CARDIOVASCULAR RISK ASSESSMENT IN HEMODIALYSIS PATIENTS

Asmita Pokhrel,[1] Buddhi Raj Pokhrel,[2] Prajwal Gyawali,[3] Deepak Regmi,[4] Madhav Khanal,[1] Ashim Bhattarai,[5] Prakash Poudel,[6] Anuj KC[6]

[1]Department of Biochemistry, Nepal Medical College & Teaching Hospital, Kathmandu, [2]Department of Biochemistry, Universal College of Medical Sciences, Bhairahawa, Nepal, [3]Priority Research Centre for Stroke and Brain Injury, School of Biomedical Sciences and Pharmacy, University of Newcastle, New South Wales, Australia, [4]Nepal Medical College & Teaching Hospital, [5]Department of Biochemistry, Institute of Medicine, Maharajgunj, [6]Hemodialysis unit, Department of Medicine, Nepal Medical College and Teaching Hospital, Kathmandu, Nepal

ABSTRACT

Cardiovascular diseases are the leading cause of death in hemodialysis patients. We aimed to evaluate non-traditional cardiovascular risk factors: homocysteine, high sensitive C-reactive protein, oxidized LDL antibodies, phosphate, and red cell distribution width in chronic kidney disease patients under maintenance hemodialysis along with traditional cardiovascular risk factors like age, hypertension, diabetes mellitus, among others. A total of 78 diagnosed chronic kidney disease patients under maintenance hemodialysis visiting a tertiary care center were included in the study, of which 59% were male. Hyperhomocysteinemia was present in 79.5% of the participants, with the median homocysteine level being 28.43µmol/L. The median hsCRP level was 4.74mg/L, and 59% and 24.4% of the total participants were at high and moderate cardiovascular risk respectively. The median oxidized LDL antibody level was 4235U/mL, which is within the reference range. The median red cell distribution width was 14.05%, which is within the normal range. Left ventricular hypertrophy, a common cardiovascular disease in such patients, was found in 55.13% of the participants. Serum homocysteine level was significantly higher in patients with left ventricular hypertrophy, whereas serum C- reactive protein level was significantly lower in patients with left ventricular hypertrophy. The mean serum phosphate was 6.23mg/dL (i.e. higher than normal) and hyperphosphatemia was seen among 76.9% of the patients. The mean age of the patients was 47.5 years, which is distinctly lower when compared to the hemodialysis patients in the Western population. The prevalence of hypertension, diabetes mellitus, and anemia were 95%, 18.25%, and 92.3%, respectively.

KEYWORDS

Hemodialysis, Homocysteine, high sensitive C-reactive protein, oxidized LDL antibody, Red cell distribution width

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CORRESPONDING AUTHOR

Dr. Asmita Pokhrel
Assistant Professor
Department of Biochemistry,
Nepal Medical College and Teaching Hospital,
Attarkhel, Gokarneshwor-8, Kathmandu, Nepal
Email: asmita.pokhrel1@nmcth.edu
Orcid No: https://orcid.org/0000-0002-5739-6056
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INTRODUCTION

Chronic kidney disease (CKD) is essentially a global epidemic, with a global prevalence of 9.1% and an estimated 697.5 million cases.[1] About 1.2 million deaths were reported in 2017, with an additional 1.4 million cardiovascular disease (CVD) mortality attributed to impaired kidney function. This makes it the 12th leading cause of death worldwide and the leading cause of death in CKD patients under hemodialysis.[2] This may be due to the higher prevalence of both traditional and non-traditional CVD risk factors among these patients when compared to the general population. The traditional risk factors include: old age, diabetes mellitus (DM), hypertension, smoking, dyslipidemia, and the non-traditional risk factors include hyperhomocysteinemia, inflammatory conditions, oxidative stress, hyperphosphatemia,and anemia.[3]

Studies suggest that hyperhomocysteinemia is associated with endothelial dysfunction, thus increasing the CVD risk. Increased homocysteine is also associated with high levels of triglycerides and cholesterol biosynthesis, as well as increased uptake of low density lipoprotein (LDL) by macrophages contributing to CVD risk. Patients with end-stage renal disease (ESRD) are often detected with it.[4] However, its role as a predictor of CVD morbidity and mortality in both the general population and ESRD has been questionable.[5,6]

