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ORIGINAL ARTICLE **OPEN ACCESS**

Histopathological evaluation of arterial wall calcification in CKD-5 patients; role of diabetes mellitus and metabolic factors.

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ABSTRACT

Majority of deaths in patients of chronic kidney disease (CKD) are due to cardiovascular diseases (CVD). Growing evidence suggests that increased risk of CVD is partly due to vascular calcifications. Traditionally vascular calcification has been associated with diabetes mellitus, advance age and CKD. The objective of the present study was the histopathological evaluation of arterial wall calcification in chronic kidney disease stage 5 patients and assessing the role of diabetes mellitus and metabolic factors in it. In this cross sectional study we included patients diagnosed with stage 5 CKD who were scheduled for creating an arterio-venous fistula (AVF). Each patient underwent complete metabolic work-up. The radial arterial wall sample was taken during AVF surgery and analyzed for calcification. Diabetic nephropathy was the cause of CKD in 17 patients (53.1%),,it was the cause of chronic interstitial nephritis//unknown in 12 (37.5%) patients, chronic glomerulo nephritis in 2 patients(6.2%) and autosomal dominant polycystic kidney disease in 1 patient . Radial artery calcification was present in 52.9% patients in diabetic group while no patient in non-diabetic group had calcification. Diabetes was associated with increased risk of vascular calcification independent of other confounding factors that may affect arterial wall calcification. Interventions to decrease vascular calcification may improve cardiovascular morbidity in diabetic nephropathy patients.

KEYWORDS-*CKD, cardiovascular diseases, Diabetes mellitus, vascular calcification*

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INTRODUCTION

The morbidity and mortality due to cardiovascular disease is high in hemodialysis (HD) patients as compared to normal population. This is true venfor younger HD patients aged less than 45 years.[1]Increased arterial stiffening and a high prevalence of atherosclerotic lesions are responsible for increased cardiovascular deaths in chronic kidney disease (CKD) population. Diabetic nephropathy is reported as the leading cause of chronic kidney disease and represents a major health problem globally. Vascular disease is rampant in diabetics and doubles the mortality as compared to non-diabetics. Cardiovascular disease (CVD) overall reduces the life expectancy in diabetics by 60% in terms of the life years lost.[2]Diabetes is also a significant independent cardiovascular risk factor even after adjusting for age, body mass index (BMI) and hypertension.[3]Chronic kidney disease – mineral bone disease (CKD-MBD) increases fracture risk, cardiovascular disease, significant reduction in quality of life and enhanced morbidity and mortality in CKD stage 3-5 patients.[4,5,6]Impaired mineral metabolism is a common finding in patients with diabetic nephropathy [7]than in general population.

Chronic kidney disease patients often do not receive adequate care in terms of risk factor modification for vascular disease. Patients suffering from chronic kidney disease are mostly been excluded from major cardiovascular trials, thus limiting the evidence for potential treatments. Extensive vascular calcification is a significant finding in arterial wall of chronic renal patients[8]and is proportional to subsequent cardiovascular mortality beyond established conventional risk factors.[9]Vascular calcification is classified into two types depending on the localization of calcium deposition: intimal calcification and medial

calcification.[10]These two forms are closely related to each other. Intimal calcification presents within fully developed atheromatous fibro fatty plaque and is a progressive lesion whereas medial calcification can occur independent of atheromatous plaques .It is usually observed in medium sized arteries in CKD/ESRD and diabetes mellitus.[11] Calcification of the intima is associated with development of atherosclerosis. Calcification of tunica media (medialcalcinosis, Monkeberg's sclerosis) is observed in patients on long term hemodialysis even without risk factors for atherosclerosis. The factors that affect the severity of vascular calcification include duration of hemodialysis therapy, the age of the patient and severity of impaired calcium-phosphate metabolism.[12] Increase serum phosphate level accounts for substantial cardiovascular morbidity and mortality amongst people on chronic dialysis therapy.[13]Hyperphosphatemia also has been independently linked with calcification of the aorta and coronary arteries .[14]and all these are responsible for causing deaths in the setting of ESRD.[15]Very few studies have attempted to correlate the morphological changes in the arterial wall with the documented laboratory findings and associated risk factors observed in patients having uremia.. Also the correlation of primary disease with the histopathological findings of the arterial wall has not been much studied. The objective of this study is to evaluate whether metabolic disturbances in calcium, phosphate or iPTH (intact parathyroid hormone) will lead to calcification of the arterial wall in diabetic patients with chronic renal disease, for this the calcification seen in wall of radial artery of CKD patients presenting with diabetes or without having diabetes was determined, also risk factors influencing the patients of these two groups were compared.

