

Cryoglobulinemia in Hemodialysis patients

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Abstract: Background: Many of HCV infected patients on regular hemodialysis are candidates for renal transplantation and receive immunosuppressive therapy that may influence cryoglobulin formation. **Aim of the study:** is studying the cryopositive cases among H.D patients and their association with clinical symptoms and assess whether the HCV patients on maintenance HD have abnormal immune response. **Methodology:** forty CRF patients receiving regular hemodialysis sessions divided according to the presence of HCV antibodies into two groups, group I (n=20) with positive HCV antibodies and group II (n=20) with negative HCV antibodies, both groups were tested for the presence of cryoglobulinemia. **Results:** Group (I) showed a higher percent (20%) of positive Cg than that of group (II) (5%) but with no statistically significant difference (p -value=0.34). In the whole studied group patients with positive Cg (n=5) have significantly greater mean AST, ALT and INR values than those with negative Cg (n=35) (p -value=0.017, 0.02 & 0.045, respectively). AST is a significant predictor to positive Cg with OR: 1.176 (95%CI: 1.031 – 1.341). Group I showed significantly higher percent of positive RF (n=4) than those of group II (n=1) (p -value=0.00). **Conclusion:** no significant difference regarding the cryoglobulin concentration between HCV positive and negative patients. The symptoms of cryoglobulinemia (purpura, arthralgia & generalized weakness) appear more frequently in HCV positive patients on HD with positive cryoglobulinemia. AST was found to be significant predictor for cryopositivity in HD patients.

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1. Introduction

Cryoglobulinemia refers to a pathologic condition caused by production of circulating immunoglobulins that precipitate on cooling and resolubilize on warming. It is associated with a variety of infections especially HCV, HBV, HGV, vascular disorders "SLE, polyarteritis nodosa, autoimmune thyroiditis, etc.", and Lymphoproliferative diseases "multiple myeloma, Waldenstrom macroglobulinemia, etc."¹

The prevalence of hepatitis C virus infection ranges from 1% to 3% in general population, but it is higher in hemodialysis patients, ranging from 3% to 23%. This difference can be related to the fact that patients on HD have risk factors for acquisition of HCV, such as receipt of blood transfusions and use of illicit intravenous drug, and may also acquire blood born infections as a result of healthcare associated transmission in HD units. Several mechanisms have been implicated in HCV transmission among HD patients, such as dialyzer reuse, contamination of hands of staff members, or items shared among patients².

It has been widely demonstrated that virus C infection causes essential cryoglobulinemia³ also Mixed cryoglobulinemia is frequently seen in chronic viral hepatitis patients⁴. The onset of ESRD in some HCV patients influences the patient's

immune status⁵. The incidence of MC is expected to be high in HCV infected patients on HD⁶. Many of these patients on regular hemodialysis are candidates for renal transplantation and receive immunosuppressive therapy that may influence cryoglobulin formation and the presence of cryoglobulins may complicate patient management both pre or post transplantation⁶.

We aimed at studying the cryopositive cases among HD patients and their association with clinical symptoms and assess whether the HCV patients on maintenance HD have abnormal immune response.

2. Patients and methods:

After a written informed consent was obtained, forty CRF patients receiving regular hemodialysis sessions (three sessions /week) were selected from the hemodialysis unit. They were divided according to the presence of HCV antibodies, detected using third generation ELISA (Murex Diagnostics, UK), into two groups, group I (n=20) with positive HCV antibodies and group II (n=20) with negative HCV antibodies. The patients of both groups were subjected to detailed medical examination, biochemical tests, test for cryoglobulin detection⁷ and RF was measured using antibody-coated sheep erythrocytes where titer below 1:16 was considered negative.

