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Platelet Factor 4-Heparin Antibody as a Predictor of Cardiovascular and Thrombotic Events in Hemodialysis

Mohamed Kamal Dewedar*, Mahmoud Abd El-Aziz Kora, Ahmed Ragheb Tawfeek, Mahmoud Mohamed Emara, Heba El-Sayed Kasem
Internal Medicine Department, Faculty of Medicine, Menoufia University, Egypt
***Corresponding author:** Mohamed Kamal Dewedar, **Mobile:** 0201006185875, **E-mail:** drmkamal2013@gmail.com

ABSTRACT

Background: Cardiovascular disease (CVD) remains the leading cause of morbidity and mortality in hemodialysis patients, with both traditional and nontraditional risk factors contributing to its high prevalence. Platelet factor 4-heparin antibody (PF4-H Ab) has been implicated in thrombotic complications, but their role in cardiovascular risk among hemodialysis patients remains unclear. **Objective:** This study aims to evaluate the association between PF4-H Ab and cardiovascular risk in end-stage renal disease (ESRD) patients on maintenance hemodialysis.

Patients and methods: This cross-sectional study included 80 hemodialysis patients recruited from Shebein El-Kom Fever Hospital, Menoufia, Egypt. Patients were classified based on cardiovascular risk and underwent enzyme-linked immunosorbent assay (ELISA) testing for PF4-H Ab.

Results: Of the 80 patients included, 36 (45%) tested positive for PF4-H Ab, while 44 (55%) were negative. 40 patients were classified as having cardiovascular risk, 22 of them (55%) tested positive for PF4-H Ab, while 18 (45%) were negative. The prevalence of congestive heart failure was significantly higher in PF4-H Ab-positive patients (36.4% vs. 0%, p = 0.005). Univariate analysis identified multiple factors associated with cardiovascular risk, but in multivariate analysis, only reduced ejection fraction (EF) remained a significant independent predictor (p = 0.024). ROC analysis demonstrated moderate discrimination for PF4-H Ab in cardiovascular risk prediction (AUC = 0.635, p = 0.038), with sensitivity and specificity of 65%.

Conclusion: The moderate diagnostic performance of PF4-H Ab suggests that it may have some clinical relevance but lacks sufficient sensitivity and specificity to be used as a standalone biomarker for cardiovascular risk stratification in hemodialysis patients. Further research is needed to clarify its role in cardiovascular complications in ESRD populations.

Keywords: Platelet factor 4-Heparin antibody, Cardiovascular risk, Hemodialysis, Chronic kidney disease, Thrombotic complications.

INTRODUCTION

Chronic kidney disease (CKD) significantly increases the risk of cardiovascular complications, making it a leading cause of morbidity and mortality. CKD promotes endothelial dysfunction, and accelerated atherosclerosis through mechanisms including dysregulated mineral metabolism, oxidative stress, and chronic inflammation [(1)]. Moreover, CKD patients often exhibit hypertension, dyslipidemia, and an overactive renin-angiotensin-aldosterone system, which further contribute to vascular injury. These nontraditional risk factors augment the prevalence of coronary artery disease (CAD), stroke, and peripheral arterial disease among CKD patients [(2)].

Hemodialysis is a lifesaving therapy for patients with ESRD, yet it is accompanied by significant complications, including a heightened risk of CVD and thrombosis. The use of unfractionated heparin to prevent clotting during hemodialysis has been implicated in the production of anti-platelet factor 4/heparin antibodies (PF4-H Ab), which are associated with adverse outcomes such as heparin-induced thrombocytopenia (HIT) and thromboembolic events [(3)]. These antibodies trigger endothelial activation and platelet aggregation, contributing to microparticle release and systemic inflammatory responses. Emerging evidence suggests that PF4-H Ab may independently elevate the risk of CAD, ischemic stroke (IS), and arteriovenous fistula thrombosis (AVFT) in hemodialysis patients [(4)].

