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Assessment of Brachial Ankle Pulse Wave Velocity in Non-Dialysis CKD Patients

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Abstract **Original Research Article**

Background: Chronic Kidney Disease (CKD) is a progressive condition that significantly affects the global population, with increasing prevalence in low- and middle-income countries like Bangladesh. Among CKD patients, cardiovascular disease remains a leading cause of morbidity and mortality, with arterial stiffness emerging as a critical indicator of cardiovascular risk. The brachial-ankle pulse wave velocity (baPWV) is a non-invasive measure of arterial stiffness, offering valuable insights into vascular health. This study explores the association between baPWV and clinical parameters in non-dialysis CKD patients in Bangladesh. ***Objective:*** To investigate the association between baPWV and various clinical and laboratory parameters in non-dialysis CKD patients in Bangladesh. ***Methods:*** A cross-sectional observational study was conducted at the Department of Nephrology, Dhaka Medical College, between September 2021 and March 2023. The study involved 100 non-dialysis CKD patients (50 with vitamin D deficiency and 50 without), aged 18 years or older, in stages 3-5. Demographic, clinical, and laboratory data were collected, and baPWV was measured. Data analysis was performed using SPSS version 26.0 with statistical tests including t-tests, ANOVA, and regression analysis. ***Results:*** Vitamin D deficiency was associated with higher baPWV compared to non-deficient patients, with significant differences observed across all CKD stages ($p<0.05$). The mean baPWV in the vitamin D deficient group was 22.5±1.75 m/s, while in the non-deficient group it was 16.86±1.82 m/s ($p<0.05$). The regression analysis identified vitamin D levels, age, and serum iPTH as significant independent predictors of baPWV. ***Conclusion:*** This study demonstrates that baPWV is significantly higher in non-dialysis CKD patients with vitamin D deficiency. The findings suggest that baPWV can serve as a reliable indicator for assessing cardiovascular risk in CKD patients, particularly in Bangladesh, where CKD prevalence is on the rise. Further research is warranted to explore the clinical implications of baPWV as a routine tool for cardiovascular risk management in CKD.

Keywords: Chronic Kidney Disease, Arterial Stiffness, Pulse Wave Velocity, Brachial-Ankle Pulse Wave Velocity, Vitamin D, Cardiovascular Risk, Non-Dialysis, Bangladesh.

INTRODUCTION

Chronic Kidney Disease (CKD) is a progressive and debilitating condition that affects a significant portion of the global population, with an increasing prevalence in low- and middle-income countries like Bangladesh. CKD is characterized by a gradual loss of kidney function, which, if left unchecked, can lead to complications such as cardiovascular disease, which remains a leading cause of morbidity and mortality among CKD patients [1-4]. A critical indicator of cardiovascular risk in CKD patients is arterial stiffness, which is associated with poor prognosis and higher rates of heart failure, stroke, and myocardial infarction. Monitoring and assessing arterial stiffness in CKD patients is, therefore, crucial for understanding and managing their cardiovascular health [5-7].

One of the most widely used non-invasive methods to assess arterial stiffness is the measurement of pulse wave velocity (PWV), which reflects the velocity at which pressure waves travel through the arteries [8]. Among the different types of PWV measurements, the Brachial-Ankle Pulse Wave Velocity (baPWV) has

gained considerable attention due to its ability to assess both central and peripheral arterial stiffness, providing a more comprehensive measure of vascular health. Studies have shown that an increased baPWV is associated with the presence of atherosclerosis, cardiovascular events, and poor kidney function in CKD patients [9, 10].

In CKD, especially in the non-dialysis stages (3-5), arterial stiffness is known to be accelerated due to factors like endothelial dysfunction, vascular calcification, and fluid retention, which are common in these patients [11, 12]. The evaluation of baPWV in non-dialysis CKD patients may provide a valuable indicator of subclinical cardiovascular changes, enabling early intervention and potentially improving patient outcomes. However, there is limited data on the use of baPWV as a diagnostic tool for assessing arterial stiffness in the Bangladeshi CKD population, which presents an opportunity for research in this area.

