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Impact of Chronic HCV Infection on Coronary Calcification in Prevalent Haemodialysis Patients

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ABSTRACT

Background: Hepatitis C virus (HCV) chronically infects an estimated 170 million people worldwide**.** Approximately 30% of patients who develop acute hepatitis C recover spontaneously, signaled by improved symptoms, normalized liver-related chemistries, loss of HCV RNA from serum, and the development of HCV antibody. Cirrhosis rates become significant after 20 years of HCV infection. Haemodialysis is a process that uses a man-made membrane (dialyzer) to clear wastes such as urea from the blood, restore the proper balance of electrolytes in the blood and eliminate extra fluid from the body. Vascular calcification is common in patients with advanced haemodialysis and is associated with poorer outcomes.

Objectives: The aim was to evaluate the possible impact of chronic HCV infection on coronary calcification in prevalent haemodialysis patients in Naval Forces hospital, Alexandria, Egypt and its relation to demographic data, haemodialysis data and other laboratory findings.

Patietns and methods: This cross-sectional study was carried out on 60 patients with at least one year duration on regular haemodialysis; 30 HCV negative prevalent haemodialysis patients and 30 HCV positive prevalent haemodialysis patients.

Results: Our study revealed that HCV negative patients included 25 (83.3%) males and 5 (16.7%) females, their mean age was 51.67 ± 6.91 years. The mean haemodialysis duration was 7.5 ± 1.89 years. There were statistically a high significant difference between HCV negative prevalent haemodialysis patients and HCV positive prevalent haemodialysis patients regarding AST, significant differences regarding ALT and albumin and non-significant differences regarding bilirubin, prothrombin time, international normalized ratio, cholesterol and total glycerides. But, there were no significant differences between HCV negative prevalent haemodialysis patients and HCV positive prevalent haemodialysis patients regarding CKD-MBD parameters (including PO_4 and iPTH), except for calcium which exhibited statistically a significant difference. Also, there were no significant differences between HCV negative prevalent haemodialysis patients and HCV positive prevalent haemodialysis patients regarding creatinine, sodium and potassium, significant differences regarding URR, C-reactive protein and a high significant difference regarding Ca score. In HCV negative prevalent haemodialysis patients, there were statistically significant correlations regarding dialysis duration and international normalized ratio and high significant correlations between Ca score and creatinine, AST and prothrombin time. In HCV positive prevalent haemodialysis patients, there were statistically significant correlations between Ca score and dialysis duration and creatinine. Other correlations were insignificant. There were non-significant relations between Ca score and gender and dialysis vascular access.

Conclusion: A very high incidence of vascular calcification was found in chronic haemodialysis patients in our study as compared to other studies. Vascular calcification is correlated with many risk factors and control of the modifiable risk factors can help to decrease prevalence of vascular calcification.

Keywords: chronic hepatitis C virus, coronary calcification, vascular calcification, haemodialysis.

INTRODUCTION

Hepatitis C virus (HCV) infection remains frequent in patient receiving long-term dialysis both in developed and less-developed countries. The natural history of HCV infection in dialysis patients remains incompletely understood. Defining the natural history of HCV remains difficult for several reasons: the disease has a very long duration, it is mostly asymptomatic, and determining its onset may be difficult. Additional factors can modify the course including coinfection with HBV, HIV, and alcohol use [1, 2].

Vascular calcification is a very common form of extra skeletal calcification in patients with chronic kidney disease (CKD). It occurs in the intimal and medial layers of the arterial wall, the most important type in CKD being calcification in the media. It is related to decrease vascular elasticity and increased mortality[3]. The mechanisms of arterial calcification are complex and are regulated by plasma components, which keep minerals dissolved and repress their deposition in tissues [4].

Patients on haemodialysis (HD) suffer from extensive cardiovascular calcifications (VCs). Vascular calcification is an independent risk factor and might explain the excessively increased cardiovascular mortality in this population[5,6].

