usefulness is obvious at third to six months among clinical and biological data in patients with severe lupus nephritis or renal flare. It seems that long term outcome on the renal function is not modified.

## CONCLUSION

The comparison between 9 month versus 6 month Cyclophosphamide induction chemotherapy in the management of Lupus Nephritisdid not show significant difference between the two in terms of changes in GFR levels. In this study, 39 subjects were included in the study predominantly female and age of onset of lupus nephritis was 13 years old. 4 dropouts was observed, 2 for each group. Patients were lost to follow up during treatment. Other parameters such as creatinine, albumin and urinalysis profile among 39 subjects did not also differ between the two groups. Creatinine levels within each group changed insignificantly over time up to 24 months, but albumin levels improved significantly post treatment, for both groups.

Proteinuria and hematuria were also markedly improved posttreatment in both groups. The proportion of subjects with renal flare ups such as increasing in creatinine by 50 % and proteinuria in urinalysis were observed in between months of protocol weresimilar between the two groups. Nausea, vomiting and headache are among side effects observed of cyclophosphamide in both $6^{th}$ and $9^{th}$ month protocol. Usually observed during first month of induction and eventually outgrow as treatment proceed. Anti emetics and pain relievers were given to lessen the symptoms. No significant difference on side effects observed in both protocols.
IVCY seems efficacious if given at the very beginning of the flare and at the start after patient was diagnosed with lupus nephritis. Diligent follow up is needed for further studies.

This study may have been limited by the lack of power due to insufficient sample size. In the estimation of sample size, we assumed a difference of 20 in GFR. However, results showed a much smaller difference, so the computed sample size may have lacked power to show significant difference. We also had 4 dropouts, 2 for each group. The data on their status post treatment could have improved the analysis of this study. The retrospective nature of the study poses a limitation to follow-up. A prospective type of study is recommended wherein serum creatinine, 24-h urinary excretion of protein, C3, and of the activity and chronicity indices on biopsy will be included pre and post treatment. A diligent followup of patients will be needed for the specificity of the results. A larger sample size is also needed to show significant difference.

**BIBLIOGRAPHY:**


1. De Bandt M, Goycochea MV, Meyer O, Delahousse M, Palazzo E, M'Bappé P, Kahn MF. Treatment of acute systemic lupus erythematosus with intravenous infusions of Cyclophosphamide.Value and limitations. Ann Med Interne (Paris). 1994;145(2):75-87.

2. David Askenazi& Barry Myones&AnkurKamdar& Robert Warren & Maria Perez & Marietta De Guzman & Anna Minta& M. John Hicks & Arundhati Kale Outcomes of children with proliferative lupus nephritis:the role of protocol renal biopsy. PediatrNephrol 10 ( 2007):

3. Austin HA 3rd, Klippel JH, Balow JE, le Richie NG, Steinberg AD, Poltz PH, Decker JL. Therapy of lupus nephritis. Controlled trial of prednisone and cytotoxic drugs. N Engl J Med (1986) 314:614–619

4. Donadio JV Jr, Holley KE, Ferguson RH, Ilstrup DM (1978) Treatment of diffuse proliferative lupus nephritis with prednisone and combined prednisone and cyclophosphamide. N Engl J Med 299:1151–115

5. Bogdanovic R, Nikolic V, Pasic S, Dimitrijevic J, Lipkovska-Markovic J, Eric-Marinkovic J, Ognjanovic M, Minic A, Stajic N. Lupus nephritis in childhood: a review of 53 patientsfollowed at a single center. PediatrNephrol(2004) 19:36–44

6. Weening JJ, D'Agati VD, Schwartz MM, Seshan SV, Alpers CE, Appel GB, Balow JE, Bruijn JA, Cook T, Ferrario F, Fogo AB,Ginzler EM, Hebert L, Hill G, Hill P, Jennette JC, Kong NC, Lesavre P, Lockshin M, Looi LM, Makino H, Moura LA, NagataM. The classification of glomerulonephritis in systemic lupus erythematosus revisited. J Am SocNephrol(2004) 15:241–25