This study aims to investigate the association between baPWV and various clinical and laboratory parameters in non-dialysis CKD patients in Bangladesh. Given that early detection of increased arterial stiffness in CKD patients can guide clinical management and reduce the risk of cardiovascular events, it is essential to explore how baPWV correlates with kidney function, age, blood pressure, and other risk factors. Identifying such associations could potentially lead to the use of baPWV as a routine clinical tool in CKD management.

The findings from this research could provide critical insights into the cardiovascular health of CKD patients in Bangladesh, where the prevalence of CKD is increasing, but comprehensive studies on arterial stiffness remain scarce. Additionally, understanding the relationship between baPWV and kidney function may help to develop more targeted therapeutic strategies aimed at improving vascular health and preventing cardiovascular complications in this vulnerable population.

By examining the association between baPWV and CKD in the Bangladeshi context, this study will contribute to the growing body of knowledge on the early detection of cardiovascular risk in CKD patients and could potentially influence clinical practice. With a more detailed understanding of this association, healthcare providers in Bangladesh may be better equipped to address the complex needs of CKD patients and mitigate the cardiovascular burden associated with the disease.

Objective

This study aims to investigate the association between baPWV and various clinical and laboratory parameters in non-dialysis CKD patients in Bangladesh.

METHODOLOGY

This study employed a cross-sectional observational design to assess the relationship between serum vitamin D levels and arterial stiffness in non-dialytic CKD patients (stage 3-5). The research was conducted at the Department of Nephrology, Dhaka Medical College. The study population comprised patients with non-dialytic Chronic Kidney Disease (CKD) in stages 3-5. The study was conducted between September 2021 and March 2023. The estimated sample size for each group was calculated to be approximately 50, resulting in a total study population of 100 patients (50 in each group).

Selection Criteria

Inclusion Criteria

- Non-dialytic Chronic Kidney Disease (stage 3-5)

Exclusion Criteria

- Age < 18 years
- Primary hyperparathyroid diseases
- Malignancy
- Peripheral vascular diseases
- Chronic liver disease (CLD)

Prior to the study, approval was obtained from the Research Review Committee (RRC) and the Ethical Review Committee (ERC) of Dhaka Medical College. Patients were selected according to the inclusion and exclusion criteria, and written informed consent was obtained. A detailed medical history and physical examination were conducted, and relevant clinical and laboratory data were recorded.

The study sample of 100 patients was enrolled using purposive sampling. All participants were informed about the study's objectives, procedures, and potential benefits and risks. The study did not involve experimental drugs or any risks. Data were collected and stored with confidentiality.

Data was collected from patients with non-dialytic CKD (stage 3-5) at the Department of Nephrology, Dhaka Medical College. The patients were enrolled based on inclusion and exclusion criteria, with written informed consent taken from each participant. Data regarding sociodemographic characteristics, clinical parameters, and laboratory results were recorded.

After data collection, the information was reviewed for completeness, accuracy, and consistency. Descriptive statistics were used to analyze the demographic and clinical characteristics. Data were analyzed using SPSS version 26.0, and statistical tests such as Student's t-test, ANOVA, and Chi-square were used to compare continuous and categorical variables. A p-value of <0.05 was considered statistically significant.

The study was approved by the Ethical Review Committee (ERC) of Dhaka Medical College. All participants were informed of the study's purpose and procedures, and written consent was obtained. The confidentiality of patient information was ensured, and participants were informed of their right to withdraw from the study at any time.

RESULTS

Table I shows the demographic variables in study groups. It was observed that almost half (44.0%) of patients belonged to age ≥60 years in Vitamin-D deficient and 21 (44.0%) of patients belonged to age <40 years in Vitamin-D non deficient. The mean age was 55.35±16.79 years in Vitamin-D deficient and 43.06±16.44 years in Vitamin-D non deficient. More than half (56.0%0 of patients were male in Vitamin-D deficient and 26(52.0%) in Vitamin-D non deficient. More than one fourth (28.0%) of patients were smoker in Vitamin-D deficient and 12(24.0%) in Vitamin-D non deficient.