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In the past, the development of vascular calcification was discovered to be actively regulated and influenced by inhibitors of calcification e.g. matrix Gla protein (MGP), and fetuin-A[7, 8].

Vitamin K may be involved in vascular calcification via its effect on post-translational modification of MGP namely gamma carboxylation to be fully active as a powerful vascular wall-based inhibitor of calcification [9].

An important question is whether oral supplementation of Vitamin K1 is able to slow the progression of vascular calcification in the coronaries and thoracic aorta in HD patients[10].

Tripepi[11] indicated that hepatitis C virus among other infectious agents may predispose patients to atherosclerosis and can result in adverse clinical events by causing inflammatory and autoimmune responses in non-dialysis patients**.** A number of non-invasive methods have been developed for detection & quantification of vascular calcification. The simplest technique is plain radiography (x-ray). Others include CT scanning both electron beam with cardiac gating to examine coronary arteries calcification and multislice helical CT for large and medium sized arteries are available[12]**.**

This work aims to evaluate the possible impact of chronic HCV infection on coronary calcification in prevalent haemodialysis patients.

PATIENTS AND METHODS

This cross-sectional study was carried out on sixty patients in Naval Forces hospital, Alexandria, Egypt. Patients with at least one year duration on regular haemodialysis were included. The study followed the ethical standard of our institute and it was approved by the ethical committee, as well as informed consent were obtained from all patients.

Patients:

The sixty patients were divided into two groups

- **Group one:** 30 HCV negative prevalent haemodialysis patients.
- **Group two:** 30 HCV positive prevalent haemodialysis patients

Inclusion criteria

Patients with the following criteria will be included in the study:

1- Males or females ≥18 years of age, and less than 60 years of age.

2- Signed informed consent.

Exclusion criteria

Patients with the following criteria were excluded:

- History of familial hyperlipidemia.
- Smoking.
- Diabetes Mellitus.
- Hepatoma.

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METHODS

The selected patients were subjected to:

- Full history taking including duration of haemodialysis, vascular access patency, other comorbidities).
- Full clinical examination including hepatic functional assessment according to Child-Pugh classification.
- Laboratory investigations including:
  - Complete blood count (CBC).
  - Renal functions (urea and creatinine).
  - Liver function tests (SGOT, SGPT, bilirubin and serum albumin).
  - PT, PTT and INR.
  - Lipid profile.
  - CRP titer.
  - Intact parathormone (iPTH).
  - S. calcium, S. phosphorus, S. sodium and S. potassium.
  - Adequacy of dialysis using urea reduction ratio.
  - HBsAg and HCV Ab by ELISA.
- Imaging studies including:
  - Chest X ray to detect calcification of arch of aorta (anteroposterior and lateral views).
  - Multislice CT on coronary with assessment of coronary calcified plaques using Agatston score to detect corneal and conjunctival calcification [13].
  - Pelvi- abdominal sonography
  - Resting ECG.
  - Echocardiography (aiming at assessing valvular calcification, myocardial function, and presence of evidence of ischemic cardiomyopathy).

Sixty adult patients with ESRD on regular haemodialysis therapy for at least 12 months were included in the study. Standard solution contained 1.75 mmol/L of calcium. Haemodialysis was performed using synthetic or semisynthetic membranes, and bicarbonate dialysate was used with 1.5 mmol/L of calcium. Weekly haemodialysis treatment duration was tailored individually between 10 and 15 hours.

Calcium-containing phosphorus binders was administered to all patients according to calcium, phosphorus, and parathyroid hormone (PTH) values. Target values were as follows: calcium, 8.4 to 9.5 mg/dL (2.10 to 2.37 mmol/L); phosphorus,

3.5 to 5.5 mg/dL (1.13 to 1.78 mmol/L); and PTH, 150 to 250 pg/dL (ng/L).

Serum levels of calcium, phosphorus,, PTH, albumin, s. creatinine, blood urea, s.cholestrol, LDH, HDL, triglycerides, AST, ALT, serum Na and serum K were collected from patients' files.

