

Serum Lipoprotein(a) Level and Apolipoprotein B/A1 Ratio in Patients of Hemodialysis and Peritoneal Dialysis: A New Approach to Predict Cardiovascular Risks in Chronic Kidney Disease

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DOI: <https://doi.org/10.3329/bccj.v8i2.50026>

Abstract:

Background: Serum lipoprotein(a) and Apolipoprotein B/A1 ratio are new, independent cardiovascular risk factors in patients on dialysis. Conversely, the choice of dialysis procedure influences the uremic dyslipidemia in chronic kidney disease (CKD) patients.

Objective: To compare lipoprotein(a) levels and apolipoprotein B/A1 ratio in patients of chronic kidney disease (CKD) stage 5 undergoing hemodialysis or peritoneal dialysis.

Methods: This cross-sectional study was conducted in Department of Nephrology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh, from September 2016 to March 2018. A total of 55 CKD stage 5 patients were included in the study – 31 in hemodialysis (HD) (group A) and 24 in continuous ambulatory peritoneal dialysis (CAPD) (group B). Group A patients were on low flux dialysis with bicarbonate dialysate 4 hours twice weekly dialysis with unfractionated heparin as anti-coagulant with a dialysis adequacy (Kt/V) >1.2. Group B patients were on 3 exchanges over 24 hours with 2 litres of 1.5% glucose fluid with a weekly measured Kt/V >1.7. Serum levels of Lipoprotein(a), Apolipoprotein B/A1 ratio were measured in both groups by using the standard laboratory technique.

Results: In group A, most of the patients were in 31-50 years age group (45.1%), while in group B, majority belonged to >50 years age group (54.16%). Patients' gender showed a male predominance in both groups, i.e. 54.83% and 70.83% respectively. Most of the patients were from urban areas, i.e. 87.1% and 70.8% group A and B respectively. Dialysis adequacy (Kt/V) was found 1.46 for HD patients (group A) and 1.81 for CAPD patients (group B). Dyslipidemia was evident more in CAPD patients than HD patients, as per raised serum Lipoprotein(a) level (83.3% vs 74.1%) and raised Apolipoprotein B/A1 ratio (100% vs. 77.4%). Moreover, comparing with HD patients, CAPD patients showed increased level of serum Lipoprotein(a) (41.4 ± 23.5 mg/dl vs 37.4 ± 25.3 mg/dl; $p > 0.05$) and Apolipoprotein B/A1 ratio (1.59 ± 0.24 vs 1.04 ± 0.22 ; $p < 0.001$).

Conclusion: The maintenance CAPD treatment is associated with more pronounced alterations of the lipoproteins and lipid metabolism than those observed during HD treatment. Besides, serum lipoprotein(a) level and apolipoprotein B/A1 ratio were found simple, accessible and effective markers of dyslipidemia in both groups.

Keywords: Chronic kidney disease, dialysis, Lipoprotein(a), Apolipoprotein B/A1 ratio, cardiovascular risks.

Introduction:

Chronic kidney disease patients on dialysis have increased cardiovascular risks and mortality (10- to 30-fold) when compared with the general population¹⁻³. Researchers have found association between chronic kidney disease and cardiovascular events, hospitalization and the risks of death, where one of the important traditional risk factors is dyslipidaemia^{3,4}. In recent research, lipoproteins, consisting of lipids and proteins (known as apolipoproteins), have drawn immense interests of the scientists in determining dyslipidemia^{1,5,6}. Lipoprotein(a) consists of an LDL-like particle and apolipoprotein(a), which is linked to apoB-100 by a disulfide bond⁶. Lp(a) is an independent genetically determined risk marker for atherosclerosis and cardiovascular disease^{5,6}. Apolipoprotein B (ApoB) is mainly present on very low-density lipoproteins (VLDL), intermediate density lipoproteins (IDL) and low-density lipoproteins (LDL). Therefore, its measurement reflects the number of potentially

atherogenic lipoprotein particles^{7,8}. Apolipoprotein A1 (ApoA1) is the major apolipoprotein of HDL particles, which is also involved in anti-inflammatory and antioxidant properties of HDL. Thus, measurement of apoA1 reflects the level of potentially anti-atherogenic lipoproteins⁸. Hence, it is assumed that the lipoprotein(a) level and ratio of apolipoproteins B and A1 are reflections of the balance between atherogenic and anti-atherogenic lipoprotein particles, and are regarded as strong lipid risk factor for predicting cardiovascular risks, morbidity and mortality^{8,9}.