MATERIAL AND METHODS

A cross sectional study was undertaken including patients with stage 5 CKD who had to undergo the formation of arterio-venous fistula (AVF). Detailed history of age, gender, duration of disease, primary etiology of renal disease, co-morbidities, and duration of hemodialysis, diabetes, hypertension and CKD was recorded. The glomerular filtration rate was determined by the serum creatinine level using CKD-EPI equation. Each patient underwent complete hemogram , liver function tests, urea, creatinine, electrolytes, uric acid estimation, serum albumin, serum calcium, serum phosphorus, lipid profile, serum vitamin D levels and intact PTH (iPTH) level. The iPTH level was determined using the chemi-luminescent micro-particle immunoassay. Small elliptical specimen of the arterial wall were taken by the surgeon during AVF surgery before making the anastomosis. For light microscopic examination of the radial artery wall, sample were fixed in 10% formalin. Specimens were then finely sectioned into 5-µm sections and staining was done with routine hepatoxylin and eosin stain. Masson's Trichrome stain was also used. For demonstrating calcification the standard Von Kossa stain was used and calcification was graded on a semi-quantitative scale (0- none, 1- mild, 2- moderate and 3- severe).

STATISTICAL ANALYSIS

Statistical analysis was done using Stata 11.0 version. The categorical variables such as sex, AVF, hypertension and diabetes were compared using the chi square test or the Fisher exact test, whichever seemed appropriate. Continuous variables were compared between the two groups by the help of the unpaired t-test.

RESULTS

Total thirty three patients were included in the study, one patient was excluded due to inadequate sample. Total 32 radial arterial wall pathological samples were assessed. **(Fig 1**) Diabetic nephropathy was the cause of CKD in 17 (53.1%) patients, it lead to chronic interstitial nephritis/unknown in 12 patients (37.5%), chronic glomerulonephritis was seen in two patients that is (6.2%) patients while one (3.1%) patient had autosomal dominant polycystic kidney disease.(Table 1)

The mean age of the study population was 49.9±12.8 years, 71.4 % of patients were male, 48.5 % patients were diabetics, 84.3 % patients were hypertensive, mean CKD duration was 1.7± 1.2 years and the mean duration of dialysis was 0.8± 0.3 years .Calcification was seen in nine (28.12%) patients. Patients were divided into diabetic and non-diabetic CKD groups. Mean age was 57.8 ± 6.9 yrs in diabetic group and 41 ± 12.1 years in non-diabetic group ($p<0.001$). Mean serum albumin was significantly low in diabetic as compared to non-diabetic group ($p<0.011$). Mean triglyceride was 153.8±78.0 mg/dl in diabetic group and 102.1 ±30.2 mg/dl in non-diabetic group ($p<0.022$).Radial artery calcification was present in 52.9% patients in diabetic group while no patient in non-diabetic group had calcification (Table 2).

DISCUSSION

High prevalence of atherosclerotic lesions and arterial stiffness in CKD patients is responsible for increased cardiovascular mortality. Vascular wall changes probably start in the early stages of kidney

disease but studies supporting this are lacking. Intimal proliferation of small and medium sized arteries increases proportionately with age. [16] These changes are proportional to changes occurring in aorta and coronary arteries.[17] Smokers and hypertensive patients have rapid intimal proliferation in intrarenal arteries.[18,19]Hypertension contributes to the pathogenesis of intrarenal vascular changes in CKD patients.

Cesar García-Canton, et al,[20]in a study conducted in CKD patients reported increased vascular wall calcification. Patients with older age, diabetes, low serum vitamin D level and history of CVD were more susceptible. Mean age of the patients in this study was 63.5 ± 13 years.Two thirds of patients were males, two third were diabetics and almost 50%had a previous episode of associated CVD. X-ray images of hands and lateral lumbar spine as well as pelvis of all the patients were taken and semi-quantitative vascular calcification scores were measured. They found a high prevalence of vascular calcification in CKD population. Normal levels of Vitamin D (>30 ng/mL) was seen in 18.5% of these patients, 53.7% of the patients showed below normal levels of Vitamin D (15–30 ng/mL),while 27.8% showed insufficient levels of vitamin D (<15 ng/mL). Multivariate analysis accounted for the fact that increase in age, diabetes and CVD have a direct relation while 25(OH)D levels were inversely related to arterial wall calcification.

Many studies have proved the relationship between metabolic changes in CKD population with arterial calcification but very few have studied direct histopathological assessment with calcification of arterial walls. In our study diabetes represented a major independent and significant risk factor for arterial wall calcification in CKD patients as prevalence of radial artery calcification were significantly high in diabetic CKD group as compare to non-diabetic CKD group. Ishimura E *et al* reported that the vascular calcification was more prevalent in hemodialysis patients with diabetes mellitus as compared to those without diabetes mellitus.[21]Findings of the present study are in accordance with previous studies which studied other sites of vascular calcification in CKD patients.[22,23,24]Diabetes may promote vascular calcification by heterogeneous metabolic processes mainly hyperglycemia, hyperlipidemia and insulin resistance. Glycation, oxidative and carbonic stress along with tissue hypoxia [25,26,27] also play a significant role. Diabetic CKD patients have advanced arteriosclerosis leading to a higher degree of vascular calcification.[28,29,30]