Detection of cryoglobulins (Cg):

Cryoglobulins were detected as described as follows, fasting blood samples were collected at least 12 hours after the last meal immediately transported at 37°C and stored in a water bath at 37°C for 30 min. Then it was cleared by centrifugation at 2000 g at the same temperature for 15 min, and then stored at 4°C for 72 hours. The cryocrit was estimated by measuring the height of the column of precipitated protein relative to the total height of the serum column after incubation at 4°C for 72 hours, Or preferably (after 7 days). The level of cryocrit was expressed as a percentage. Depending on the cryocrit level, the Cg level was evaluated as follows:

- Cg negative, low level Cg: cryocrit 1–2%,
 - Moderate Cg: cryocrit 2–5%,
 - High Cg: cryocrit 5–10%,
 - Very high Cg: cryocrit >10%, and Cg in gel form.
- The result consider positive if >2%

Statistical analysis:

Patients' data were analyzed using SPSS 17.0 for windows 7. Quantitative variables were expressed by mean and SD (Standard deviation), compared using unpaired t-student test and Mann-Whitney U test. Qualitative variables were expressed by numbers (Frequency) and percent compared between groups using Chi-square test. Logistic regression analysis was performed and accuracy, sensitivity, specificity, PPV and the NPV were calculated. P value was considered to be significant if less than 0.05.

3. Results:

Data of the studied groups is represented in table (1). Clinical diagnosis showed that the causes of renal failure in the patients on hemodialysis are: obstructive uropathy in 3 (5%), pyelonephritis in 1 (2.5%), hypertension and nephrosclerosis in 20 (50%), diabetic nephropathy in 9 (22.5%), polycystic kidney disease in 1 (2.5%), cry. nephropathy in 1 (2.5%), S.L.E in 1 (2.5%), drug nephropathy in 2 (5%), and diabetic nephropathy + hypertension in 2 (5%). In the cryoglobulin +ve patients (5 cases): Two patients had hypertension and nephrosclerosis (40%), 2 had diabetic nephropathy (40%), while one had cry. nephropathy (20%). In the cryoglobulin -ve patients (35 cases): obstructive uropathy was diagnosed in 3 patients (8.6%), pyelonephritis in 1 (2.9%), hypertension and nephrosclerosis in 18 (51.4%), diabetic nephropathy in 7 (20%), polycystic kidney disease in 1 (2.9) & S.L.E nephropathy in another 1 (2.9%). 2 patients (5.7%) had diabetic nephropathy + hypertension and nephrosclerosis. Drug nephropathy was evident in 2 patients (5.7%).

The causes of renal failure in the HCV +ve group (20 cases) are: obstructive uropathy (2) 10%, pyelonephritis (1) 5%, hypertension and nephrosclerosis (11) 55%, diabetic nephropathy (2) 10%, cry. nephropathy (1) 5%, drug nephropathy (1) 5% and diabetic nephropathy + hypertension and nephrosclerosis in 2 cases (10%). In cryoglobulin +ve subgroup (4 cases) hypertension and nephrosclerosis (2) 50%, diabetic nephropathy (1) 25% and cry. nephropathy in one case (25%). In cryoglobulin -ve subgroup (16 cases), obstructive uropathy (2) 12.5%, pyelonephritis (1) 6.3%, hypertension and nephrosclerosis (9) 55.3%, diabetic nephropathy (1) 6.3%, drug nephropathy (1) 6.3% and diabetic nephropathy + hypertension and nephrosclerosis in 2 cases (12.5%). The causes of renal failure in the HCV -ve group (20 cases) are: obstructive uropathy (1) 5%, hypertension and nephrosclerosis (9) 45%, diabetic nephropathy (7) 35%, polycystic kidney disease (1) 5%, S.L.E nephropathy (1) 5% and drug nephropathy in one case (5%). In Cryoglobulin +ve subgroup only one case of diabetic nephropathy. In Cryoglobulin -ve subgroup (19 cases) obstructive uropathy (1) 5.3%, hypertension and nephrosclerosis (9) 47.4%, diabetic nephropathy (6) 31.6%, polycystic kidney disease (1) 5.3%, S.L.E nephropathy (1) 5.3%, and drug nephropathy in one case (5.3%). Duration of dialysis in HCV +ve group was 4.43 ± 1.8 years. while in Cryoglobulin +ve subgroup (4 cases) was 4.25 ± 1.71 years. In Cryoglobulin -ve subgroup (16 cases) the mean duration of dialysis was 4.47 ± 1.88 years. Duration of dialysis in HCV -ve group was 3.6 ± 1.11 years. Duration of dialysis in Cryoglobulin +ve subgroup (only one case) was 2 years while in Cryoglobulin -ve subgroup (19 patients) was 3.68 ± 1.07 years.

