ORIGINAL ARTICLE ASIAN JOURNAL OF MEDICAL SCIENCES

Prevalence of coronary artery calcification in patients with end-stage renal disease undergoing dialysis and the association of various risk factors with the development of coronary artery calcification in this patient population

Avisek Maity

Associate Professor, Department of Nephrology, Nil Ratan Sircar Medical College and Hospital, Kolkata, West Bengal, India

Submission: 14-06-2024 Revision: 20-08-204 Publication: 01-10-2024

ABSTRACT

Background: Patients with end-stage renal disease (ESRD) on dialysis exhibit a significantly higher risk of coronary artery calcification (CAC) than age-matched normal individuals, contributing to elevated cardiovascular morbidity and mortality. **Aims and Objectives:** This study designed to assess the prevalence of CAC in ESRD patients on dialysis and identify associated risk factors. **Materials and Methods:** Fifty ESRD patients undergoing maintenance dialysis and twenty normal subjects were included in this cross-sectional observational study. Serum calcium, phosphate, and parathyroid hormone were measured in all ESRD patients and normal controls. Multi Row Spiral Computed Tomography was performed to determine CAC scoring (CACS). **Results:** CACS was significantly higher in ESRD patients compared to normal subjects (mean CACS: 91.4 ± 32.7 vs. 7.75 ± 9.5 Agatston units, $P < 0.05$). Elevated levels of calcium phosphate products, serum leptin, intact parathyroid hormone (iPTH), presence of diabetes mellitus, and longer duration of dialysis were correlated with increased incidence of CACS in ESRD patients, as indicated by higher odd's ratios ranged from 1.10 to 6.93. **Conclusion:** CAC is highly prevalent in ESRD patients on dialysis, emphasizing the need for stringent risk factor management. Our findings suggest that controlling calcium phosphate product, serum leptin, age, iPTH levels, and duration of dialysis may reduce CAC burden in this population, potentially mitigating cardiovascular risk and improving outcomes.

Key words: Coronary artery calcification; End-stage renal disease; Dialysis; Cardiovascular complications; Serum leptin; Parathyroid hormone

Access this article online

Website: http://nepjol.info/index.php/AJMS

DOI: 10.3126/ajms.v15i10.66798

E-ISSN: 2091-0576

P-ISSN: 2467-9100

INTRODUCTION

End-stage renal disease (ESRD) represents a critical stage in renal dysfunction where the ability of kidney to function adequately is severely compromised, necessitating renal replacement therapy, commonly in the form of dialysis. Patients grappling with ESRD are confronted not only with the challenges of renal insufficiency but also with an increased risk of cardiovascular complications.[1] Among these, coronary artery calcification (CAC) emerges as a significant concern, as evidenced by the staggering 2–5-fold increase in prevalence compared to age-matched counterparts without renal impairment.[2] The burgeoning recognition of ESRD as a formidable risk factor for cardiovascular disease (CVD) has led to recent calls by the American Heart Association to designate individuals with chronic kidney disease (CKD), including those with ESRD, as being within the highest-risk category for cardiovascular events.[3] This categorization advocates for intensified preventative strategies aimed at mitigating the

Address for Correspondence:
Dr. Avisek Maity, Associate Professor, Department of Nephrology, Nil Ratan Sircar Medical College and Hospital, Kolkata - 700 014, West Bengal, India. **Mobile:** +91-9163653344. **E-mail:** avisek.maity@yahoo.com

prevalence and severity of cardiovascular complications in this vulnerable population.

While traditional risk factors for CVD are certainly pertinent in this context, non-traditional risk factors characteristic of CKD patients play an equally pivotal role in the genesis of arterial calcification. Among these nontraditional factors, the duration of dialysis and disturbances in mineral metabolism stand out prominently.[4,5] The multifaceted interplay of these factors creates a milieu ripe for the development and progression of CAC. A breadth of research has illuminated various factors contributing to the elevated risk of CAC in ESRD patients.[4] Dyslipidemia, hyperhomocysteinemia, serum leptin, and oxidative stress, compounded by the presence of diabetes mellitus (DM) and advanced age, have all been implicated.[4,6,7] Moreover, investigations have increasingly scrutinized the roles of hyperphosphatemia, elevated calcium × phosphate (Ca^{+2}×P) product, and hyperparathyroidism in the intricate pathogenesis of CVD in the context of ESRD.[8] In past, several studies have elucidated the dynamics of CAC progression and its association with diverse risk factors in ESRD patients.[9-11] In addition, research by Barreto et al., underscored the contribution of both traditional and uremia-related risk factors to coronary calcification in hemodialysis patients.[12] Similarly, Bundy et al., demonstrated the incremental risk posed by serum calcification propensity and its association with CAC in CKD patients.[13]