In CKD patients, measurement of apolipoproteins is advantageous over traditional lipid profiling in predicting cardiovascular risks. Firstly, it is a direct measurement, in contrast to LDL cholesterol measured using the Friedewald formula which often fails to show its accuracy (especially in hypertriglyceridemia i.e. >400 mg/dl¹⁰ or in subjects with low LDL cholesterol levels i.e. <70 mg/dl¹¹; both may be observed in advanced CKD). Secondly, calculated LDL cholesterol

does not reflect the true value in patients with metabolic syndrome, diabetes mellitus, nephrotic syndrome or liver disease¹². Thirdly, apolipoprotein B gives a better insight into the number of small dense LDL cholesterol particles which are believed to be highly atherogenic and often increased in CKD^{7,8,10}. Fourthly, fasting condition is not mandatory for apolipoprotein measurement, which seems an advantage especially for CKD patients with diabetes mellitus¹⁰. Last but not the least, the tests for measuring lipoprotein(a), apoB and apoA1 are simple, widely available, internationally standardized and automated; the assay can be performed even on frozen samples, with less technical errors¹³. Hence, by measuring the above-mentioned markers the pattern and magnitude of dyslipidemia can be effectively identified followed by appropriate intervention to the risk group merely reducing mortality and morbidity.

However, to our knowledge, no such study has been done in our country to date. Therefore, the present study was designed to compare the effect of hemodialysis and peritoneal dialysis on lipoprotein(a) level and apolipoprotein B/A1 ratio in chronic kidney disease stage 5 patients in a tertiary level facility in our country. It is expected that the study results will help the clinicians to identify the risk group, predict future cardiovascular events, choose appropriate dialysis management plan for the patients, and prevent a substantial number of morbidities and mortalities.

Methods:

This cross-sectional study was conducted in the Department of Nephrology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh, from September 2016 to March 2018. Our study population was all the patients admitted in the BSMMU hospital with CKD stage 5 either under hemodialysis or under continuous ambulatory peritoneal dialysis (CAPD) for at least 3 months during that

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study period. However, we adopted convenient purposive sampling method and study participants were selected based on the following inclusion and exclusion criteria:

Inclusion criteria are the following

- 1) Age 18 or above of both sexes;
- 2) CKD stage 5 patients (as defined by K/DOQI clinical practice guidelines for chronic kidney disease)¹⁴ having either HD or CAPD for at least 3 months; and
- 3) Patients on adequate dialysis (Kt/V >1.2 for HD patients¹⁵ and weekly Kt/V >1.7 for CAPD patients¹⁶).

Exclusion criteria are the following .

- 1) Patients receiving statin therapy;
- 2) Patients having chronic liver diseases;
- 3) Patients declined to participate in the study.

Finally, a total of 55 patients were included in the study – 31 in hemodialysis (HD) (group A) and 24 in continuous ambulatory peritoneal dialysis (CAPD) (group B).

Assessment of dialysis adequacy was done in terms of Kt/V. In hemodialysis (HD), probable study patient was asked to come in fasting state in the morning. They were on low flux dialysis with bicarbonate dialysate 4 hours twice weekly dialysis with unfractionated heparin as anti-coagulant. Pre-dialysis and post-dialysis blood samples were taken for assessing urea. Post dialysis weight with ultrafiltrate amount also noted in that session. They were assessed for measuring dialysis adequacy using standard formula (Kt/V) for HD patients¹⁷ and finally, 31 patients were enrolled for the study group A (HD) having required Kt/V >1.2.

$$Kt/V = - \ln \left[\frac{U_{\text{post}}}{U_{\text{pre}}} - 0.008t \right] + \left[4 - 3.5 \frac{U_{\text{post}}}{U_{\text{pre}}} \right] \times \left(\frac{W_{\text{post}} - W_{\text{pre}}}{W_{\text{post}}} \right)$$

where U_{post} = post-dialysis urea in mg/dl; U_{pre} = pre-dialysis urea in mg/dl; t = time of dialysis session in hour; W_{post} = post-dialysis weight in kilograms; W_{pre} = pre-dialysis weight in kilograms; $(W_{\text{post}} - W_{\text{pre}})$ is the UF volume i.e. ultrafiltrate in kilograms.