Table I: Demographic variables in study groups (N=100)

Demographic variables	Vitamin-D Deficient (n=50)		Vitamin-D Non-deficient (n=50)		P-value
	n	(%)	n	(%)	
Age in years					
<40	8	16.0	21	42.0	
40-49	10	20.0	10	20.0	
50-59	10	20.0	10	20.0	
≥60	22	44.0	9	18.0	
Mean± SD	55.35±16.79		43.06±16.44		[a]0.001[s]
Range (Min-Max)	19-80		15-76		
Sex					
Male	28	56.0	26	52.0	[b]0.688[ns]
Female	22	44.0	24	48.0	

Table II shows the brachial ankle pulse wave velocity in study groups. The mean brachial ankle pulse wave velocity was 22.5±1.75 m/s in Vitamin-D deficient and 16.86±1.82 m/s in Vitamin-D non deficient. The differences of brachial ankle pulse wave velocity were statistically significant (p<0.05) between Vitamin-D deficient and non deficient.

Table II: Association of brachial Ankle pulse wave velocity in study groups (N=100)

	Vitamin-D Deficient (n=50)	Vitamin-D Non-deficient (n=50)	P-value
	Mean± SD	**Mean± SD**	
baPWV (m/s)	22.5±1.75	16.86±1.82	0.001[s]

Table III shows the brachial ankle PWV according to cause of CKD in study groups. In stage 3A, the mean brachial ankle pulse wave velocity was 18.21±0.96 m/s in Vitamin-D deficient and 15.07±1.72 m/s in Vitamin-D non deficient. In stage 3B, the mean brachial ankle pulse wave velocity was 20.1±0.32 m/s in Vitamin-D deficient and 16.51±0.49 m/s in Vitamin-D non deficient. In stage 4, the mean brachial ankle pulse wave velocity was 20.21±1.17 m/s in Vitamin-D deficient and 17.82±0.93 m/s in Vitamin-D non deficient. In stage 5, the mean brachial ankle pulse wave 0velocity was 23.16±1.3 m/s in Vitamin-D deficient and 19.12±1.14 m/s in Vitamin-D non deficient. The differences of brachial ankle pulse wave velocity in stage 3A, 3B, 4 and 5 were statistically significant (p<0.05) between Vitamin-D deficient and non deficient.

Table III: Comparison of Brachial Ankle PWV according to stages of CKD in study groups (N=100)

CKD (Stage)	Brachial Ankle PWV in Vitamin-D Deficient group	Brachial Ankle PWV in Vitamin-D Non-deficient group	P-value
	Mean± SD	**Mean± SD**	
3A	18.21±0.96	15.07±1.72	0.028[s]
3B	20.1±0.32	16.51±0.49	0.001[s]
4	20.21±1.17	17.82±0.93	0.001[s]
5	23.16±1.3	19.12±1.14	0.001[s]

Table IV shows the brachial ankle PWV according to CKD stage. The mean brachial ankle PWV was 15.1±1.72 m/s in stage 3A, 17.1±1.4 m/s in stage 3B, 18.6±1.5 m/s in stage 4, 22.62±1.88 m/s in stage 5. Brachial ankle PWV was statistically significant ($p<0.05$) among CKD stages.

Table IV: Comparison of Brachial Ankle PWV according to stages of CKD (N=100)

	Stage 3A (n=15)	Stage 3B (n=13)	Stage 4 (n=27)	Stage 5 (n=45)	P-value
	Mean± SD	Mean± SD	Mean± SD	Mean± SD	
baPWV (m/s)	15.1±1.72	17.1±1.4	18.6±1.5	22.62±1.88	0.001[s]

s=significant
p value reached from ANOVA test

Table V shows the comparison of Brachial Ankle PWV according to demographic variables in study groups. The differences of brachial ankle pulse wave velocity in demographic variables (age, sex and smoking status) were statistically significant ($p<0.05$) between Vitamin-D deficient and non deficient.