Building upon this foundation, our study endeavors to delve deeper into the prevalence of CAC in ESRD patients on maintenance dialysis, providing insights into the magnitude of this burden within this high-risk cohort. In addition, we aim to delineate the risk factors intricately linked with CAC development in this population, shedding light on the clinical correlates that underpin this ominous cardiovascular complication. By elucidating the prevalence and risk factors associated with CAC in ESRD patients, our study not only contributes to the expanding body of knowledge concerning CVD in CKD but also highlights the imperative for targeted interventions aimed at curtailing the burden of CAC in this vulnerable patient population. Through rigorous examination and understanding of these factors, we aim to pave the way for enhanced risk stratification and personalized therapeutic approaches, ultimately striving to mitigate the substantial cardiovascular morbidity and mortality faced by individuals grappling with ESRD.

Aims and objectives

This cross-sectional study was conducted to investigate the prevalence of CAC in patients with ESRD undergoing dialysis and to identify and analyze the association of various traditional and non-traditional risk factors with the development of CAC in this patient population.

MATERIALS AND METHODS

Study design and patients

This study employed a cross-sectional design to investigate the prevalence of CAC in patients with ESRD undergoing maintenance dialysis, compared to a control group of normal subjects. The study was conducted at Mission of Mercy Hospital between, focusing on patients attending the dialysis unit. In this single-center, cross-sectional observational study, we analyzed the medical charts or records of patients who had been diagnosed with CKD, having ESRD undergoing dialysis and admitted to critical care unit as part of their routine clinical care and followed up from May 2017 to July 2018. Inclusion criteria for the study group comprised individuals diagnosed with ESRD undergoing dialysis for more than 3 months. Exclusion criteria encompassed patients with active infection, inflammation, or those unwilling to participate in the study.

Laboratory parameters and CAC assessment

Blood samples were collected or obtained from all CKD-ESRD patients and normal controls, before dialysis sessions to ensure accurate biochemical profiling, for the assessment of serum calcium, phosphate, and parathyroid hormone levels. Furthermore, all patients with ESRD and normal subjects underwent Multi Row Spiral Computed Tomography to detect CAC scoring (CACS). The extent of coronary calcification was quantified and recorded.

Data source, collection, and management

Patient information was meticulously recorded using a prepared pro forma and questionnaire method, supplemented by comprehensive patient histories and physical examinations. Data collection involved enrolling 50 patients diagnosed with ESRD and undergoing maintenance dialysis, along with 20 normal subjects, and entering their information into a dedicated software system. Supervision by a cardiologist and nephrologist oversaw the data entry process at each center, with a team comprising a medical officer and a nurse. Regular monitoring and verification of data entry were conducted by the study center, ensuring data quality. Data editing capabilities were disabled after 1 week of entry, and only the principal investigator of study center had access to the data. The data management system, developed by Inmenzo Technologies LLP, provided password-protected access and allowed for the export of de-identified encrypted data files. Subsequently, the collected data, including patient values, underwent thorough statistical analysis.

Ethics statement

The study was conducted after due approval from institutional ethics committee of nil Ratan Sircar Medical College and Hospital, Kolkata, India, in accordance with the ethical principles that have their origin in the Declaration of Helsinki,[14] International Council for Harmonization Good Clinical Practice guidelines,[15] any other applicable regulatory requirements and in compliance with the submitted study protocol. All the study patients provided signed written informed consent before enrolment as this was a cross-sectional observational study where patients undergoing dialysis as a part of natural course of their treatment. In addition, the data presented in the current study was analyzed data without identifying any patient. Throughout the data analysis and manuscript preparation, patient confidentiality was completely maintained and data were anonymized.

Statistical analysis

Statistical analysis was performed using both non-parametric and parametric tests. The chi-square (χ^2) test with an alpha error of 5% was employed for categorical data, while parametric tests such as the Z-test were utilized for continuous variables. A $P<0.05$ was considered statistically significant. In addition, odds ratios were calculated to assess the strength of association between risk factors and CAC. Higher odds ratios indicated a greater association between the risk factor and the outcome.