CKD patients under hemodialysis are predisposed to different inflammatory processes and systemic inflammation is believed to promote CVD in them. Many recent studies have demonstrated high sensitive C-reactive protein (hsCRP), a potential biomarker of inflammation, as a strong independent predictor of future cardiovascular events.[6,7] It has also been suggested that chronic inflammation and hyperhomocysteinemia lead to lipid peroxidation, thereby promoting atherogenesis particularly in this group of participants.[8]

Various CKD related factors such as advanced age, high prevalence of DM, and uremia related factors lead to high oxidative stress in hemodialysis patients. This leads to increased oxidized LDL (ox-LDL) formation which again leads to the formation of ox-LDL antibodies as an immunological response to oxidative stress. The roles of ox-LDL and ox-LDL antibodies in different aspects of CVDs have been demonstrated. It has been suggested that ox-LDL and ox-LDL antibodies might be valuable markers of assessing CVD risks in CKD patients.[6,9]

Hyperphosphatemia has been suggested as an independent risk factor of cardiovascular mortality in CKD patients since long. It is believed to induce vascular calcification, endothelial dysfunction, myocardial hypertrophy, and cardiac malfunctions.[10] However, many studies did not find any significant correlation between these two.

Patients with CKD are often anemic and have high red cell distribution width (RDW) levels. Apart from its role in anemia, RDW has been recently found to be a novel and independent predictor for mortality in the general population, as well as in patients with chronic heart failure, peripheral artery disease, and kidney transplants. However, the role of RDW has not been sufficiently elucidated in CKD patients.[11]

The present study aimed to investigate some novel CVD risk factors along with some traditional factors among CKD patients undergoing maintenance hemodialysis and analyze how these risk factors are associated with CVD such as left ventricular hypertrophy (LVH). Traditional CVD risk factors in this group of study population has been discussed in our previous study.[12]

MATERIALS AND METHODS

Ethical approval was obtained from the Institutional Review Committee of Nepal Medical College Teaching Hospital (NMCTH), Kathmandu, Nepal before the study. A hospital-based cross-sectional study of 78 hemodialysis patients was carried out for 24 months from July 2018 to July 2020. Patients were explained about the study and verbal consent was taken. A convenient sampling technique was used.

Sample collection and laboratory analysis: Pre-dialysis venous blood samples were drawn in the morning during fasting conditions following standard aseptic protocol. Serum samples were obtained after centrifuging and were frozen at –70°C until examination.

Serum homocysteine was assessed by chemiluminescent microparticle immunoassay (Abbott architect i2000sr instrument, USA). According to American Heart Association, serum homocysteine less than 15 mmol/L was considered normal.[13] Serum hsCRP levels were measured by nephelometry (miSPA-i3, Agappe Diagnostics Limited, Ernakulam, Kerala). Serum hsCRP values were categorized into 3 groups: Normal (<1 mg/L), moderate risk (1-3 mg/L)

and high risk (>3mg/L).[14] Ox-LDL antibody was assessed by measuring ox-LDL antibody titers using sandwich ELISA technique (DRG anti ox-LDL, Germany). The reference range of 4000-12,000 U/mL was considered normal as per the manufacturer's instructions.

The serum levels of Ca, P, total protein, and albumin were analyzed by dry chemistry using Vitros 250. Hb was estimated in the coulter counter, Sysmex XS-500i. Serum Ca level between 9-11mg/dL was considered normal. Similarly, serum P >4.5mg/dL was considered high. Hb level <10gm/dL was considered low, serum total protein <6gm/dL and albumin <3.5gm/dL were considered low. Other demographic and medical details were noted from the patients' medical records on the day of sample collection.

Data collection and analysis: Data were entered into Microsoft Excel and analyzed using Statistical Package for the Social Sciences (SPSS vs. 16). Descriptive statistical tools were employed. The mean and standard deviation of numerical data were calculated, both overall and gender-wise. The prevalence of CVD risk factors was expressed as percentages. Data were expressed as either mean ± standard deviation (SD) or median (interquartile range) based on the distribution (Shapiro-Wilk test). Independent sample t-test, Mann-Whitney test, and Kruskal-Wallis test were used to compare the mean/mean rank between the groups. Pearson's and Spearman's correlation analyses were performed to see the association between numerical variables.