Biochemical parameters were measured using an autoanalyzer (Olympus AU 800; Olympus Diagnostica GmhH, Hamburg, Germany). PTH levels were determined by means of chemiluminescent immunoassay (Liaison N-tact; DiaSorin Inc, Stillwater, MN).

Chest x-ray (anteroposterior view) was performed to assess for the presence of vascular calcifications. One single experienced radiologist who was blinded to patient information evaluated all radiographic films.

The study was done after approval of ethical board of Ain Shams university and an informed written consent was taken from each participant in the study.

Statistical Methods

Data was collected, tabulated, then analyzed using IBM© SPSS© Statistics version 22 (IBM© Corp., Armonk, NY).

Normally distributed numerical data was presented as mean and SD, and skewed data as median and interquartile range. Qualitative data was presented as number and percentage. Comparison of normally distributed numerical data was done using the unpaired student t test. Skewed data was compared using the Mann-Whitney U test. Categorical data was compared using the chi-squared test or Fisher's exact test, when appropriate. A two-sided p-value <0.05 was considered statistically significant.

RESULTS

Table (1) shows the baseline characteristics of the study population. There were no significant differences between HCV negative prevalent haemodialysis patients and HCV positive prevalent haemodialysis patients regarding age, gender, access and dialysis duration. HCV negative patients included 25 (83.3%) males and 5 (16.7%) females, their mean age was 51.67 ± 6.91 years. The mean haemodialysis duration was 7.5 ± 1.89 years.

In table (2), there were statistically a high significant difference between HCV negative prevalent haemodialysis patients and HCV positive prevalent haemodialysis patients regarding AST, significant differences regarding ALT and albumin and non-significant differences regarding bilirubin, prothrombin time, international normalized ratio, cholesterol and total glycerides.

In table (3), there were no significant differences between HCV negative prevalent haemodialysis patients and HCV positive prevalent haemodialysis patients regarding CKD-MBD parameters (including PO_4 and iPTH), except for calcium which exhibited statistically a significant difference.

In table (4), there were no significant differences between HCV negative prevalent haemodialysis patients and HCV positive prevalent haemodialysis patients regarding creatinine, sodium and potassium and a statistical significant difference regarding URR.

In figure (1), there was statistically a significant difference between HCV negative prevalent haemodialysis patients and HCV positive prevalent haemodialysis patients regarding C-reactive protein as an inflammatory marker.

In figure (2), there was statistically a high significant difference between HCV negative prevalent haemodialysis patients and HCV positive prevalent haemodialysis patients regarding Ca score.

In order to explore the different factors affecting valvular calcification, a correlation was done between Ca score and other demographic and laboratory data. In all cases, there were statistically significant correlations between Ca score and URR and ALT and a high significant correlation regarding AST. In HCV negative prevalent haemodialysis patients, there were statistically significant correlations regarding dialysis duration and international normalized ratio and high significant correlations between Ca score and creatinine, AST and prothrombin time. In HCV positive prevalent haemodialysis patients, there were statistically significant correlations between Ca score and dialysis duration and creatinine. Other correlations were insignificant (table 5).

In table (6), there were non-significant relations between Ca score and gender and dialysis vascular access.

A multiple stepwise linear regression analysis was performed to determine the independent factors of Ca score. The most significant independent predictors of valvular calcification were AST, PO_4, iPTH and Hepatitis C virus (table 7).