RESULTS

Prevalence of CAC in ESRD patients on dialysis

CACS was conducted in 50 patients diagnosed with ESRD undergoing dialysis, alongside 20 normal subjects. In the cohort of ESRD patients on dialysis, the mean CACS was determined to be 91.4±32.7 Agatston units. Comparatively, normal subjects exhibited a significantly lower mean CACS of 7.75±9.5 Agatston units ($Z=14.3$, $P<0.05$). Out of the 50 patients undergoing dialysis, a striking 48 individuals (96%) manifested CAC. The observed CACS in this group ranged from a minimum of 8 Agatston units to a maximum of 204.0 Agatston units. The sharp disparity in the prevalence of CACS between ESRD patients on dialysis and normal subjects underscores the heightened burden of CAC in this high-risk population. Figure 1 illustrates the prevalence of CAC in ESRD patients undergoing dialysis compared to normal subjects.

Association of risk factors with CAC in ESRD patients

A comprehensive analysis of risk factors associated with CAC in ESRD patients revealed notable determinants contributing to increase CACS. Simple odd's ratios were calculated to gauge the strength of association between each risk factor and elevated CACS. Among the identified determinants, serum calcium phosphate product (Ca × Pi) exhibited a simple odd's ratio of 2.67, indicating a considerable association with elevated CACS. Leptin, a marker of adipose tissue, demonstrated the highest simple odd's ratio of 6.93, suggesting a robust association with increased CACS. Age exceeding 50 years conferred a simple odd's ratio of 2.2, while the duration of dialysis yielded a simple odd's ratio of 1.84. Interestingly, DM and parathyroid hormone (PTH) levels also displayed associations with elevated CACS, with simple odd's ratios of 1.10 and 1.95, respectively. These findings suggest the multifactorial nature of CAC in ESRD patients, with various metabolic, demographic, and clinical factors exerting discernible influences on its pathogenesis. Table 1 provides a summary of the determinants associated with CAC in ESRD patients, highlighting the calculated simple odd's ratios for each risk factor.

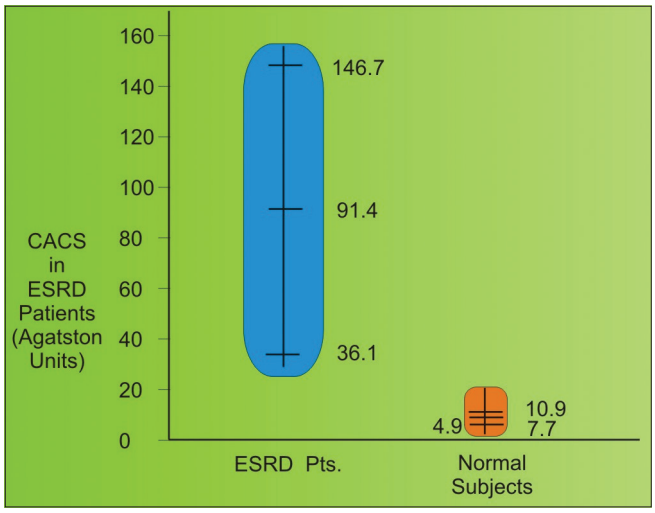

Figure 1: Prevalence of coronary artery calcification in end-stage renal disease patient undergoing dialysis
The graph depicts the distribution of CACS among ESRD patients on dialysis and normal subjects. ESRD patients' exhibit a significantly higher prevalence and severity of coronary artery calcification compared to normal subjects

DISCUSSION

CVD remains a leading cause of mortality among individuals with ESRD, accounting for more than 50% of deaths in this population. Remarkably, ESRD patients exhibit a higher burden of CAC than age-matched individuals with angiographically proven coronary artery disease.[16] This pronounced calcification in ESRD patients underscores the complex interplay between traditional and nontraditional risk factors in the pathogenesis of cardiovascular complications. Beyond conventional risk factors, ESRD patients contend with a myriad of nontraditional risk factors, including disturbances in mineral metabolism, hyperphosphatemia,

Table 1: Determinants of CAC in ESRD patients

Determinants	Simple odd's ratio Increased CACS in ESRD patients
Calcium phosphate product (Ca × Pi)	2.67
Leptin	6.93
Age >50 years	2.2
Duration of dialysis	1.84
DM	1.10
PTH	1.95

PTH: Parathyroid hormone, DM: Diabetes mellitus, CAC: Coronary artery calcification, ESRD: End-stage renal disease, CACS: Coronary artery calcification score

hyperparathyroidism, and hyperlipidemia, which contribute to the disproportionate burden of CAC observed in this population.[4] The present study contributes to the growing body of literature elucidating the intricate relationship between ESRD and the development of CAC in patients undergoing maintenance dialysis. Our findings underscore the substantial burden of CAC in this high-risk population and shed light on the diverse array of risk factors implicated in its pathogenesis. Moreover, our study also contributes to the understanding of CAC in ESRD patients by investigating the association of serum leptin levels with CAC in this vulnerable population.