12.5% of the whole studied group of patients (n=40) proved to be positive Cg with a higher percent (20%) in group (I) than that of group (II) (5%) but this difference is not statistically significant with p-value=0.34. No statistically significant difference detected regarding mean age and duration of dialysis between positive (n=4) and negative (n=16) Cg cases in group I. Patients with positive Cg in both groups and those with negative Cg in group I showed more obvious symptoms than those with negative Cg in group, where it is was obvious that the most prominent symptoms in patients with negative Cg either in group I or II are arthralgia and generalized weakness while in patients with positive Cg arthralgia and purpura are more prevalent. In all patients (n=40) patients with positive cryoglobulin (n=5) two (40%) presented with purpura, 4 (80%) presented with arthralgia, 2 (40%) presented with generalized weakness, 1 (20%) presented with Raynaud's

phenomenon, and 2 (40%) presented with rash. But in cryoglobulin -ve subgroup (n=35) 2 (5.7%) presented with purpura, 10 (28.6%) presented with arthralgia, 7 (20%) presented with generalized weakness, 2 (5.7%) presented with Raynaud's phenomenon, and 4 (11.4%) presented with rash. In HCV +ve patients (n=20). In cryoglobulin +ve subgroup (n=4) two (50%) presented with purpura, 4 (100%) presented with arthralgia, 2 (50%) presented with generalized weakness, 1 (25%) presented with Raynaud's phenomenon, 2 (50%) presented with rash. But in cryoglobulin -ve subgroup (n=16) 2

(12.5%) presented with purpura, 7 (43.8%) presented with arthralgia, 4 (25%) presented with generalized weakness, 1 (6.3%) presented with Raynaud's phenomenon, and 2 (12.5%) presented with rash.

In HCV-ve patients (n=20) patients with cryoglobulin +ve only one case presented with purpura. But in cryoglobulin -ve subgroup (n=19) 3 (15.8%) presented with arthralgia, 3 (15.8%) presented with generalized weakness, 1 (5.3%) presented with Raynaud's phenomenon, and 2 (10.5%) presented with rash.

Table (1): comparison of data of both studied groups.