Despite significant progress in understanding the pathophysiological mechanisms underlying these complications, the clinical significance of PF4-H Ab in the hemodialysis population remains incompletely understood. This study seeks to further elucidate the relationship between PF4-H Ab and cardiovascular morbidity.

PATIENTS AND METHODS

Study subjects:

The study included 80 hemodialysis patients collected from Shebein El-Kom Fever Hospital - Menoufia-Egypt from January 2023 to May 2024. After clinical, radiological and laboratory investigation, they were classified into: Group A included 40 chronic hemodialysis patients with cardiovascular risk and Group B included 40 chronic hemodialysis patients without cardiovascular risk.

Ethical considerations:

The Faculty of Medicine at Menoufia University granted ethical approval for this study's research design, which followed the Declaration of Helsinki's requirements (N. 199191/NTM2). Before commencing the investigation, written informed assent was obtained from every patient participated in the study.

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The study included patients with chronic kidney disease (CKD) who were undergoing hemodialysis three times a week for at least three months and receiving unfractionated heparin as anticoagulant therapy. Patients were classified as having cardiovascular risk if they had a history of any cardiac disease, positive findings on electrocardiogram (ECG), abnormal echocardiography results, or abnormal carotid duplex scans. Additional cardiovascular risk factors included a history of myocardial infarction (MI), coronary artery bypass grafting (CABG), percutaneous coronary intervention (PCI), or congenital heart disease (CHD). Patients with a history of stroke, arteriovenous fistula (AVF) thrombosis, or catheter occlusion, as well as those with dyslipidemia, were also considered to be at risk. The study excluded patients with acute renal failure or CKD patients who were not treated with hemodialysis or were not receiving high-molecular-weight (HMW) heparin. Other exclusion criteria included patients with atrial fibrillation, malignancies, or those younger than 18 years of age.

METHODS

Sampling

All study individuals were subjected to personal history taking, physical examinations and routine laboratory tests as follow: venous blood (9 ml) was collected and divided into 3 aliquots. three ml of blood were withdrawn in EDTA vacutainer tube and used for complete blood count (CBC), The CBC were done using Swelab's fully automatic Auto counters, AC970EO, from Buele Medical AB, Stockholm, Sweden. The second blood portion (3 ml) was put in a plain vacutainer and serum was separated for testing of liver function, hepatitis virus markers (HBsAg and HCV antibodies), renal function and lipid profile.

The estimation of anti-platelet factor 4/heparin antibody by ELISA:

Measurement of PF4-H Ab was done by ELISA technique using (BZEK2420-48, China) kit manufactured by Chongqing Biospes Co. intended for quantitative detection of PF4-H Ab in human serum. The test method was based on standard sandwich enzyme-linked immune-sorbent assay technology. The recombinant human PF4-H antigen was pre-coated onto 48-well plates. And the HRP conjugated anti- PF4-H Ab antibody was used as detection antibodies. The standards, test samples and HRP conjugated detection antibody were added to the wells subsequently, mixed and incubated, then, unbound conjugates were washed away with wash buffer. TMB substrates (A and B) were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the PF4-H Ab amount of sample captured in plate. Read the O.D. absorbance at 450 nm in a microplate reader, and then the concentration of PF4-H Ab can be calculated [(5)].

Following ELISA testing for PF4-H Ab, patients were reclassified based on their test results. Group I included 44 chronic hemodialysis patients who tested negative for PF4-H Ab, while Group II included 36 chronic hemodialysis patients who tested positive for PF4-H Ab. For further statistical analysis, we specifically focused on the subgroup of patients with cardiovascular risk to investigate potential associations between PF4-H Ab positivity and cardiovascular complications.

Statistical analysis

The statistical analysis was carried out using IBM SPSS software version 20.0. Categorical data were summarized using numbers and percentages and were compared by Chi-square test or Fisher's exact test. Normality in continuous data was determined using the Shapiro-Wilk test. The quantitative data were provided as range, mean, standard deviation, median, and interquartile range (IQR). To compare two sets of regularly distributed quantitative data, the Student t-test was employed, and the Mann Whitney test was used for non-normally distributed quantitative variables. The significance level for all statistical tests was set to 5%.