In present study, the prevalence of CAC in ESRD patients on dialysis was strikingly high, with 96% of patients demonstrating evidence of CAC. This prevalence far exceeds that observed in normal subjects, highlighting the disproportionate burden of CAC in ESRD patients and in a line with findings from a meta-analysis conducted by Wang et al.[17] The mean CACS in ESRD patients was notably elevated, ranging from 8 to 204.0 Agatston units, compared to normal subjects, reinforcing the heightened risk of cardiovascular complications in this population. These findings align with previous studies by Stompor group[18] and Merjanian group,[19] which reported similarly high incidences of CAC in ESRD populations.

Our analysis of risk factors associated with CAC in ESRD patients revealed several notable determinants contributing to its development. Serum calcium phosphate product (Ca × Pi) emerged as a significant predictor of elevated CACS, with a simple odd's ratio of 2.67, indicating a substantial association. This finding underscores the importance of mineral metabolism disturbances in the pathogenesis of vascular calcification in ESRD patients.[20,21] In addition, we found that DM, elevated serum leptin levels, elevated PTH levels, elderly age (>50 years), and longer duration of dialysis were associated with increased CACS in ESRD patients. These findings underscore the multifactorial nature of CAC in ESRD, with metabolic, demographic, and clinical factors exerting discernible influences on its pathogenesis.[22]

Notably, leptin, a marker of adipose tissue, demonstrated the highest simple odd's ratio of 6.93, suggesting a robust association with increased CACS. This finding is supported by previous research by Shih et al., and Ciccone et al., implicating adipose tissue dysfunction in the development of vascular calcification and underscores the importance of considering nontraditional risk factors in assessing cardiovascular risk in ESRD patients.[23,24] Age exceeding 50 years and longer duration of dialysis were also identified as significant risk factors for elevated CACS, with simple odd's ratios of 2.2 and 1.84, respectively. These findings underscore the cumulative effect of aging and prolonged exposure to dialysis on the progression of vascular calcification in ESRD patients. Interestingly, DM and PTH levels displayed associations with elevated CACS, although with lower Simple odd's ratios of 1.10 and 1.95, respectively. While the associations were less pronounced, they highlight the multifactorial nature of CAC in ESRD patients, with metabolic derangements and hormonal disturbances contributing to its pathogenesis.[25]

The multifactorial etiology of CAC in ESRD patients underscores the complexity of CVD in this population. Metabolic, demographic, and clinical factors interact in intricate ways to promote vascular calcification and exacerbate cardiovascular risk. Our study adds to the growing body of evidence supporting the importance of aggressive risk factor management in ESRD patients to mitigate the burden of CAC and reduce cardiovascular morbidity and mortality. The implications of our findings extend beyond the realm of clinical research to clinical practice. Health-care providers caring for ESRD patients must be vigilant in assessing and managing risk factors for CAC, including mineral metabolism abnormalities, adipose tissue dysfunction, and aging. Strategies aimed at optimizing mineral metabolism, glycemic control, and cardiovascular risk factor modification are paramount in reducing the incidence and progression of CAC in this vulnerable population.[4,25,26]

In a nutshell, our study highlights the high prevalence of CAC in ESRD patients undergoing dialysis and identifies several important risk factors associated with its development. These findings underscore the urgent need for aggressive risk factor management in this vulnerable population to mitigate the burden of CAC and reduce cardiovascular morbidity and mortality. Further research is warranted to elucidate the underlying mechanisms driving vascular calcification in ESRD patients and to develop targeted interventions aimed at preventing its progression.

Limitations of the study

Limitations of our study warrant consideration. The cross-sectional design precludes establishment of causality, and longitudinal studies are needed to elucidate the temporal relationship between risk factors and CAC progression.

In addition, our sample size may limit generalizability, and larger multicenter studies are warranted to validate our findings across diverse patient populations.