In present study no significant difference was observed in duration of hemodialysis, systolic and diastolic blood pressure, calcium, Phosphorus, Ca×Pi product &iPTH levels between the diabetes and non-diabetes CKD groups. Many studies have demonstrated a direct correlation between increased serum calcium levels and vascular calcification in the CKD patients.[31,32] A significant correlation has also been documented between severity of calcification and a raised Ca × P product.[33] In a study by **Ibels *et al*.**[34] arterial wall samples of patients suffering from uremia were collected and compared with control patients from the non-uremic group. Arterial wall from the uremic patients demonstrated a fibrous or fibro elastic thickening of tunica intima, calcification, disruption and even reduplication of the internal elastic lamina. Lipid deposition wasseen in only few cases. The arterial wall of these patients had high calcium concentration as compared to the controls. Intimal thickening was correlated with the duration of renal disease. Similarly arterial wall calcification, duration of hypertension as well as the time duration of kidney disease were also correlated. Itwas reported that the control group showed a positive correlation between age and arterial calcium, but it was also seen that the calcium levels were high in the aorta than in any other artery regardless of the age. Teresa Adragao, *et al* [35] in 123 chronic HD patients showed that diabetes (P=0.01), male gender (P<0.001), age (P=0.02), HD duration (P=0.02) and MAP (P=0.03) had increased calcification score in the blood vessels. Calcium, phosphate and Ipth concentration were measured and analyzed for a period of six months before the arterial wall calcification score evaluation. Increase in calcification was associated with significant iliac calcifications (P=0.03) and with PAD (P=0.01) phosphorus levels were also associated with CAD (P=0.01)), but iPTH values were not correlated with vascular calcification. It is reported that the disturbances in calcium and phosphate metabolism has no effect on vascular calcification in non-dialyzed patients having diabetic nephropathy.[36] Similar studies have failed to establish a correlation among serum calcium-phosphate product and iPTH levels with severity of vascular calcification.[37]

CONCLUSION

In conclusion, our study clearly demonstrates the role of diabetes as a significant independent risk factor for enhanced arterial calcification in CKD patients and is independent of other confounding variables that may influence arterial wall calcification. Further studies should be conducted to identify unconventional and minor metabolic factors that may contribute to arterial calcification in diabetic CKD patients.

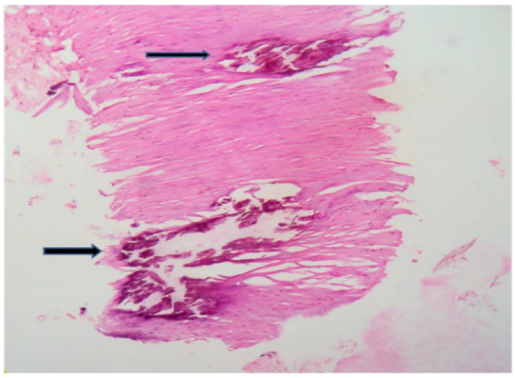
Basic Disease	No of patients (%)
DN(%)	17 (53.1%)
CIN/Unknown (%)	12(37.5%)
CGN(%)	2(6.2%)
ADPKD(%)	1 (3.1%)

Table I Etiology of CKD

Table 2- Clinical, laboratory parameters and radial artery calcification in diabetic Vs non-diabetic CKD patients (n-32)

	Diabetic CKD (n-17) (Mean ± SD)	Non-diabetic CKD (n-15)(Mean ± SD)	P Value
Age(yrs)	57.8±6.9	41± 12.1	<0.001
Male(%)	14(82.3%)	11(73.3%)	0.538
AntiHT drugs	2.1±1.3	2.2±1.7	0.880
HTN(%)	15/17(88.2%)	12/15(80%)	0.522
HD duration(months)	0.8± 0.3	0.8±0.3	0.747
SBP (mm of hg)	143.2±21.0	141.6±24.9	0.848
DBP(mm of hg)	86.7±9.8	86.4±14.9	0.945
LVH(%)	9/17(52.9%)	6/15(40%)	0.464
HB (g/l)	8.5±1.3	8.26±1.3	0.539
Ca(mg/dl)	8.3±0 .9	8.07±1.1	0.400
Po4(mg/dl)	8.0±2.1	7.9±2.4	0.862
Ca*po4	67.1±19.7	63.8±21.9	0.645
Uric acid(mg/dl)	8.01± 2.8	8.6±2.4	0.521
iPTH(pg/ml)	462.2±281.4	397.6±212.7	0.474
S.albumin(g/l)	3.2 ± 0.5	3.7±0 .4	0.011
Vitamin D(ng/mL)	28.6 ± 10.9	27.6 ±12.5	0.817
Triglyceride (mg/dl)	153.8±78.0	102.1±30.2	0.022
T. cholesterol(mg/dl)	140.4± 48.9	113.1±24.5	0.059
Calcification	9/17 (52.9%)	0/15 (0%)	0.001

Figure 1-Von Kossa staining showing medial calcification (arrow) of the radial arterial wall specimen. (High resolution image, magnification x 200)



CONFLICT OF INTEREST

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