RESULTS

PF4-H antibodies and their association with demographic, clinical and laboratory parameters:

The study included 80 patients, of whom 36 (45%) tested positive for PF4-H Ab and 44 (55%) tested negative. The gender distribution in the two groups did not differ significantly. However, individuals with +ve PF4-H Ab were substantially younger than those with –ve PF4-H Ab **(Table 1)**.

Table (1): Demoghraphic data among PF4-H Ab neggative and PF4-H Ab positive patients in all study participants (80 patients):

	PF4-H Ab		Test of Sig.	P
	Negative (n = 44)	Positive (n = 36)		
Sex				
Male	32 (72.7%)	24 (66.7%)	χ^2= 0.346	0.556
Female	12 (27.3%)	12 (33.3%)		
Age				
Min – Max.	40.0 – 72.0	22.0 – 61.0	t= 4.416	<0.001*
Mean ± SD.	57.82 ± 9.99	47.33 ± 11.23		

*: Significant

The group with cardiovascular risk included 40 patients, of whom 22 (55%) tested positive for PF4-H Ab and 18 (45%) tested negative. The gender distribution in the two groups did not differ significantly. However, individuals with +ve PF4-H Ab were substantially younger than those with –ve PF4-H Ab. Regarding hematological characteristics, the median WBC count was significantly lower in PF4-H Ab-positive patients than in the negative group. The PF4-H Ab-positive group's platelet counts were lower, although they were not statistically significant. The levels of parathyroid hormone (PTH), calcium, phosphate, renal function markers (creatinine, urea), and liver function tests did not differ statistically significantly between the two groups. Hepatitis B surface antigen (HBsAg) positivity, however, was considerably lower in individuals with PF4-H Ab than in the negative group **(Table 2)**.

Table (2): Comparison between PF4-H Ab neggative and PF4-H Ab positive patients in the group of ESRD patients with cardiovascular risk (n = 40)

	PF4-H Ab		Test of Sig.	P
	Negative (n = 18)	Positive (n = 22)		
Sex Male Female	 14 (77.8%) 4 (22.2%)	 16 (72.7%) 6 (27.3%)	χ^2= 0.135	^{FE}p=1.000
Age (Years) Min – Max. Mean ± SD. Median (IQR)	 55.0 – 72.0 62.67 ± 6.88 65.0 (55.0 – 68.0)	 32.0 – 61.0 50.55 ± 8.46 54.0 (45.0 – 57.0)	t= 4.896	<0.001*
Hb (g/dL) Mean ± SD.	 10.60 ± 1.33	 10.58 ± 1.47	t= 0.041	0.968
WBCs (mcL) Mean ± SD.	 6.66 ± 1.66	 5.78 ± 0.97	U= 124.00	0.045*
PLT (mcL) Min – Max. Median (IQR)	 95.0 – 413.0 148.0 (130.0 – 219.0)	 81.0 – 208.0 135.0 (115.0 – 187.0)	U= 132.00	0.075
HCV-Ab	4 (22.2%)	10 (45.5%)	χ^2=2.349	0.125
HBsAg	8 (44.4%)	2 (9.1%)	χ^2=6.599	^{FE}p=0.025*
ALT (U/L) Min – Max. Median (IQR)	 13.0 – 48.0 41.0 (30.0 – 47.0)	 23.0 – 55.0 32.0 (24.0 – 45.0)	U= 182.00	0.677
Urea (mg/dL) Mean ± SD.	 102.2 ± 23.53	 104.8 ± 25.90	U= 194.00	0.925
Creatinine (mg/dL) Mean ± SD.	7.82 ± 1.74	8.45 ± 1.33	U= 160.00	0.312
Calcium (mg/dl) Mean ± SD.	8.42 ± 1.55	8.20 ± 0.75	U= 184.00	0.717
PTH (pg/ml) Min – Max. Median (IQR)	 177.0 – 945.0 269.0 (237.0 – 809.0)	 142.0 – 1902.0 344.0 (248.0 – 619.0)	U= 196.00	0.968
Pho (mg/dl) Mean ± SD.	4.47 ± 0.90	4.43 ± 1.10	U= 188.00	0.798