CONCLUSION

Our study highlights the significant burden of CAC among ESRD patients undergoing dialysis. We found a high prevalence of CAC in this population, with the majority of patients exhibiting evidence of CAC. The heightened prevalence of CAC observed in ESRD patients on dialysis highlights the urgent need for proactive risk factor management in this population. Our findings identify several key determinants, including calcium phosphorus product, serum leptin levels, age, PTH levels, and duration of dialysis, which are significantly associated with increased CAC incidence in ESRD patients. These results emphasize the importance of targeted interventions aimed at controlling these risk factors to alleviate the burden of coronary artery calcium and improve cardiovascular outcomes in ESRD patients undergoing dialysis.

ACKNOWLEDGMENT

Authors are thankful to Dr. Jaykumar Sharma (Intas Pharmaceutical Ltd, Gujarat, India) for critically reviewed the manuscript and fruitful suggestions. Authors also acknowledge Dr. Mehul R. Chorawala and Ms. Sakshi Srivastava, Intas Pharmaceutical Ltd, Gujarat, India, for medical writing assistance and additional editorial communication. The authors also grateful to the study patients for their participation in this study.

DECLARATION AND STATEMENT

Data availability statement

The datasets generated during study are available from corresponding author upon reasonable request.

Consent to participate

All participants provided a written informed consent form to participate in the study.

Consent for publication

Consent for publication was obtained from all the participants or legally authorized representatives involved in this study.

REFERENCES

1. Wouk N. End-stage renal disease: Medical management. Am Fam Physician. 2021;104(5):493-499.
2. United States Renal Data System. USRDS 2023 Annual Report. Bethesda, Maryland: National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases. Available from: https://usrds-adr.niddk.nih.gov/2023 [Last accessed on 2024 Mar 22].
3. Sarnak MJ, Levey AS, Schoolwerth AC, Coresh J, Culleton B, Hamm LL, et al. Kidney disease as a risk factor for development of cardiovascular disease: A statement from the American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure Research, Clinical Cardiology, and Epidemiology and Prevention. Circulation. 2003;108(17):2154-2169.
https://doi.org/10.1161/01.CIR.0000095676.90936.80
4. Jankowski J, Floege J, Fliser D, Böhm M and Marx N. Cardiovascular disease in chronic kidney disease: Pathophysiological insights and therapeutic options. Circulation. 2021;143(11):1157-1172.
https://doi.org/10.1161/CIRCULATIONAHA.120.050686
5. Kendrick J and Chonchol MB. Nontraditional risk factors for cardiovascular disease in patients with chronic kidney disease. Nat Clin Pract Nephrol. 2008;4(12):672-681.
https://doi.org/10.1038/ncpneph0954
6. Vallianou NG, Mitesh S, Gkogkou A and Geladari E. Chronic kidney disease and cardiovascular disease: Is there any relationship? Curr Cardiol Rev. 2019;15(1):55-63.
https://doi.org/10.2174/1573403X14666180711124825
7. Rapa SF, Di Iorio BR, Campiglia P, Heidland A and Marzocco S. Inflammation and oxidative stress in chronic kidney disease-potential therapeutic role of minerals, vitamins and plant-derived metabolites. Int J Mol Sci. 2019;21(1):263.
https://doi.org/10.3390/ijms21010263
8. Shaman AM and Kowalski SR. Hyperphosphatemia management in patients with chronic kidney disease. Saudi Pharm J. 2016;24(4):494-505.
https://doi.org/10.1016/j.jsps.2015.01.009
9. Goodman WG, Goldin J, Kuizon BD, Yoon C, Gales B, Sider D, et al. Coronary-artery calcification in young adults with end-stage renal disease who are undergoing dialysis. N Engl J Med. 2000;342(20):1478-1483.
https://doi.org/10.1056/NEJM200005183422003
10. Guérin AP, London GM, Marchais SJ and Metivier F. Arterial stiffening and vascular calcifications in end-stage renal disease. Nephrol Dial Transplant. 2000;15(7):1014-1021.
https://doi.org/10.1093/ndt/15.7.1014
11. Milliner DS, Zinsmeister AR, Lieberman E and Landing B. Soft tissue calcification in pediatric patients with end-stage renal disease. Kidney Int. 1990;38(5):931-936.
https://doi.org/10.1038/ki.1990.293
12. Barreto DV, Barreto FC, Carvalho AB, Cuppari L, Cendoroglo M, Draibe SA, et al. Coronary calcification in hemodialysis patients: The contribution of traditional and uremia-related risk factors. Kidney Int. 2005;67(4):1576-1582.
https://doi.org/10.1111/j.1523-1755.2005.00239.x
13. Bundy JD, Cai X, Scialla JJ, Dobre MA, Chen J, Hsu CY, et al. Serum calcification propensity and coronary artery calcification among patients with CKD: The CRIC (chronic renal insufficiency cohort) study. Am J Kidney Dis. 2019;73(6):806-814.
https://doi.org/10.1053/j.ajkd.2019.01.024
14. Dixon JR Jr. The International Conference on harmonization good clinical practice guideline. Qual Assur. 1998;6(2):65-74.
https://doi.org/10.1080/105294199277860
15. World Medical Association. World Medical Association Declaration of Helsinki: Ethical principles for medical research

involving human subjects. JAMA. 2013;310(20):2191-2194.
https://doi.org/10.1001/jama.2013.281053