Table (1): Comparison between groups as regard demographic and clinical data

		HCV		P Value
		Negative (30)	Positive (30)	
Age (Years)		1.67 .91	).30 ł.14	0.357
Gender	**Male**	25 (83.3%)	24 (80%)	1
	Female	5 (16.7%)	6 (20%)	
Access	**AVF**	28 (93.3%)	23 (76.6%)	0.145
	AVG	2 (6.7%)	7 (23.3%)	
Dialysis Duration (Yrs)		7.50 1.89	7.53 ± 2.29	0.951

Table (2): Comparison between groups as regard liver function tests

	HCV						alue
	Negative (30)			Positive (30)			
AST (iu/L)	17.37	±	5.96	27.73	±	10.87	**<0.001**
ALT (iu/L)	18.03	±	9.32	25.53	±	8.85	**0.002**
Bilirubin (mg/dl)	0.44	±	0.17	0.44	±	0.15	1
Albumin (mg/dl)	3.07	±	0.43	2.76	±	0.53	**0.018**
PT (sec)	13.10	±	3.13	13.97	±	5.07	0.429
INR	1.14	±	0.30	1.29	±	0.47	0.148
Cholesterol (mg/dl)	164.83	±	56.57	142.40	±	30.46	0.061
TGs (mg/dl)	109 (94 – 149)			111 (95 – 152)			1

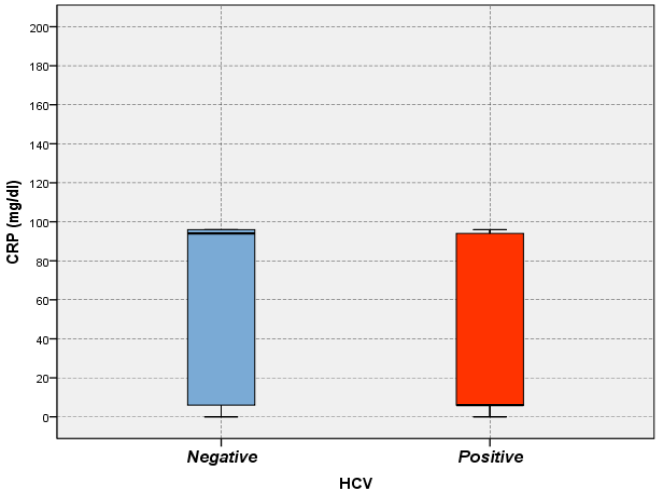
Table (3): Comparison between groups as regard CKD-MBD parameters

	HCV				Value
	Negative (30)		Positive (30)		
Ca (mg/dl)	42	1.77	6.35	± 1.79	**0.023**
PO4 (mg/dl)	30	2.01	5.58	± 2.07	0.176
iPTH (pg/ml)	172 (85 – 438)		387 (201 – 565)		0.099

Table (4): Comparison between groups as regard RFTs, and adequacy of dialysis

	HCV						P Value
	Negative (30)			Positive (30)			
URR (%)	71.87	±	5.86	66.53	±	7.25	**0.003**
Creat (mg/dl)	11.01	±	4.08	10.44	±	3.35	0.562
Na (mEq/L)	135.57	±	3.86	135.77	±	2.50	0.812
K (mEq/L)	5.06	±	1.04	4.97	±	0.79	0.707