Table V: Comparison of Brachial Ankle PWV according to demographic variables in study groups (N=100)

Demographic variables	Brachial Ankle PWV in Vitamin-D Deficient group	Brachial Ankle PWV in Vitamin-D Non-deficient group	P-value
Age in years			
<40	21.88±2.28	16.49±1.93	0.001[s]
40-49	21.86±1.31	16.93±1.86	0.001[s]
50-59	22.29±1.10	16.64±1.87	0.001[s]
≥60	23.11±1.84	17.9±1.19	0.001[s]
Sex			
Male	22.44±1.53	16.68±2.15	0.001[s]
Female	22.57±2.0	17.1±1.3	0.001[s]
Smoking			
Smoker	22.85±1.28	16.95±2.22	0.001[s]
Non smoker	22.36±1.90	16.82±1.70	0.001[s]

Table VI shows the multiple linear regression analysis: independent predictors of baPWV in non dialysis CKD. It was observed that Vit-D, age and S.iPTH were independent significant predictors of baPWV. Others were not significantly associated with baPWV.

Table VI: Multiple linear regression analysis: independent predictors of baPWV in non dialysis CKD (Stage 3-5).

	Unstandardized Coefficients		Standardized Coefficients	P value
	B	Std. Error	Beta	
Vitamin D	-0.213	0.029	-0.587	0.001[s]
DM	-0.755	0.653	-0.102	0.250[ns]
Systolic Blood pressure	0.005	0.017	0.046	0.787[ns]
Diastolic Blood pressure	0.001	0.041	0.005	0.979[ns]
S.phosphate	-0.224	0.191	-0.105	0.245[ns]
Calcium	-.238	.250	-.101	0.345 [ns]
iPTH	0.003	0.001	0.188	0.037[s]
Age	0.032	0.018	0.168	0.017[s]
Smoking	0.169	0.603	0.022	0.780[ns]

DISCUSSION

In this study, regarding the demographic variables in study groups, it was observed that almost half (44.0%) of patients belonged to age ≥60 years in Vitamin-D deficient and (16.0%) of patients belonged to age <40 years in Vitamin-D non deficient. The mean age was 55.35±16.79 years in Vitamin-D deficient and 43.06±16.44 years in Vitamin-D non deficient. More than half of patients were male in both Vitamin-D deficient (56%) and Vitamin-D non deficient (52%). One fourth (28.0%) of patients were smoker in Vitamin-D deficient and 12 (24.0%) in Vitamin-D non deficient. The differences of age was statistically significant between Vitamin-D deficient and non-deficient. Which is comparable with a study done by Lee *et al*., (2015) [1]. It was observed that more than half patients belonged to age of 62± 16 years in vitamin D deficient group. They also reported that the prevalence of vitamin D deficiency 25(OH)D 20ng/ml was 58.3%, but was not statistically significant between male and female (57.8% vs 58.8%).

This indicates that vitamin D deficiency is associated with older age which is due to decrease dietary intake, diminished sunlight exposure, reduced skin thickness, impaired intestinal absorption and impaired hydroxylation in the liver and kidneys [2].

In another study, Akdam H and Alp A (2017) reported that CKD patients, who were not on dialysis, the mean age was 56.7±11.3 years and 49.5% were female which is comparable to our study [3]. They also observed that there was a significant gender difference in mean 25(OH) D levels: 12.2±7.8 ng/mL among female patients and 16.0±7.7 ng/mL for male patients which is not consistent with our study. Vitamin D deficiency is common in female is due to decreasing duration of sun exposure and reduce dietary intake [1].

In this study, the brachial ankle PWV according to stage of CKD in study groups showed that in stage 3A, the mean brachial ankle pulse wave velocity was 18.21±0.96 m/s in Vitamin-D deficient and 15.07±1.72 m/s in Vitamin-D non deficient. In stage 3B, the mean brachial ankle pulse wave velocity was 20.1±0.32 m/s in Vitamin-D deficient and 16.51±0.49 m/s in Vitamin-D non deficient. In stage 4, the mean brachial ankle pulse wave velocity was 20.21±1.17 m/s in Vitamin-D deficient and 17.82±0.93 m/s in Vitamin-D non deficient. In stage 5, the mean brachial ankle pulse wave velocity was 23.16±1.3 m/s in Vitamin-D deficient and 19.12±1.14 m/s in Vitamin-D non deficient. The differences of brachial ankle pulse wave velocity in stage 3A, 3B, 4 and 5 were statistically significant between Vitamin-D deficient and non deficient. Another study done by Yoon HE *et al*., (2013) stated that baPWV was independently associated with the decline in renal function and short-term cardiovascular events [4]. Which showed PWV increases as stage of CKD increase. Which is consistent with our study. This is because that serum 25-dihydroxyvitamin D levels is associated with peripheral arterial stiffness (PAS) in patients with stage 3–5 CKD. This indicates that 25-dihydroxyvitamin D could play a role in the progress of PAS [1].