RESULTS

The present study involved 78 CKD patients undergoing hemodialysis, of which 46 (59%) were male. The median homocysteine level was 28.43µmol/L, and hyperhomocysteinemia was recorded in 79.5% of the participants. The median hsCRP level was also above the reference range at 4.74mg/L. When categorized according to the risk of developing CVDs, 59% were at high risk (>3mg/L) and 24.4% were at

Table 1: Distribution of numerical variables between Sex

Parameters	Total Study Population (n = 78)	Sex		P-Value
		Male (n= 46)	Female (n= 32)	
Age (Years)	47.46 ± 15.92a	48.35 ± 17.76	46.19 ± 13.01	0.537[b]
Homocysteine (µmol/L)	28.43 (17.39 - 42.35)	25.27 (18.30 – 43.07)	21.87 (15.92 – 41.65)	0.387
hsCRP(mg/L)	4.74 (1.34 - 10)	4.32 (1.52 – 10)	6.60 (1.05 – 10)	0.664
Ox-LDLantibodies (U/mL)	4235 (2582.5 - 412.5)	3535 (2832.5 – 8880)	4480 (2500 – 8045)	0.955
RDW(%)	14.05 (13.20 - 15.52)	13.85 (13.10 – 15.20)	14.30 (13.30 –15.67)	0.512
Hb(gm/dL)	8.93 ± 1.69[a]	8.71 ± 1.76)	9.25 ± 1.83	0.194[b]
Albumin (gm/dL)	3.65 ± 0.44[a]	3.66 ± 0.44	3.63 ± 0.45	0.812[b]
Ca (mg/dL)	8.05 (7.30 - 8.80)	8.05 (7.45 – 8.70)	8 (7.20 – 8.90)	0.703
P (mg/dL)	6.23± 1.96[a]	6.28± 2.01	6.16 ± 1.93	0.785[b]
SBP (mm of Hg)	140 (130 -150)	140 (130 – 160)	140 (120 – 140)	0.266
DBP (mm of Hg)	80 (80 - 90)	80 (80 – 90)	80 (80 – 90)	0.665
CKD Duration (Years)	4 (2 - 7)	4 (2.87 – 5.25)	4.75 (2 - 7)	0.589
Dial. Duration	3 (0.9 - 4)	2.75 (0.8 – 4)	3 (1 – 5)	0.330

Abbreviation: oxLDL- Oxidized LDL antibody titre; hsCRP-High Sensitive C reactive protein; RDW-Red Blood Cell Distributiuon width; Hb- Hemoglobin; Ca- Calcium; P- Phosphate; SBP- Systolic Blood Pressure; DBP- Diastolic Blood Presure; CKD- Chronic Kidney Disease; Dial.-Dialysis.

a- Normally distributed variables expressed as Mean ± S.D. (Rest are expressed as median with 25th and 75th percentile values in parentheses); b- P-values obtained from independent sample t-test (Rest were analyzed by Mann-Whitney U test)

moderate risk (1-3mg/L). Median ox-LDL titre was 4235 U/mL. The mean serum phosphate was 6.23mg/dL with hyperphosphatemia being present in 76.9% of the patients. The mean Hb level was 8.93gm/dl, where 92% (n=72) had anemia. The median RDW was 14.05% where 44.9% had a higher RDW percentage.

Table 2: Prevalence of cardiovascular risk factors

Prevalence of cardiovascular risk factors %, (n)	
Early age (< 40 years)	61% (n = 48)
Hypertension	95% (n = 74)
DM	18.2% (n = 22)
Past smokers	18.2 % (n = 22)
Past alcohol users	51.3% (n = 40)
Hyperhomocysteinemia	79.5% (n = 62)
High hsCRP	59% (n = 46) high risk 24.4% (n = 19) moderate risk
Hyperphosphatemia	76.9% (n = 60)
Low Hb	92.3% (n = 72)
High RDW	44.9% (n = 35)

Abbreviation: DM- diabetes mellitus; hsCRP- high sensitive C-reactive protein; Hb; hemoglobin; RDW- red cell distribution width

The mean age of the patients was 47.46 ± 15.92 years, with 61% (n=48) being 40 years and below. Of the total participants, 95% (n=74) were hypertensives, 18.2% (n=22) had DM, 51.3% (n=40) were past alcohol users, and 18.2% (n=22) were past smokers.

The baseline characteristics and numerical variables are summarized in table 1. The prevalence of CVD risk factors are summarized in Table 2.

LVH was present in 55.13% (n = 43) patients. Significantly high homocysteine levels were found in the dialysis patients with LVH compared to the patients without LVH. Serum hsCRP levels were, however, significantly low in patients with LVH. The details of the results are presented in Table 3.