Range and median (IQR): non paramtric test, *: Significant, IQR: Interquartile range

The relationship between PF4-H Ab status and iron metabolism, lipid profile, and cardiovascular parameters revealed that serum iron levels were significantly higher in PF4-H Ab-positive patients than in the negative group. However, serum ferritin and albumin levels did not differ significantly between the groups. Moreover, patients with +ve PF4-H Ab had higher levels of triglycerides and cholesterol, but the differences were not statistically significant. The use of statin therapy was significantly more frequent in PF4-H Ab-positive patients **(Table 3)**.

There was no significant difference in left carotid thickness, but carotid artery measurements showed that the right carotid artery thickness was significantly higher in PF4-H Ab-positive patients than in PF4-H Ab-negative patients. Echocardiographic results showed that the ejection fraction (EF) was significantly lower in PF4-H Ab-positive patients, and the prevalence of congestive heart failure was significantly higher in PF4-H Ab-positive patients. Other cardiovascular events, such as the history of myocardial infarction, stroke, and arteriovenous fistula (AVF) thrombosis, did not significantly differ between the groups **(Table 3)**.

Table (3): Comparison between PF4-H Ab neggative and PF4-H Ab positive patients in the group of ESRD patients with cardiovascular risk (n = 40)

	PF4-H Ab		Test of Sig.	P
	Negative (n = 18)	Positive (n = 22)		
Serum iron (mcg/dL) Min – Max. Median (IQR)	 21.60 – 109.9 65.80 (34.70 – 91.20)	 51.98 – 156.0 70.60 (55.80 – 122.0)	U= 122.00	0.039
Serum ferritin (ng/mL) Min – Max. Median (IQR)	 47.80 – 360.0 240.0 (66.56 – 293.0)	 23.80 – 665.0 174.0 (78.40 – 219.0)	U= 164.00	0.366
Serum albumin (g/dL) Mean ± SD.	3.77 ± 0.47	3.86 ± 0.33	U= 172.00	0.492
Cholesterol (mg/dL) Mean ± SD.	175.2 ± 19.45	186.9 ± 24.24	t= 1.655	0.106
Triglycerides (mg/dl) Mean ± SD.	154.7 ± 22.64	158.7 ± 30.10	t= 0.473	0.639
Statin ttt	6 (33.3%)	16 (72.7%)	χ^2=6.208	0.013*
Right carotid thickness Min – Max. Mean ± SD. Median (IQR)	 0.70 – 1.0 0.79 ± 0.10 0.80 (0.70 – 0.80)	 0.60 – 1.20 0.94 ± 0.21 1.0 (0.80 – 1.10)	U= 108.00*	0.014*
Left carotid thickness Min – Max. Mean ± SD. Median (IQR)	 0.60 – 1.20 0.82 ± 0.19 0.80 (0.70 – 0.90)	 0.60 – 1.20 0.92 ± 0.19 1.0 (0.70 – 1.10)	U= 138.00	0.106
ECG arrhythmia	2 (11.1%)	4 (18.2%)	χ^2=0.388	FEp=0.673
ECG ischemic changes	8 (44.4%)	14 (63.6%)	χ^2=1.473	0.225
Echo arrhythmia	2 (11.1%)	4 (18.2%)	χ^2=0.388	FEp=0.673
Echo EF Min – Max. Mean ± SD. Median (IQR)	 52.0 – 72.0 62.89 ± 6.94 64.0 (58.0 – 67.0)	 35.0 – 76.0 54.27 ± 13.42 53.0 (44.0 – 63.0)	t= 2.614	0.013*
History of MI, CHD, CABG, PCI	6 (33.3%)	8 (36.4%)	χ^2=0.040	0.842
Cong heart failure	0 (0.0%)	8 (36.4%)	χ^2=8.182	FEp=0.005*
History of stroke	6 (33.3%)	2 (9.1%)	χ^2=3.636	FEp=0.110
AVF thrombosis	3 (16.6%)	9 (40.9%)	χ^2=3.252	0.071
Catheter occlusion	6 (33.3%)	8 (36.4%)	χ^2=0.040	0.842