Figure (1): Comparison between groups as regard CRP as an inflammatory marker



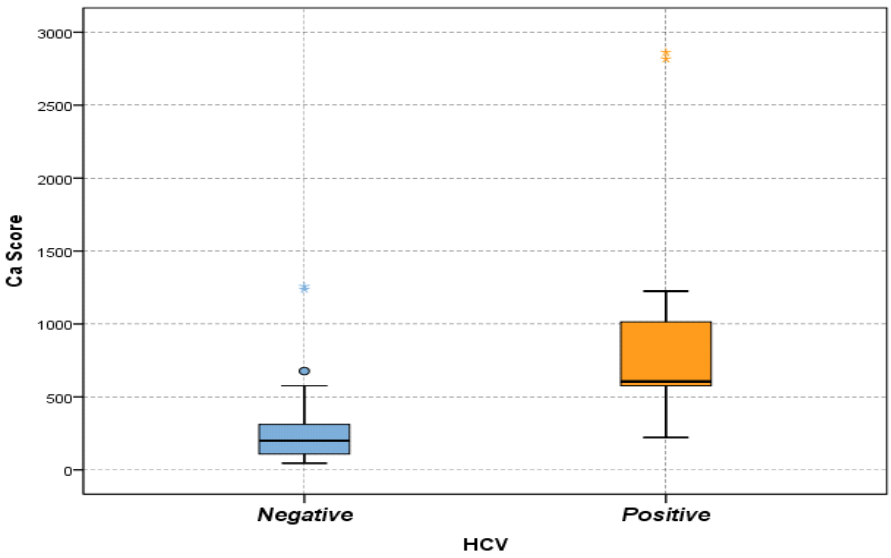


Figure (2): Comparison between groups as regard Ca score

Table (5): Correlations between Ca score and other parameters in all patients, and in subgroups divided according to HCV seropositivity

	All (60)		HCV -ve (30)		HCV +ve (30)	
	Ca Score		Ca Score		Ca Score	
	r	P Value	r	P Value	r	P Value
Age (Years)	-0.062	0.637	0.034	0.859	0.379	0.039
Dialysis Duration (Yrs)	-0.022	0.866	0.457	**0.011**	-0.474	**0.008**
HGB (gm/L)	-0.045	0.731	0.262	0.162	-0.316	0.089
WBCs (x10^3/mm3)	-0.166	0.204	-0.257	0.171	0.081	0.671
PLT (x10^3/mm3)	-0.127	0.335	-0.207	0.273	0.056	0.768
URR (%)	-0.260	**0.045**	0.264	0.158	-0.192	0.310
Creat (mg/dl)	0.107	0.414	0.600	**< 0.001**	-0.502	**0.005**
AST (iu/L)	0.577	**< 0.001**	0.634	**< 0.001**	-0.205	0.278
ALT (iu/L)	0.389	**0.002**	0.295	0.113	-0.254	0.175
Bilirubin (mg/dl)	0.168	0.198	0.357	0.053	-0.087	0.647
Albumin (mg/dl)	-0.213	0.103	0.230	0.222	-0.071	0.709
Cholesterol (mg/dl)	-0.137	0.298	0.032	0.867	-0.009	0.962
TGs (mg/dl)	0.095	0.469	0.325	0.080	-0.056	0.769
CRP (mg/dl)	-0.109	0.430	0.053	0.789	0.277	0.162
Ca (mg/dl)	-0.249	0.055	-0.132	0.487	0.094	0.623
Na (mEq/L)	0.219	0.093	0.287	0.124	0.022	0.910
K (mEq/L)	-0.020	0.878	-0.031	0.871	0.131	0.490
PO4 (mg/dl)	-0.264	**0.042**	-0.146	0.442	-0.323	0.081
PT (sec)	0.338	**0.008**	0.628	**< 0.001**	0.127	0.503
INR	0.394	**0.002**	0.404	**0.027**	0.309	0.096
iPTH (pg/ml)	0.429	**0.001**	0.307	0.099	0.350	0.058

Table (6): Relation between Ca score and gender and dialysis vascular access

		Ca Score			P Value
		Median	Percentile 25	Percentile 75	
Gender	**Male**	551	239	677	0.395
	Female	226	46	1021	
Vascular Access	**AVF**	292	164	598	0.096
	AVG	738	715	1014	

Table (7): Multivariate linear regression analysis of independent predictors of Ca score

Model	Unstandardized Coefficients		Sig.
	B	Std. Error	
(Constant)	-287.656	731.073	0.696
URR (%)	16.555	11.198	0.145
AST (iu/L)	-23.959	11.683	**0.045**
ALT (iu/L)	2.931	9.151	0.750
PO4 (mg/dl)	-120.562	28.645	**< 0.001**
PT (sec)	82.152	50.579	0.110
INR	-723.569	534.676	0.182
iPTH (pg/ml)	.683	.162	**< 0.001**
HCV	785.888	184.599	**< 0.001**