	Group I (positive)		Group II (negative)		p-value
Age	49.45±11.12		56.55±9.25		0.034
Sex (M:F)	11:9		13:7		0.5
Cg +/-	4/16		1/19		0.3
Hb (Cg+/-)	8.3±1.61/9.59±1.69		8.2/9.99±1.99		0.3
platelets (Cg+/-)	163±34.81/211.31±83.08		235/291.58±54.53		0.00
INR (Cg+/-)	1.18±0.05/1.06±0.07		1/1.06±0.07		0.2
s.k (Cg+/-)	4.63±0.61/4.94±0.69		5/5.05±0.81		0.46
AST (Cg+/-)	35.25±4.11/23.31±8.06		13/12.21±3.88		0.00
ALT (Cg+/-)	52.5±8.19/36.75±14.64		24/22.68±6.36		0.00
S. cr (Cg+/-)	7.75±2.63/7.84±2.06		9.3/9.07±3.03		0.12
s. BUN (Cg+/-)	39.75±10.63/48.19±34.66		52/51.58±16.54		0.52
s. Alb (Cg+/-)	3.18±0.43/3.58±0.42		4/3.74±0.46		0.08
S.ca (Cg+/-)	9.4±0.36/9.89±1.15		9.2/9.29±0.63		0.07
S.Na (Cg+/-)	135.5±3.32/133.25±4.09		133/132.3±3.4		0.23
S.phos (Cg+/-)	4.85±0.79/5.58±1.72		5/5.99±1.4		0.28
Symptoms					
-Palpable purpura	-4 (20%)		-0		
-Arthralgia	-11 (55%)		-3 (15%)		
-Generalized weakness	-6 (30%)		-3 (15%)		
-Raynaud's ph.	-2 (10%)		-1 (5%)		
-Rash	-4 (20%)		-2 (10%)		
Cause of renal failure					
-DM	-2 (10%)		-7 (35%)		
-Drug nephropathy	-1 (5%)		-1 (1%)		
-HTN	-1 (5%)		-0		
-Obst. Uropathy	-11 (55%)		-9 (45%)		
-Polycystic kidney disease	-2 (10%)		-1 (5%)		
-0	-0		-1 (5%)		
-S.L nephropathy	-0		-1 (5%)		
-DM+HTN	-2 (10%)		-0		
-Chronic pyelonephritis	-1 (5%)		-0		
-Cry. Nephropathy					
Duration of Dialysis	4.43±1.8		3.6±1.1		0.089
RF +	4 (20%)		1 (5%)		0.00
Cryopositive cases	Case 1	Case 2	Case 3	Case 4	Case 1
Age	(52.5±10.47)				69
Sex	M	M	M	F	F
Symptoms					
-Palpable purpura	-Yes	Yes	No	No	-yes
-arthralgia	-Yes	Yes	Yes	Yes	-No
-G. weakness	-Yes	Yes	No	No	-No
-Raynaud's ph	-Yes	No	No	No	-No
-Rash	-Yes	Yes	No	No	-No
Cause of renal failure	Diabetic nephropathy	Cry. Nephropathy	HTN nephrosclerosis	& HTN nephrosclerosis	Diabetic nephropathy
HD duration	(4.25±1.7)		2		
RF+	Yes	Yes	No	No	No

No statistically significant difference reported between patients with positive Cg (n=5) and patients with negative Cg (n=35) in the whole studied group regarding age, sex, duration of dialysis, cause of renal failure or laboratory tests results (Hb%, BUN, Creatinine, s. Na, s. K, s.Ca, s. phosphorous, and albumin) while patients with positive Cg have significantly greater mean AST, ALT and INR values than those with negative Cg (p -value=0.017, 0.02 & 0.045 respectively), but patients with negative Cg have greater mean platelets than those with positive Cg and this difference is statistically significant (p -value =0.032). By applying logistic regression analysis it was found that AST is a significant predictor to positive Cg (Figure 1) with OR: 1.176 (95%CI: 1.031 – 1.341) with an overall accuracy of 90%, sensitivity 20%, specificity 100%, PPV: 100%, NPV: 89.7% and this model is statistically significant with p -value=0.016 with a negative 2 log likelihood of 21.45.

Five patients in our study had positive RF where group I showed higher percent of positive RF (n=4) than those of group II (n=1) and this difference is statistically significant with p -value=0.00. Regarding the association between Cg and RF it was found that 40% of the HD cryopositive patients had RF activity positive where two patients of group I with positive Cg were positive for RF while the other three patients were negative for Cg.