Range and median (IQR): non paramtric test, *: Significant, IQR: Interquartile range

The correlation between PF4-H Ab levels and different clinical and laboratory variables in all patients of the study showed that PF4-H Ab positivity had significant negative correlation with age and ALT. Additionally, PF4-H Ab levels showed a significant positive correlation with serum calcium and right carotid thickness. However, no significant correlations were found with hemoglobin, WBC count, platelet count, renal function markers, lipid profile, or left carotid artery thickness **(Table 4)**.

The correlation between PF4-H Ab levels and different clinical and laboratory variables in ESRD patients with cardiovascular risk showed that PF4-H Ab positivity had significant negative correlation with age. Additionally, PF4-H Ab levels showed a significant positive correlation with serum iron and right carotid thickness. However, no significant correlations were found with hemoglobin, WBC count, platelet count, renal function markers, lipid profile, or left carotid artery thickness **(Table 4)**.

While the correlation between PF4-H Ab levels and different clinical and laboratory variables in ESRD patients without cardiovascular risk showed that PF4-H Ab positivity had significant negative correlation with age. Additionally, PF4-H Ab levels showed a significant positive correlation with serum calcium and echo ejection fraction. However, no significant correlations were found with hemoglobin, WBC count, platelet count, renal function markers, lipid profile, or right and left carotid artery thickness **(Table 4)**.

Table (4): Correlation between PF4-H Ab with different parameters

	Total patients (n = 80)		With cardiovascular risk (n = 40)		Without cardiovascular risk (n = 40)	
	r_s	p	r_s	p	r_s	p
Age (Years)	-0.269	0.016*	-0.531	<0.001*	-0.343	0.030*
Hb (g/dL)	-0.058	0.611	-0.212	0.190	0.047	0.775
WBCs (mcL)	-0.178	0.114	-0.223	0.167	-0.137	0.399
PLT (mcL)	-0.173	0.124	-0.197	0.223	-0.280	0.080
ALT (U/L)	-0.273	0.014*	-0.260	0.105	-0.230	0.153
Urea (mg/dL)	-0.035	0.757	0.034	0.835	0.071	0.665
Creatinine (mg/dL)	-0.138	0.221	0.082	0.615	-0.147	0.364
Calcium (mg/dL)	0.266	0.017*	0.148	0.361	0.355	0.025*
Pho (mg/dL)	0.034	0.766	0.152	0.350	0.013	0.938
PTH (pg/ml)	-0.126	0.264	-0.194	0.230	-0.100	0.539
Serum iron (mcg/dL)	0.193	0.087	0.352	0.026*	0.235	0.144
Serum ferritin (ng/mL)	-0.100	0.379	-0.017	0.919	-0.186	0.251
Serum albumin (g/dL)	0.204	0.070	0.189	0.244	0.253	0.115
Cholesterol (mg/dL)	0.047	0.679	0.112	0.491	-0.053	0.747
Triglycerides (mg/dL)	0.149	0.186	0.017	0.919	0.114	0.482
Right Carotid thickness	0.248	0.027*	0.343	0.030*	-0.038	0.814
Left Carotid thickness	0.171	0.128	0.220	0.173	-0.106	0.514
Echo EF	0.139	0.218	-0.206	0.203	0.770	<0.001*

r_s: Spearman coefficient, *: Significant

Diagnostic performance and factors associated with cardiovascular risk in ESRD patients:

To evaluate the parameters influencing cardiovascular risk in ESRD patients, univariate and multivariate logistic regression analyses were conducted. Univariate logistic regression analysis identified several factors associated with increased cardiovascular risk, including older age, lower creatinine levels, lower serum phosphate levels, higher triglyceride levels, increased carotid artery thickness, and statin therapy. However, after adjusting for potential confounders in the multivariate model, only a reduced ejection fraction (EF) remained a significant independent predictor of cardiovascular risk. PF4-H Ab positivity showed a trend toward an association with cardiovascular risk, but this did not reach statistical significance **(Table 5)**.

Table (5): Univariate and multivariate logistic regression analysis for the parameters affecting cardiovascular risk (n = 40 vs. 40)

	Univariate		#Multivariate	
	p	**OR (LL – UL 95%C.I)**	**p**	**OR (LL – UL 95%C.I)**
Sex [Female]	0.331	0.619 (0.235 – 1.627)		
Age (years)	0.031*	1.046 (1.004 – 1.090)	0.282	1.037 (0.971 – 1.108)
Hb	0.057	1.373 (0.990 – 1.904)		
WBCs	0.092	0.825 (0.660 – 1.032)		
PLT	0.462	0.998 (0.991 – 1.004)		
HCV-Ab	0.137	2.154 (0.784 – 5.920)		
HBsAg	0.617	0.778 (0.291 – 2.082)		
ALT	0.505	0.988 (0.955 – 1.023)		
Urea	0.488	0.994 (0.978 – 1.010)		
Creatinine	0.015*	0.746 (0.590 – 0.945)	0.281	0.281 (0.606 – 1.157)
Calcium	0.350	1.287 (0.759 – 2.183)		
PTH	0.703	1.000 (0.999 – 1.001)		
Pho	0.024*	0.690 (0.500 – 0.953)	0.335	0.784 (0.478 – 1.286)
Serum iron	0.151	0.994 (0.985 – 1.002)		
Serum ferritin	0.172	0.998 (0.996 – 1.001)		
Serum albumin	1.000	1.000 (0.390 – 2.564)		
Cholesterol	0.227	1.012 (0.992 – 1.033)		
Triglycerides	0.001*	1.039 (1.016 – 1.061)	0.268	1.022 (0.984 – 1.061)
Statin ttt	<0.001*	6.926 (2.380 – 20.157)	0.280	2.353 (0.499 – 11.106)
Positive PF4-H Ab	0.074	2.270 (0.923 – 5.583)		
PF4-H Ab	0.100	2.105 (0.867 – 5.115)		
Right carotid thickness	0.001*	872.1 (15.60 – 48752.7)	0.894	0.639 (0.001 – 455.168)
Left carotid thickness	<0.001*	1114.4 (26.36 – 47114.3)	0.323	23.165 (0.046 – 11776.6)
Echo EF	0.001*	0.907 (0.855 – 0.961)	0.024*	0.928 (0.870 – 0.990)
History of stroke	0.059	4.750 (0.941 – 23.983)		
AVF thrombosis	0.304	1.714 (0.613 – 4.794)		
Catheter occlusion	0.137	2.154 (0.784 – 5.920)		

Model 1#: Hosmer and Lemeshow Test (χ^2=25.014; p=0.002*), #: All variables with p<0.05 were included in the multivariate, OR: Odd`s ratio, C.I: Confidence interval, LL: Lower limit, UL: Upper Limit, *: Significant

The diagnostic performance of PF4-H Ab in differentiating ESRD patients with cardiovascular risk from those without cardiovascular risk was evaluated by the receiver operating characteristic (ROC) curve analysis, which revealed an area under the curve (AUC) of 0.635, indicating a moderate ability of PF4-H Ab to discriminate between the two groups. A cutoff value of >0.335 was identified, yielding a sensitivity and specificity of 65% each. The positive predictive value (PPV) and negative predictive value (NPV) were also 65%, suggesting that while PF4-H Ab has some predictive value, it is not a highly sensitive or specific biomarker for cardiovascular risk stratification in ESRD patients **(Table 6 and Figure 1).**