DISCUSSION

Cardiovascular disease is the leading cause of death in chronic dialysis patients. The underlying diseases such as diabetes, hypertension, advanced age, and chronic inflammation are all strongly related to survival in the dialysis population. More recently, both the presence as well as the extent of cardiovascular calcification has been demonstrated to be predictors of cardiovascular and all-cause mortality in dialysis patients[14]. Cardiovascular calcification are divided into three types, depending on the locations involved: arterial intimal calcification (AIC), which is characteristic of atherosclerosis and causes plaque formation and significant luminal stenosis; arterial medial calcification (AMC), which is the nonendochrondral ossification process of the arterial tunica media and is highly characteristic of diabetes mellitus and chronic renal failure and vascular calcification (VC), which occurs in response to mechanical stressors and inflammation, and thus recruit dystrophic mineralization and nonenchondral ossification processes to deposit calcium[15].

Lee *et al.*[16] have demonstrated that VC represents a marker of atherosclerosis and arterial calcification in dialysis patients. Additionally, hemodialysis patients with AIC were reported to have higher mortality than those with AMC. Mechanisms of cardiovascular calcification in dialysis patients are multiple. A variety of biomarkers circulate in blood stream can be determined as a method of early detection of calcification. However, the role of these biomarkers in different types of calcification has rarely been compared. In this study, the aim was to evaluate the possible impact of chronic HCV infection on coronary calcification in prevalent haemodialysis patients in Naval Forces hospital, Alexandria, Egypt and its relation to demographic data, haemodialysis data and other laboratory findings. This cross-sectional study was carried out on 60 patients with at least one year duration on regular haemodialysis; 30 HCV negative prevalent haemodialysis patients and 30 HCV positive prevalent haemodialysis patients.

Our study revealed that HCV negative patients included 25 (83.3%) males and 5 (16.7%) females, their mean age was 51.67 ± 6.91 years. The mean haemodialysis duration was 7.5 ± 1.89 years. **Lee *et al.*[17]** investigated the distribution of vascular calcification in haemodialysis patients. The mean age of the study population was 60.9 ± 10.2 years, and male patients accounted for 49.4% of enrolled patients. Their mean haemodialysis duration was 75.8 ± 47.1 months.

In our study, there were statistically a high significant difference between HCV negative prevalent haemodialysis patients and HCV positive prevalent haemodialysis patients regarding AST, significant differences regarding ALT and albumin and non-significant differences regarding bilirubin, prothrombin time, international normalized ratio, cholesterol and total glycerides. But, there were no significant differences between HCV negative prevalent haemodialysis patients and HCV positive prevalent haemodialysis patients regarding CKD-MBD parameters (including PO_4 and iPTH), except for calcium which exhibited statistically a significant difference.

Also, there were no significant differences between HCV negative prevalent haemodialysis patients and HCV positive prevalent haemodialysis patients regarding creatinine, sodium and potassium, significant differences regarding URR, C-reactive protein and a high significant difference regarding Ca score.

Lee *et al.*[17] stated that the levels of serum calcium, phosphate, calcium phosphate products, total cholesterol, triglycerides, and parathyroid hormone were similar among the groups.

Our study found that in HCV negative prevalent haemodialysis patients, there were statistically significant correlations regarding dialysis duration and international normalized ratio and high significant correlations between Ca score and

creatinine, AST and prothrombin time. In HCV positive prevalent haemodialysis patients, there were statistically significant correlations between Ca score and dialysis duration and creatinine. Other correlations were insignificant. There were non-significant relations between Ca score and gender and dialysis vascular access.

Although several studies have confirmed the high prevalence of valvular calcification in HD patients, there was no consistent agreement on the risk factors of valvular calcification[18]. In our study, the most significant independent predictors of valvular calcification were AST, PO_4, iPTH and Hepatitis C virus.