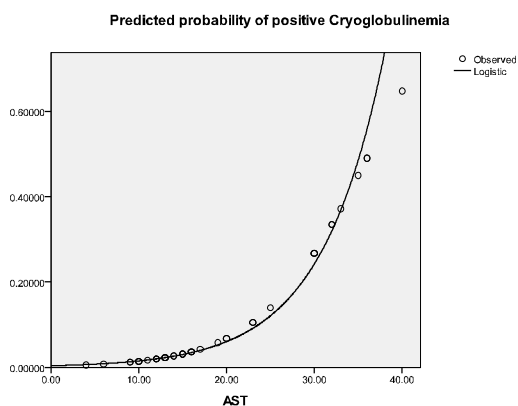


Figure (1): Predicted probability of positive cryoglobulinemia using AST.

4. Discussion:

In our pilot study the HCV infected patients on HD showed a relatively low percent of cryopositivity compared to other studies⁶, with no significant association reported between Cg positivity and gender, age or dialysis duration in contrast to what was concluded in other studies^{8, 9} this discrepancies may be explained by the different Cg detection methods used and the difference in

demographic features of the studied groups as regional difference, Genetic factors such as HLA may also be involved in the pathogenesis of Cg¹⁰. Also, there is evidence that other environmental factors may play a role, MC is most prevalent in southern Europe and its frequency diminishes in more northern locations¹¹. While the anti-HCV negative HD patients showed a relatively very close percent of cryopositivity to the previously reported ones¹², but relatively low compared to others⁶, however, these findings adds to the increasing evidence for a role of HCV infection in cryoglobulin pathogenesis. Also, patients on HD are immunocompromised, with increased risk of acquiring other viral and non-viral infections that may induce cryoglobulinaemia⁶.

Although no significant differences in the cryoglobulin concentrations between both anti-HCV positive and negative patients reported previously⁶ but in our study, two anti-HCV positive patients had high cryoglobulin levels at 10% and. Both were with classical symptoms of palpable purpura and arthralgia.

RF activity positivity of HD cryopositive patients of our study was similar to that reported in some studies¹², but is greater than that reported by others^{6, 5}. These differences may reflect variable patient selection criteria, besides genetic and environmental factors.

No significant difference detected between both Cg positive and Cg negative patients regarding many studied parameters and this is in agreement with the results of some studies¹² but differ from others^{6, 10, 12, 14}. While there was significant increase in the AST, ALT and INR levels and a significant decrease in the platelets levels in the Cg positive patients compared to the Cg negative patients of our study although it is well known that serum aminotransferases activity is generally low in patients with chronic renal failure, The possible explanations for this are suppression of ALT and AST synthesis from the hepatocyte, inhibition of their release from the hepatocyte into circulation or their accelerated clearance from serum⁵, So any elevation in these enzymes might be of concern.

The significant predictor to Cg positivity in our patients is the AST and not the platelets count and INR as reported by other studies¹⁵.

Regarding clinical symptoms they were highly represented in the cryoglobulin positive patients of HCV positive cases while in the HCV negative group purpura was the only presentation confirming what was reported by previous studies. Moreover it is known that there is an established direct correlation between MC severity and the Frequency and degree of main clinical manifestations¹⁶.

Conclusion:

The patients on regular HD showed a number of cases with positive cryoglobulinemia, where hepatitis C positive patients on regular HD showed a higher percent than that of hepatitis C negative patients but this difference was not statistically significant, also there is no significant difference in the cryoglobulin concentrations of both anti-HCV positive and negative patients, however, two anti-HCV positive patients had high cryoglobulin levels at 10% & both were represented with classical symptoms of palpable purpura and arthralgia. RF activity positivity is (40%) in HD cryopositive patients. The symptoms of cryoglobulinemia (purpura, arthralgia & generalized weakness) appear more frequently in HCV positive patients on HD with positive cryoglobulinemia. AST was found to be significant predictor for cryopositivity in HD patients.

Recommendation

Screening of cryoglobulinemia in chronic hepatitis C patients on regular hemodialysis should be taken into consideration as many of these patients are candidates for renal transplantation, as well as screening of HCV in cryoglobulin +ve patients should also be considered. Further studies are needed to clarify the effect of haemodialysis on cryoglobulinemia with larger sample size.

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