DISCUSSION

The present study demonstrated the high prevalence of cardiovascular risk factors among hemodialysis patients. We measured serum homocysteine, hsCRP, ox-LDL antibodies, phosphate, and Hb along with RDW in our patients. Homocysteine, hsCRP, ox-LDL antibodies are the markers of inflammation and oxidative stress which are deemed as the important pathophysiological basis of developing CVD in CKD.[6]

Table 3. Numerical variables according to LVH

PARAMETERS	LVH		P-Value
	No (n = 35)	Yes (n = 43)	
Homocysteine(µmol/L)	18.23 (14.58 – 32.52)	27.49 (20.53 – 44.47)	**0.004**
hsCRP (mg/dL)	9.36 (2.49 – 10)	2.77 (1.24 – 9.60)	**0.035**
Ox-LDLantibodies(U/mL)	4460 (2680 – 8840)	3560 (2520 – 7370)	0.612
RDW (%)	14.10 (13.20 – 15.70)	14 (13.20 – 15.20)	0.944
Hb (gm/dL)	9.05 ± 1.80[a]	8.84 ± 1.81	0.608[b]
Albumin (gm/dL)	3.68 ± 0.36[a]	3.63 ±0.50	0.611[b]
SBP (mm of Hg)	140 (120 – 140)	140 (130 – 160)	0.311
DBP (mm of Hg)	80 (80 – 90)	80 (80 – 90)	0.775
Ca (mg/dL)	8.20 (7.60 – 9.10)	7.90 (7.30 – 8.60)	0.138
P (mg/dL)	6.12 ± 2.11[a]	6.32 ± 1.84	0.668[b]
CKD Duration (Years)	4 (2 – 8)	4 (3 – 6)	0.751
Dial. Duration (Years)	2 (0.8 – 4)	3 (1 – 4)	0.359
Age (Years)	47.83 ± 15.36[a]	47.16 ± 16.54	0.856[b]

Abbreviation: LVH- Left Ventricular Hypertrophy (Other similar as above table 1) a- expressed as Mean ± S.D. (Rest expressed as median with 25th and 75th percentile values in parentheses); b- P-values obtained from independent sample t-test (Rest analyzed by Mann-Whitney test). P-values <0.05 were considered statistically significant, and are expressed in bold typing.

Approximately 79.5% of the patients showed hyperhomocysteinemia (≥15µmol/L) in our study with the median homocysyteine level (28.43µmol/L) being higher than the reference range. Persistent mild hyperhomocysteinemia is common in patients with ESRD. It is an emerging risk factor for CVD in the general population and in patients with ESRD.[4] The possible explanation for this high prevalence of hyperhomocysteinemia could also be due to folic acid, vitamins B12 and B6 deficiency which is common among the hemodialysis patients. These vitamin supplements are believed to reduce the concentration of homocysteine in hemodialysis patients but they do not normalize it.[6] In our study, the median level of homocysteine was significantly higher among patients with LVH compared to those without LVH. However, the association of hyperhomocysteinemia and CVD is still conflicting. Some studies had established positive association[4,15] while some had entailed no association.[16,17]

The median hsCRP level (4.74mg/L) in our patients was above the recommended reference range (< 1mg/dL) like many other studies.[6,18] When hsCRP was categorized according to the risk of developing CVD, 59% of the patients were at high risk and 24.4% of the patients were at moderate risk. Despite this, the level of hsCRP was seen to be significantly lower in the LVH group when compared to the patients without LVH, in contrast to our expectation. This possibly could be explained in two different ways: firstly, irrespective of the presence of cardiac disease, our patient population might already have systemic low-grade inflammation from several other potential reasons such as hypertension or dyslipidemia; and secondly, some undiagnosed cardiac complications might be present among hemodialysis patients without LVH. Further large-scale studies are warranted in this respect. Taheri *et al*[19] have also reported no difference in the mean level of inflammatory markers hsCRP and interleukin-6 among hemodialysis patients with or without CVD. However, there was no significant difference in the median level of ox-LDL-antibodies between hemodialysis patients with and without LVH.