In this study, the comparison of Brachial Ankle PWV according to demographic variables in study groups showed that the differences of brachial ankle pulse wave velocity in demographic variables (age, sex and smoking status) were statistically significant between vitamin-D deficient and non-deficient. It showed baPWV is more in advanced age and in case female and smoker group it is slightly higher. Similar result observed in He D *et al*., (2022) showed baPWV was more strongly associated with male patients, participants aged ≥65 years, and those with other cardiovascular risk factors which is in accordance with our study [5]. PWV increases in older age because arterial stiffness increases as age increased which is because, with older age elastin content decreases, elastin elongates and loses some of the elastic recoil properties (Julie C *et al*., 2015). Tobacco smoking leads to changes in hemodynamic parameters such as heart rate, systolic and diastolic blood pressure. It has a direct influence on the elasticity of blood vessels and increase arterial stiffness and PWV, which can result in development of atherosclerosis (Azra mahmud *et al*., 2003) [6]. Female sex is associated with high BMI and higher susceptibility of weight related arterial stiffness compared to male [7].

In this study shows the multiple linear regression analysis: independent predictors of baPWV in non dialysis CKD. It was observed that Vit-D, age and S.iPTH were independent significant predictors of baPWV. Others were not significantly associated with baPWV. Similar findings were observed by Lee *et al*., (2015) [1]. Where they observed that Vitamin D, intact parathyroid hormone associated with high pulse wave velocity in study group which is consistent with our study.

In this study, the brachial ankle pulse wave velocity in study groups showed that the mean brachial ankle pulse wave velocity was 22.5±1.75 m/s in Vitamin-D deficient and 16.86±1.82 m/s in Vitamin-D non deficient. The differences of brachial ankle pulse wave velocity were statistically significant between Vitamin-D deficient and non-deficient. The similar range of baPWV observed by Lee *et al*., (2015) [7]. They showed that the brachial-ankle pulse wave velocity (baPWV) (m/s) were 17.99± 4.31 inpatients with vitamin-D deficient group and were 15.77± 3.61 in vitamin-D non deficient group. In another study done by Anandabaskar N *et al.*, (2017) reported that the right brachial-ankle pulse wave velocity (14.46 ± 2.4 vs. 13.50 ± 1.7) [8], and left brachial-ankle pulse wave velocity (14.93 ± 2.1 vs. 13.67 ± 2.2) showed a significant reduction following Vitamin D supplementation. High baPWV is associted with low vitamin D level which may be due to Vitamin D has a key role in arterial stiffness as it regulates the renin-angiotensin system, suppresses proliferation of vascular smooth muscle cell, improves insulin resistance and endothelial cell-dependent vasodilation, inhibits anticoagulant activity and myocardial cell hypertrophy and modulates macrophage activity and cytokine generation. In addition, vitamin D deficient patients also tended to have higher intact parathyroid hormone levels, which strongly associated with vascular calcification progression [1].