Table (6): Diagnostic performance for PF4-H Ab to discriminate ESRD patients with cardiovascular risk (n = 40) from those without cardiovascular risk (n = 40)

	AUC	**p**	**95% C.I**	**Cut off#**	**Sensitivity**	**Specificity**	**PPV**	**NPV**
PF4-H Ab	0.635	0.038*	0.512 –0.758	>0.335	65.0	65.0	65.0	65.0

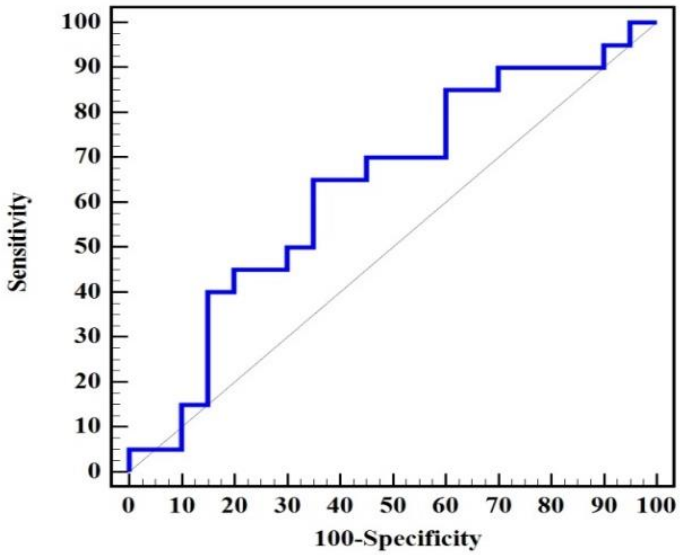

Figure (1): ROC curve for PF4-H Ab to discriminate ESRD patients with cardiovascular risk (n = 40) from those without cardiovascular risk (n = 40).

DISCUSSION

Cardiovascular disease is the leading cause of morbidity and mortality in hemodialysis patients, with significantly high rates. Both traditional and nontraditional risk factors contribute to this increased burden, including hypertension, diabetes, dyslipidemia, and left ventricular hypertrophy, alongside CKD-specific factors such as hyperphosphatemia, oxidative stress, anemia, and chronic inflammation [6, 7]. Despite advances in dialysis techniques and CVD management, hemodialysis patients remain at heightened risk for vascular complications due to endothelial dysfunction, arterial stiffness, and prothrombotic states that develop as a consequence of renal impairment and dialysis-related factors [8].

Recent studies highlight the role of novel biomarkers such as homocysteine, oxidized LDL antibodies, and high-sensitivity C-reactive protein (hs-CRP) in predicting cardiovascular risk in hemodialysis patients, emphasizing the need for a more comprehensive approach to risk stratification beyond traditional risk factors [6]. As cardiovascular mortality remains disproportionately high in this population, targeted interventions that address both conventional and CKD-related risk factors are critical in improving long-term outcomes. The presence of HPF4 Ab may serve as an additional marker for identifying hemodialysis patients at higher cardiovascular risk, warranting further investigation into its potential clinical utility.

The current study's demographic data revealed that individuals with +ve PF4-H Ab were substantially younger than those with –ve PF4-H Ab (mean age: 50.55 ± 8.46 vs. 62.67 ± 6.88 years, $p < 0.001$). The significant association between younger age and PF4-H Ab positivity is an intriguing finding. While age-related immune modulation might typically suggest higher antibody prevalence in older patients, some studies indicate that younger individuals on hemodialysis may have a more reactive immune system and a greater likelihood of developing antibodies against heparin-platelet complexes. Additionally, younger patients may have a longer cumulative exposure to heparin therapy due to extended dialysis dependence, increasing their risk of sensitization [9].