The mean serum phosphate level was 6.12mg/dL in the study with around 77.0% of patients having hyperphosphatemia. The reason behind this could be the altered calcium -phosphorus metabolism in CKD patients and such a high prevalence of hyperphosphatemia in the present study is alarming and needs serious attention as hyperphosphatemia is considered as an independent risk CVD risk factor.[10] A similar study showed only 39.0% prevalence of high serum phosphate among the dialysis patients.[20]

Various studies have predicted high RDW to be a predictor of high mortality from all causes in CKD patients.[11,21] In our study, the median RDW was 14.05 % which is within the normal range. However, 44.9% participants had a high RDW percentage (>14.5%). Various studies have reported RDW percentage in hemodialysis patients to be either within the reference range or increased.[22,23] A study conducted in Japan among non-dialysis dependent CKD patients concluded that higher RDW was independently associated with worse renal outcome and RDW could be an additional prognostic marker of the progression of CKD.[21] We did not find a significant difference in RDW values between the LVH groups.

More than 90.0% of the patients in our study were anemic with the mean Hb level of 8.93 gm/dL. Majority of the patients are found to be incapable of affording erythropoietin treatment or disease specific balanced diet. The socioeconomic status of CKD patients registered in one of the government tertiary healthcare centers in Kathmandu- National Kidney Centre, was shown to be so poor that 37.0% of the patients (out of 96) had to sell some sort of their property to afford the treatment.[24] Similarly, very poor dietary knowledge and practice are found among Nepalese hemodialysis patients. A study reported that although 70.0% of hemodialysis patients knew about renal diet; only 60.0% of them believed in it. Moreover, after having kidney disease, the majority of the patients consumed a regular diet like their other family members.[25] Poor socioeconomic status of the patients, lack of knowledge and awareness explains higher prevalence of anemia in our patients compared to patients from developed nations. More proactive approach to anemia management in advanced CKD patients seems to be necessary so as to reduce the cardiovascular burden in these patients. However, long-term prospective studies of CKD patients are needed.

Of the matter of grave concern, in the present study, the mean age of the hemodialysis patients was found to be only 47.5 years, with 61% of patients being 40 years or below.This figure is much less than reported by several middle- and high-income countries.[26,27] This has been discussed in our previous study as well.[12] A study from Africa also reported a mean age of CKD patients of only 43 years (n=217; 111 stage 5 CKD).[20] A study from India reported the mean age of hemodialysis patients around 50 years,[28] which is higher than that of our study, but

still lower than that of high-income countries. This clearly shows that even the middle-aged population of the low-income country is highly vulnerable to developing CKD. Other than the unhealthy lifestyle and poor national Non Communicable Disease (NCD) health services, the unregulated use of pesticides for farming in Nepal might be the other potential reason for developing CKD at a younger age.[29] Early screening, prevention, detection, and management of CKD and its complications, including CVD, are, without doubt, an urgent necessity in Nepal.

In our study, most of the study participants (94.8%, n=74) had hypertension. Likewise, the higher figure (90.0% prevalence) has also been reported by another low-income country Uganda.[20] Although the general prevalence of hypertension in hemodialysis ranges from 50 to 90.0%,[30] several reports from the developed nations have shown prevalence towards the lower side of this given range. A large scale study in the United States, the prevalence of hypertension was shown to be only 40% among chronic hemodialysis patients.[31] Similarly, a study conducted in Iran reported a much less percentage of hypertensive patients (35.0%) in their study.[32] The almost universal presence of hypertension in our cohort is alarming.

The prevalence of DM was 28.2% in our study while it was 14.8% and 16.2% in studies conducted in Uganda and Nigeria respectively.[20,33] This higher prevalence of diabetes among CKD patients in our study may reflect the significance of diabetes as an etiological factor for CKD in Nepal.

Higher prevalence of cardiovascular risk factors reflects poor national NCD policy. This suggests a lack of an early risk factors intervention in our health system. Our findings strongly emphasize the need of focusing on an early preventive approach rather than a late curative approach for the management of CKD. CKDs are multi-factorial in origin, and their control should therefore (also) be multisectoral. Health system strengthening for the early detection and intervention of cardiovascular risk factors, awareness, and advocacy on healthy lifestyle, disease surveillance, research, monitoring, and evaluation along with leadership development are the identified capacity-building strategies that would be useful to tackle cardiovascular risk factors and the development of CKD.[34]

Limitations: Serum homocysteine level is influenced by folic acid, vitamin B12, and B6 levels which were not considered in our study. A prospective study with an assessment of morbidities and mortalities with suitable statistical tools would have increased the impact of our study. The small sample size and the lack of a control group were the other major limitations of our study.

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