CONCLUSION

In conclusion, this study reveals a significant association between brachial ankle pulse wave velocity (baPWV) and the presence of Vitamin-D deficiency in non-dialysis CKD patients. The findings indicate that Vitamin-D deficient patients exhibit higher baPWV, which is a marker of increased arterial stiffness, compared to their non-deficient counterparts. This association was observed across various stages of CKD, with significant differences in baPWV between the two groups at each stage. Additionally, demographic factors

such as age, sex, and smoking status were found to further influence baPWV levels, with older age and smoking being linked to higher arterial stiffness. The study also identified Vitamin-D levels, age, and intact parathyroid hormone (iPTH) as independent predictors of baPWV. These results suggest that Vitamin-D deficiency contributes to the progression of arterial stiffness in non-dialysis CKD patients, highlighting the potential role of Vitamin-D in managing cardiovascular risk in this population. The findings emphasize the importance of monitoring baPWV as a marker for vascular health and considering Vitamin-D supplementation as part of CKD management to reduce cardiovascular complications.

REFERENCE

1. Lee, J. H., & Suh, H. S. (2017). Association of serum 25-hydroxy-vitamin D concentration and arterial stiffness among Korean adults in single center. *Journal of Bone Metabolism*, *24*(1), 51-58.
2. Hasan, K., & Housam, E. Department of Geriatric Medicine, Hospital if Pierre Oudot, Bourgoin-jallieu City, Isere Department, Region of Auvergne-Rhone-Alpes, Franch, Department of Opthalmology and visual science, University of British colombia,vancouver,british columbia, Canada.
3. Akdam, H., & Alp, A. (2017). Arterial stiffness and 25-hydroxyvitamin D levels in chronic kidney disease patients. *Revista da Associação Médica Brasileira*, *63*(10), 910-916.
4. Yoon, H. E., Shin, D. I., Kim, S. J., Koh, E. S., Hwang, H. S., Chung, S., & Shin, S. J. (2013). Brachial-ankle pulse wave velocity predicts decline in renal function and cardiovascular events in early stages of chronic kidney disease. *International Journal of Medical Sciences*, *10*(11), 1430.
5. He, D., Gao, L., Yang, Y., Jia, J., Jiang, Y., Sun, P., ... & Huo, Y. (2022). Brachial-ankle pulse wave velocity as a measurement for increased carotid intima-media thickness: A comparison with carotid-femoral pulse wave velocity in a Chinese community-based cohort. *The Journal of Clinical Hypertension*, *24*(4), 409-417.
6. Azra, M., John, F., Thankappan, K. R., & Sylaja, (2003). Department of Pharmacology and Therapeutics, Trinity College Dublin, and the Hypertension Clinic, *St James Hospital, Dublin, Ireland*, *41*(1), 183-7.
7. Zuo, J., Qian, K., He, Y., Tan, I., Butlin, M., Avolio, A., & Chen, P. (2019). Serum 25-Hydroxyvitamin D Deficiency and Insufficiency are Associated with Ankle-Brachial Index but not Arterial Stiffness in an Elderly Community-dwelling Chinese Population. *Artery Research*, *25*(3), 113-119.
8. Anandabaskar, N., Selvarajan, S., Dkhar, S. A., Kamalanathan, S. K., Tamilarasu, K., & Bobby, Z. (2017). Effect of vitamin D supplementation on vascular functions and oxidative stress in type 2 diabetic patients with vitamin D deficiency. *Indian Journal of Endocrinology and Metabolism*, *21*(4), 555-563.
9. Brøndum-Jacobsen, P., Benn, M., Jensen, G. B., & Nordestgaard, B. G. (2012). 25-hydroxyvitamin d levels and risk of ischemic heart disease, myocardial infarction, and early death: population-based study and meta-analyses of 18 and 17 studies. *Arteriosclerosis, thrombosis, and vascular biology*, *32*(11), 2794-2802.
10. Campbell, M. J., & Adorini, L. (2006). The vitamin D receptor as a therapeutic target. *Expert Opinion on Therapeutic Targets*, *10*(5), 735-748.
11. Chen, S. C., Chang, J. M., Liu, W. C., Tsai, Y. C., Tsai, J. C., Hsu, P. C., ... & Chen, H. C. (2011). Brachial-ankle pulse wave velocity and rate of renal function decline and mortality in chronic kidney disease. *Clinical Journal of the American Society of Nephrology*, *6*(4), 724-732.
12. Fehr, A. R., & Perlman, S. (2015). Coronaviruses: an overview of their replication and pathogenesis. *Coronaviruses: methods and protocols*, 1-23.