16. Aoki J and Ikari Y. Cardiovascular disease in patients with end-stage renal disease on hemodialysis. Ann Vasc Dis. 2017;10(4):327-337.
https://doi.org/10.3400/avd.ra.17-00051
17. Wang XR, Zhang JJ, Xu XX and Wu YG. Prevalence of coronary artery calcification and its association with mortality, cardiovascular events in patients with chronic kidney disease: A systematic review and meta-analysis. Ren Fail. 2019;41(1):244-256.
https://doi.org/10.1080/0886022X.2019.1595646
18. Stompor T, Pasowicz M, Sułłowicz W, Dembińska-Kieć A, Janda K, Wójcik K, et al. An association between coronary artery calcification score, lipid profile, and selected markers of chronic inflammation in ESRD patients treated with peritoneal dialysis. Am J Kidney Dis. 2003;41(1):203-211.
https://doi.org/10.1053/ajkd.2003.50005
19. Merjanian R, Budoff M, Adler S, Berman N, Mehrotra R. Coronary artery, aortic wall, and valvular calcification in nondialyzed individuals with type 2 diabetes and renal disease. Kidney Int. 2003;64(1):263-271.
https://doi.org/10.1046/j.1523-1755.2003.00068.x
20. Moe SM and Drüeke TB. Management of secondary hyperparathyroidism: The importance and the challenge of controlling parathyroid hormone levels without elevating calcium, phosphorus, and calcium-phosphorus product. Am J Nephrol. 2003;23(6):369-379.
https://doi.org/10.1159/000073945
21. Block GA, Hulbert-Shearon TE, Levin NW and Port FK. Association of serum phosphorus and calcium x phosphate product with mortality risk in chronic hemodialysis patients: A national study. Am J Kidney Dis. 1998;31(4):607-617.
https://doi.org/10.1053/ajkd.1998.v31.pm9531176
22. Nasri H and Kheiri S. Effects of diabetes mellitus, age, and duration of dialysis on parathormone in chronic hemodialysis patients. Saudi J Kidney Dis Transpl. 2008;19(4):608-613.
23. Shih YL, Shih CC, Chen SY and Chen JY. Elevated serum leptin levels are associated with lower renal function among middle-aged and elderly adults in Taiwan, a community-based, cross-sectional study. Front Endocrinol (Lausanne). 2022;13:1047731.
https://doi.org/10.3389/fendo.2022.1047731
24. Ciccone M, Vettor R, Pannacciulli N, Minenna A, Bellacicco M, Rizzon P, et al. Plasma leptin is independently associated with the intima-media thickness of the common carotid artery. Int J Obes Relat Metab Disord. 2001;25(6):805-810.
https://doi.org/10.1038/sj.ijo.0801623
25. Stompór T. Coronary artery calcification in chronic kidney disease: An update. World J Cardiol. 2014;6(4):115-129.
https://doi.org/10.4330/wjc.v6.i4.115
26. Kim JS and Hwang HS. Vascular calcification in chronic kidney disease: Distinct features of pathogenesis and clinical implication. Korean Circ J. 2021;51(12):961-982.
https://doi.org/10.4070/kcj.2021.0995

Authors Contribution:
AM- Conceptualization, design of study, clinical protocol, definition of intellectual content, literature survey, prepared first draft of manuscript and subsequent edition/revision, implementation of study protocol, data collection, statistical analysis and interpretation, created tables and figures.

Work attributed to:
Mission of Mercy Hospital and Research Centre, Mother Teresa Sarani, Dharmatala, Taltala, Kolkata, West Bengal-700016, India.

Orcid ID:
Avisek Maity - https://orcid.org/0000-0003-1300-2243

Source of Funding: Nil, **Conflicts of Interest:** None declared.

Remark: Reprint request should be directed to corresponding author Dr. Avisek Maity (avisek.maity@yahoo.com).