In continuous ambulatory peritoneal dialysis (CAPD), patients were on 3 exchanges over 24 hours with 2 litres of 1.5% glucose fluid and asked to bring all the 3 dialysate bags used in last 24 hours in a pre-fixed date and aliquots were taken from each bag. According to the multiple-aliquot method (as described by Lyon et al.)¹⁸, net weight of fluid was determined in each bag. A small aliquot (10ml) from each bag was collected for urea determination. Urea level was analyzed from each bag and fluid volume per bag was noted. Mean dialysate urea level was determined for each patient. Patients were also asked to bring last 24-hour urine collection. Total urine volume and urinary urea level were measured. At the same day, blood sample was collected (between the gap of the fluid exchange) for measuring urea level. Patients' weight was also measured. After collecting all data peritoneal and renal Kt/V were measured according standard formula¹⁹:

$$\text{Peritoneal Kt/V} = \left[\frac{D_{\text{urea}}}{P_{\text{urea}}} \times \text{Dialysate Volume} \right] \div V_{d_{\text{urea}}} \times 7 \text{ days}$$

$$\text{Renal Kt/V} = \left[\frac{U_{\text{urea}}}{P_{\text{urea}}} \times \text{Urine Volume} \right] \div Vd_{\text{urea}} \times 7 \text{ days}$$

where D_{urea} = Dialysate urea in mg/dl; P_{urea} = Plasma urea in mg/dl; U_{urea} = urine urea in mg/dl; Vd = volume of distribution = (Total body weight \times 0.6).

Then total Kt/V was calculated by summation of peritoneal and renal Kt/V for CAPD patients. Finally, 24 patients with total Kt/V >1.7 were included in our study.

Estimation of Lipoprotein (a), Apolipoprotein A1 and Apolipoprotein B are as follows.

Centrifuged serum of the two groups of patients were stored in -20°C. Serum lipoprotein(a) was measured by immunoturbidimetric method using monoclonal antibodies against Lp(a) combined with latex particles; apolipoprotein A1 and apolipoprotein B were also measured using automated immunoturbidimetric method standardized against the WHO-IFCC International Reference Materials (in Mindray BS-230 Automated Clinical Chemistry Analyzer; Made in Shenzhen, China). Then, apolipoprotein B/A1 ratio was calculated. Having serum level of lipoprotein(a) >30 mg/dl²⁰ and apolipoprotein B/A1 ratio >1²¹ were considered as risk factors in both groups.

The results were presented in tables. Quantitative data were expressed as mean and standard deviation and qualitative data were expressed as frequency and percentage. Statistical analyses were performed using SPSS (Statistical Packages for Social Sciences) version 20.0 (SPSS Inc, Chicago, IL, USA). Association between variables were done by unpaired Student 't' test and Mann-Whitney test. P value <0.05 was considered as statistically significant.

The present study was approved by the Institutional Review Board (IRB) of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh.

Results:

In the present study, 31 patients were under hemodialysis (HD) (group A), while another 24 patients were under continuous ambulatory peritoneal dialysis (CAPD) (group B). In group A, most of the patients are from 31-50 age group (45.1%), while in group B, majority are from >50 age group (54.16%). Patients' gender shows male predominance in both groups, which is more marked i.e. 54.83% and 70.83% in group A and B respectively. Most of the patients are from urban areas i.e. 87.1% and 70.8% group A and B respectively (Table I). The difference in duration of CKD in between groups was not significantly significant (p>0.05); however, the duration of dialysis was quite different between the CAPD and HD patients and was significant (p<0.01). Dialysis adequacy in the form of Kt/V was found 1.46 for HD patients and 1.81 for CAPD patients (Table II). More CAPD patients showed presence of dyslipidemia than that of HD patients, as per raised serum Lipoprotein(a) level (83.3% vs 74.1%) and raised Apolipoprotein B/A1 ratio (100% vs. 77.4%) (Table III). CAPD patients (group B) had a raised Lipoprotein(a) level than HD patients (41.4±23.5 mg/dl vs 37.4±25.3 mg/dl); however, the difference was not statistically significant (p>0.05). Apolipoprotein B/A1 ratio was more in CAPD

patients (group B) than HD patients (group A) (1.59±0.24 vs 1.04±0.22), which was statistically significant (p<0.001) (Table IV).