7. Steinberg AD, Steinberg SC (1991) Long-term preservation of renal function in patients with lupus nephritis receiving treatmentthat includes cyclophosphamide versus those treated with prednisone only. Arthritis Rheum 34:945–950

8. Tangnararatchakit K, Tapaneya-Olarn C, Tapaneya-Olarn W.The efficacy of intravenous pulse cyclophosphamide in the treatment of severe lupus nephritis in children.J Med Assoc Thai. 1999 Nov;82Suppl 1:S104-10.

9. Hagelberg S, Lee Y, Bargman J, Mah G, Schneider R, LaskinC,Eddy A, Gladman

10. D, Urowitz M, Hebert D, Silverman E Longtermfollowup of childhood lupus nephritis. J Rheumato (2002) l29:2635–2642

11. Niaudet P. Treatment of lupus nephritis in children. PediatrNephrol (2000) 14:158–166

12. McCurdy DK, Lehman TJ, Bernstein B, Hanson V, King KK,Nadorra R, Landing BH Lupus nephritis:prognosticfactors in children. Pediatrics (1992) 89:240–246

13. Yang LY, Chen WP, Lin CY Lupus nephritis in children—a review of 167 patients. Pediatrics 94:335–340

14. Barbano G, Gusmano R, Damasio B, AlpigianiMG, BuoncompagniA, Gattorno M, Perfumo F. Childhood-onset lupus nephritis: asingle-center experience of pulse intravenous cyclophosphamidetherapy. J Nephrol(2002) 15:123–129

15. Keisha L. Gibson et.al Predictors of Relapse and End Stage Kidney Disease in Proliferative Lupus Nephritis: Focus on Children, Adolescents, and Young Adults.October 2009, doi: 10.2215/CJN.00490109

16. S. K. Annavarajula. The outcome of proliferative lupus nephritis with pulse cyclophosphamide therapy. Arthritis Rheum. 2002 Apr;46(4):995-1002.

17. Esdaile JM, Levinton C, Federgreen W, Hayslett JP, Kashgarian M. The clinical and renal biopsy predictors of long-term outcome in lupus nephritis: a study of 87 patients and review of the literature. 1989 Sep;72(269):779-833.