The significantly higher frequency of statin use in PF4-H Ab-positive patients (72.7% vs. 33.3%, $p = 0.013$) suggests a potential link between antibody presence and dyslipidemia or increased cardiovascular risk, which warrants closer investigation. Statins are widely used in hemodialysis patients to reduce lipid levels and cardiovascular risk; the association observed in this study may indicate that PF4-H Ab-positive patients have underlying vascular dysfunction or higher baseline cardiovascular risk, leading to a greater likelihood of being prescribed statins.

The finding that congestive heart failure was significantly more prevalent in PF4-H Ab-positive patients (36.4% vs. 0%, $p = 0.005$) is particularly concerning. Previous studies have shown that the presence of PF4-H Ab is associated with an increased risk of thrombotic complications, arterial stiffness, and vascular dysfunction, which can contribute to the pathogenesis of CHF [8]. PF4-H Ab has been implicated in endothelial activation and pro-inflammatory processes, which may exacerbate left ventricular dysfunction and promote heart failure progression. Additionally, studies have suggested that hemodialysis patients with PF4-H Ab may have a higher incidence of cardiovascular mortality, further supporting the hypothesis that these antibodies contribute to adverse cardiac outcomes [10]. However, conflicting reports indicate that not all studies have found a direct correlation between PF4-H Ab and CHF, suggesting that additional factors, including preexisting cardiac disease and dialysis-related complications, may play a role in this relationship.

The association between PF4-H Ab positivity and cardiovascular risk ($p = 0.074$) aligns with previous research suggesting a link between these antibodies and adverse cardiovascular events, although not all studies have confirmed its independent predictive value [3]. In contrast, other studies have reported a stronger association between PF4-H Ab positivity and vascular complications, particularly in patients with thrombotic risk factors such as atherosclerosis and hypercoagulability [11]. The diagnostic performance of PF4-H Ab, as evaluated by ROC curve analysis, showed an AUC of 0.635 ($p = 0.038$), indicating moderate discrimination between ESRD patients with and without cardiovascular risk. While a cutoff value of

>0.335 yielded a sensitivity and specificity of 65%, these values suggest that PF4-H Ab is not a highly sensitive or specific biomarker for cardiovascular risk stratification in ESRD patients. Previous studies have similarly reported limited diagnostic accuracy of PF4-H Ab for predicting cardiovascular events in dialysis patients, highlighting the need for additional biomarkers to improve risk assessment [(10)]. However, other studies have shown a more pronounced association between PF4-H Ab positivity and mortality, suggesting that these antibodies may still play a role in long-term cardiovascular risk [(12)].

This study has several limitations that should be considered, the sample size was relatively small, which may limit the generalizability of the results, in addition, this study was conducted at a single center, which may introduce selection bias and limit the applicability of the findings to broader populations of hemodialysis patients with varying demographic and clinical characteristics. Another limitation is that other potential confounders such as inflammation markers, dialysis adequacy, and other prothrombotic factors were not extensively evaluated. Also, the study relied on a single measurement of PF4-H Ab, which may not fully capture the dynamic nature of antibody formation over time.

Larger multicenter studies with more diverse patient populations are necessary to validate our results with incorporation of other confounders such as inflammation markers, dialysis adequacy, and other prothrombotic factors to provide a more comprehensive understanding of the role of PF4-H Ab in cardiovascular risk stratification. Additionally, serial antibody measurements could help determine whether persistent or fluctuating PF4-H Ab levels have stronger clinical relevance.

CONCLUSION

While PF4-H Ab was more prevalent in younger patients and those receiving statin therapy, its association with overall cardiovascular risk was not statistically significant in multivariate analysis. This suggests that, although these antibodies are frequently present in hemodialysis patients, they may not independently contribute to the development of CVD when other risk factors are accounted for. However, the significant correlation between PF4-H Ab positivity and congestive heart failure indicates that these antibodies may still play a role in cardiac dysfunction, possibly through mechanisms related to endothelial activation, vascular inflammation, and prothrombotic states.

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