Table I: Demographic characteristics of study subjects (n=55)

	Group A (CKD 5 with HD) (n=31)		Group B (CKD 5 with CAPD) (n=24)	
	Frequency	Percentage	Frequency	Percentage
Age (years)				
18-30	4	12.9	3	12.5
31-50	14	45.1	8	33.33
> 50	13	41.9	13	54.16
Sex				
Male	17	54.83	17	70.83
Female	14	45.17	7	29.16
Residence				
Rural	4	12.9	7	29.1
Urban	27	87.1	17	70.8

Table II: Duration of dialysis and chronic kidney disease (CKD) & dialysis adequacy

	Group A (CKD 5 with HD) (n=31)		Group B (CKD 5 with CAPD) (n=24)		P value
	Median	Range	Median	Range	
Duration of Dialysis (months)	10.0	3-24	9.0	3-12	0.009
Duration of CKD (months)	24.0	8-60	18.0	10-60	0.333
Dialysis Adequacy (Kt/V)	1.46	1.2-1.86	1.81	1.71-2.00	-

Table III: Comparison of dyslipidemia by Lipoprotein(a) and Apolipoprotein B/A1 ratio

	Group A (CKD 5 with HD) (n=31)		Group B (CKD 5 with CAPD) (n=24)	
	Frequency	Percentage	Frequency	Percentage
Lipoprotein(a)				
>30 mg/dl	23	74.1	20	83.3
Apolipoprotein B/A1 ratio				
>1	24	77.4	24	100

Table IV: Lipoprotein (a) and Apolipoprotein panel with Apolipoprotein B/A1 ratio

	Group A (CKD 5 with HD) (n=31) Mean ± SD	Group A (CKD 5 with CAPD) (n=24) Mean ± SD	P value
Lipoprotein (a) (mg/dl)	37.4±25.3	41.4±23.5	>0.05
Apolipoprotein A1 (g/L)	0.85±0.15	0.74±0.14	<0.05
Apolipoprotein B (g/L)	0.89±0.20	1.22±.26	<0.001
Apolipoprotein B/A1 Ratio	1.04±0.22	1.59±0.24	<0.001

Discussion:

Lp(a) levels were reported to be markedly increased in CAPD as well as in HD patients²². The possible explanation behind the phenomenon is enhanced Lp(a) synthesis in the liver in response to high urinary protein loss²³. In the present study, we found that CAPD patients have higher lipoprotein(a) level than HD patients, though it was not statistically significant ($p>0.05$). Similarly, higher Lp(a) levels were found in CAPD group in comparison to HD group by Yilmaz et al.²⁴ (43.1±36.7 mg/dl vs. 32.5±31.5 mg/dl) and Kanbay et al.²⁵ (46.0±42.7 mg/dl vs. 43.1±40.6 mg/dl), but the difference was not statistically significant. However, Siamopoulos et al.²⁶ reported that CAPD patients had higher serum Lp(a) levels than HD patients, which was marginally statistically significant ($p=0.056$). In a large multicenter study done by Kronenberg et al.¹ found that 37% of CAPD patients and 30% of HD patients had elevated serum Lp(a) than healthy control group and overall, higher serum Lp(a) was observed in PD patients in comparison to HD patients (34.6±38.4 mg/dl vs. 23.4±25.0 mg/dl; $p<0.001$). Similar result was reported by Borazan, Ustün & Yilmaz²⁷ (58.65±19.67 mg/dl vs. 38.72±12.25 mg/dl; $p<0.05$).

Apo B/A1 ratio is an efficient marker over traditional markers for dyslipidemia in assessing CVD risks⁸. In the present study, we found that CAPD patients have higher Apo B/A1 ratio than HD patients ($p<0.001$), which is supported by the previous studies done by Siamopoulos et al.²⁶ and Samouilidou et al.²⁸. In contrast, Sato et al.²⁹ and Liu & Rosner³⁰ reported that the apo B/apo A1 ratio was higher in patients on HD than patients receiving PD. Nonetheless, Grützmacher et al.³¹ reported that Apo B and A1 were unaffected by the degree of renal insufficiency.

In summary, increased Lp(a) level and Apo B/A1 ratio (as determined by the reduced levels of apo A-containing

lipoproteins and increased TG-rich apo B-containing lipoproteins) indicated a clear atherogenic pattern in patients of advanced CKD under dialysis – both CAPD and HD. These findings are of great importance for predicting dyslipidemia as well as selection and monitoring of appropriate preventive measures for normalizing dyslipidemia in dialysis patients.

However, this study had some limitations that it was conducted in a single center, study population was relatively small, and it was non-randomized and might be subjected to selection bias. Hence, we recommend further studies in the same population with larger samples and longer duration, in multiple sites with randomized sampling and ensuring availability of a better emergency treatment facility.

Conclusion:

The results of this study showed that the maintenance CAPD treatment is associated with more pronounced alterations of the lipoproteins and lipid metabolism than those observed during HD treatment. Besides, serum lipoprotein(a) level and apolipoprotein B/A1 ratio were found simple, accessible and effective markers of dyslipidemia in both groups.

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