**FIG. 1 Box and Whisker Plots of GFR Pre-treatment, and at 6 and 24 months**

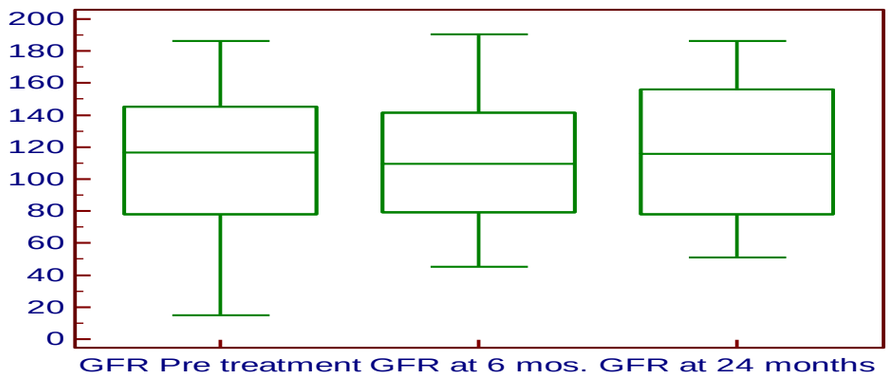


**FIG. 2. Box and Whisker Plots of GFR Pre-treatment, and at 9 and 24 months**

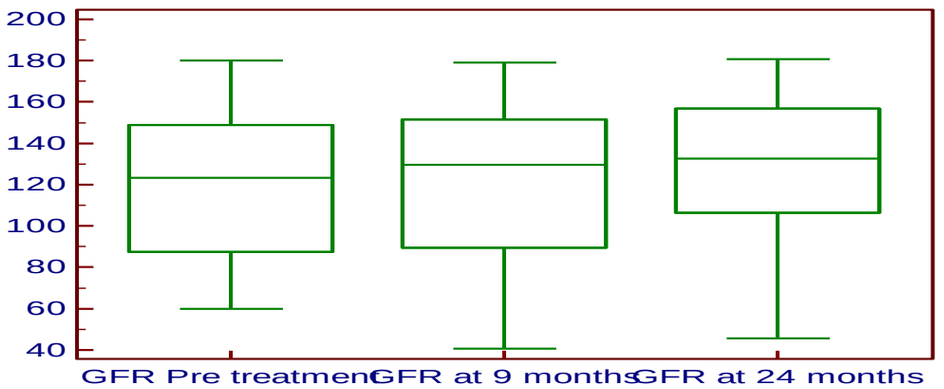

**FIG. 3. Serum Creatinine Pre and Post Treatment for Protocol 1 ( 6 months)**

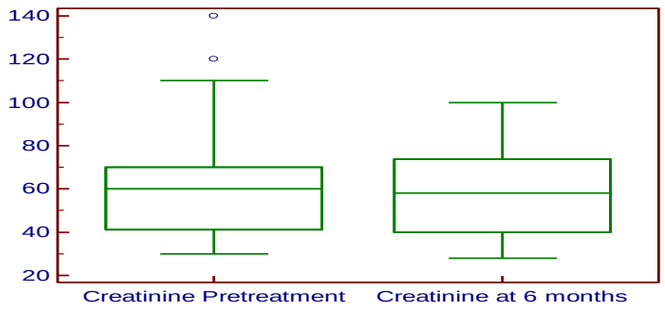


**FIG. 4. Serum Creatinine Pre and Post Treatment for Protocol 1 ( 9 months)**

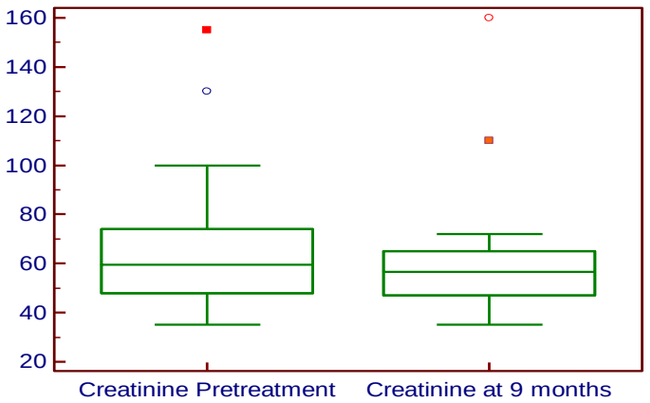


**FIG. 6. Serum Albumin Pre-treatment and at 6 months**

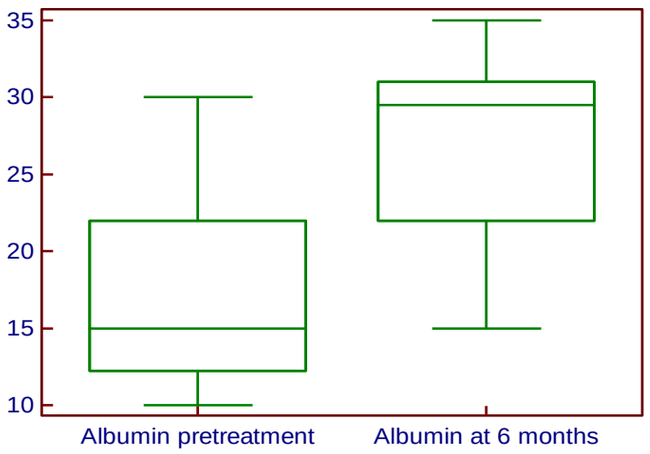


**Fig. 7. Serum Albumin Pre-treatment and at 9 months.**