Lee *et al.*[19] have shown a strong association between chronic inflammation and disturbance of bone mineral metabolism in chronic hemodialysis patients. The link between dysregulation of calcium, phosphate, and the parathyroid hormone, and cardiovascular calcification is controversial. **Lee *et al.*[17]** revealed that age, diabetes, and uric acid levels were strongly related to AIC. Diabetes, uric acid, and OPG levels were independent factors associated with AMC. They concluded that the prevalence of cardiovascular calcification in chronic HD patients was high with cardiac valve involvement more frequent. Factors associated with different Vitamin K of calcification were not identical. Changes in biomarkers may represent clinical clues for assessment of cardiovascular calcification in HD patients.

Finally, we can say that vascular calcification is common in HD patients. It is related to many factors , control of the modifiable factors is necessary to control the degree of calcification. **Chertow *et al.*[20]** found that 17% of HD subjects had no coronary calcifications and 20% had no aortic calcifications using EBCT.

Valvular calcifications were detected in 44% patients (mitral and aortic valve). **Ix *et al.*[21]** detected mitral valve calcifications in 20% cardiovascular patients without renal disease.

Muntner *et al.*[22] revealed the importance of simple method including demographic information, dialysis vintage, abdominal aorta calcification and mitral and aortic valve calcification in predicting of coronary artery calcifications with very good accuracy. Furthermore, they concluded even simpler method warrants consideration and that omitting the echocardiogram would result in substantially reduced test cost and feasibility. It seems that every dialysis center search for the most available, feasible, less costly and, on the first place, most accurate diagnostic method for VC.

Krasniak *et al.*[23] evaluated several risk factors for coronary artery calcifications in univariate analysis (age, BMI, serum iPTH, CRP, interleukin-6, 25-OH-vitamin D3, transforming growth factor-β, plateled derived growth factor and carotid artery IMT), but in multivariate regression analysis only age and carotid artery IMT remained as independent predictors of coronary artery calcifications.

Hermans *et al.*[24] evaluated the relation between serum fetuin-A and arterial stiffness, as a feature of predominant VC. Fetuin-A, well known inhibitor of VC, appeared not to be an independent predictor of aortic stiffness in a dialysis population with a low level of inflammatory activity.

Oyake *et al.*[25] examined whether HCV infection is associated with increased aortic stiffness and cardiovascular events in chronic dialysis patients. They indicated that among dialysis patients, persistent HCV infection is closely associated with increased aortic stiffness, which may result in left ventricular hypertrophy, cardiac overload and cardiovascular events.

Damjanovic *et al.*[26] screened VC in patients undergoing chronic HD using sensitive, non-invasive radiographic methods and evaluation of the risk factors for their appearance. 214 patients aged 4.59 years were studied. VC were scored based on to plain radiographs. Potential risk factors were assessed. Out of the 214 patients studied, only 14% did not display any detectable VC. Using plain radiographs calcifications could be detected in 136 (63.6%) patients. Calcified plaques on carotid arteries were detected in 168 (78.4%) patients. There was the highest frequency of patients with the most pronounced calcifications. Calcifications of heart valves were detected in 89 (44.1%) patients. Risk to develop VC is present in older patients, patients with longer dialysis vintage, thicker intima media, higher lumen diameter and mitral valve calcifications. They revealed these factors as independent predictors of VC in dialysis patients. They confirmed a high prevalence of VC in HD patients, their association with older ages, longer dialysis vintage, and presence of valvular calcifications and early markers of atherosclerosis. **Roed *et al.*[27]** provided an overview of the literature on the association between chronic HCV infection and the risk of CAD. They revealed a tendency towards a higher risk of CAD among patients with HCV infection. As the majority of studies published on this topic are of poor quality, firm conclusions are hard to reach.

CONCLUSION

A very high incidence of vascular calcification was found in chronic haemodialysis patients in our study as compared to other studies.

Vascular calcification is correlated with many risk factors and control of the modifiable risk factors can help to decrease prevalence of vascular calcification. We have to revise our policy towards haemodialysis treatment using calcium supplement and Alphacalcidol treatment as they are also considered as modifiable risk factors.

Further studies are needed for better understanding calcification process and its consequences.

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