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Apolipoprotein E and plasminogen activator inhibitor 1 gene polymorphism in children with chronic renal insufficiency associated with cardiovascular disease

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Abstract

Background: Cardiovascular disease (CVD) is considered a major cause of death in renal insufficiency (RI). Contributing genetic factors is a recent focus of research. This study aims to elucidate apolipoprotein E (APO-E) and plasminogen activator inhibitor 1 (PAI-1) gene polymorphisms in RI children associated with CVD.

Methods: We studied 50 cases with chronic kidney disease (CKD) associated with CVD, and 30 healthy controls. Study sample was grouped as one on conservative treatment, the second on hemodialysis and the third was posttransplant. PAI-1 and APO-E gene polymorphisms were investigated using allele-specific polymerase chain reaction (AS-PCR) and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) respectively.

Results: 4G4G and 4G5G were the most common PAI-1 polymorphism denoting high association of 4 G allele in renal insufficiency associated with CVD with absent link to dyslipidemia, echocardiography changes or thrombosis. E3E3 was the most common among APO-E polymorphism without relation to dyslipidemia or thrombosis. Dyslipidemia was significantly linked to thrombosis. The study confirmed the role of dyslipidemia and hemodialysis in promoting thrombosis.

Conclusion: Although PAI 4G Genotyping did not show significant association with echocardiography severity or thrombotic severity, yet genetic expression for high levels of PAI in plasma is expected in response to CRI factors known to trigger its release, in addition to those related to dialysis. APO-E3E3 genotyping showed a significant association with echocardiography severity as it enhances APO-A which contributes to CVD. The current study confirmed a significant association between dyslipidemia and CVD; however, the prevalent patterns 4G and E3E3 did not show a significant association with dyslipidemia. The genetic role for APO-A, B, O, or even other isomers for APO-E should be further studied as well.

Keywords: Apolipoprotein E gene, Plasminogen activator inhibitor 1 gene, Chronic renal failure, Cardiovascular disease

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Background

Cardiovascular disease is considered a major cause of death in children with chronic renal failure. Unlike adults, atherosclerosis and coronary ischemia are questionable or subclinical in children whereas, hypertension, impaired cardiac systolic, and diastolic functions and vascular thrombosis are more frequent presentations for CVD. The role of traditional factors related to RF in promoting CVD include hypertension, anemia, dyslipidemia, hypoalbuminemia, and hyperparathyroidism. Patient gene pattern may contribute to the progress of renal damage as well as the development of accelerated CVD through altering some biochemical and biological processes that are reflected in the kidney and cardiovascular system. Plasminogen activator inhibitor-1 (PAI-1) plays a critical role in the regulation of intravascular fibrinolysis [1].

Since plasminogen activators (PA) and their inhibitors are an important role for fibrinolysis and in extracellular matrix degradation, a diminished synthesis of renal PAs and/or increased production of renal (PAI-1) may result in progression of glomerular sclerotic lesions [2]. Coronary heart disease (CHD) and atherosclerosis [1] are the leading cause of mortality and morbidity in the dialysis population [3]. Interstitial fibrosis and tubular atrophy (IFTA) is a clinicopathological entity characterized by fibrosclerosis of the different renal structures leading to the progressive decline of renal function after kidney transplantation [4]. The molecular mechanisms that underlie the pathophysiology of IFTA remain unclear [5]. The upregulation of PAI-1 has been demonstrated in human IFTA accompanied by persistent fibrin deposition in the graft [6]. PAI-1 levels are related to the rate of renal failure progression after kidney transplantation [7]. A common 4G/5G polymorphism in the promoter region of the human PAI-1 gene has been described and is associated with different levels of serum PAI-1 activity [8]. Patients with the 4G4G genotype have the highest levels of PAI-1 activity, whereas those with the 5G5G genotype have the lowest plasma PAI-1 activity [9]. The 5G variant binds the E2F transcription repressor, whereas 4G fails to do so and is associated with the higher PAI1 plasma level [10].

Apolipoprotein E (APO-E) is a lipid carrier protein mainly sensitized in the liver. It is important for uptake, transport, metabolism, and distribution of cholesterol. There are three different alleles for the APO-E gene (E2, E3, and E4) which show a variable association with different kidney and cardiovascular diseases. APO-E polymorphisms have a major role in the pathogenesis of renal diseases they influence the serum lipid profile of patients with end-stage renal disease (ESRD) and consequently the risk for atherosclerotic vascular disease and is associated with development and progress of glomerulopathesis especially with the E2 allele as a predisposing factor in most

Table 1 Echocardiography severity according to our discretionary grading

Degree	Echocardiography findings
Mild	Diminished systolic and diastolic function
Moderate	Diminished systolic and diastolic function with cardiac dilatation
Severe	Diminished systolic and diastolic function with cardiac dilatation and functional mitral regurge.

studies. E4 is associated with a more atherogenic profile in patients with ESRD and is considered a genetic marker for coronary artery disease and global atherosclerosis in post-transplant graft [11]. This study investigated the pattern for both PAI-1 4G/5G and APO-E gene polymorphisms in patients with ESRD associated with cardiovascular complications and tried to find the possible link(s) between the different allelic frequencies to the atherogenic lipid profile and cardiovascular morbidity in these patients.

Methods

Patients

Fifty patients with end-stage renal disease associated with cardiovascular disease (CVD) were included in this study. The patients were followed in our university's children hospital and were classified according to current treatment into three groups. Group I: 20 patients on conservative treatment. Group II: 20 patients on maintenance hemodialysis. Group III: 10 patients who had been transplanted. The reported signs of CVD included hypertension, echocardiographic changes, and vascular thrombosis. Thirty age and sex-matched apparently healthy controls were included in the study. Informed consents were taken from parents of the children (being incompetent patients) after full explanation of the laboratory tests done. This study protocol and the consents were approved and deemed sufficient by "The Postgraduate Clinical Research and Ethical Committee of Pediatric Department, Faculty of Medicine in our University."

Methodology

All patients (50 cases) were subjected to good history taking for both renal and cardiovascular clinical data, hemodialysis sessions, posttransplantation follow- up, and vascular access failure due to repeated thrombosis. Laboratory investigations (serum creatinine, BUN, CBC,

Table 2 Severity of thrombosis according to our discretionary grading

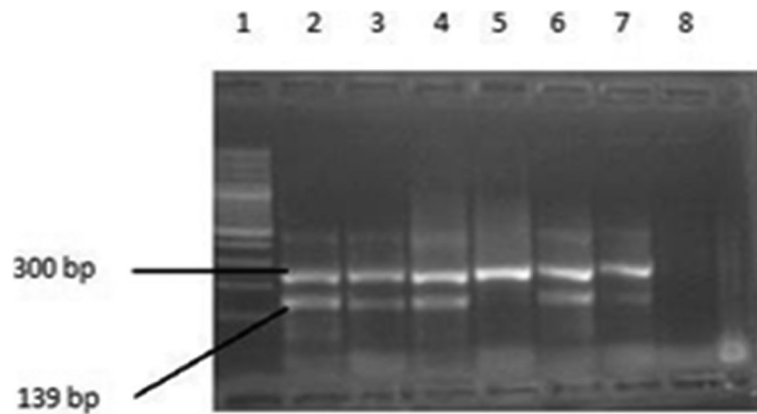| Degree	Findings Group 1 and 2	Group 3
Mild	Fistula failure reported < 2	At anastomotic site
Moderate	Fistula failure reported 2–5	Graft thrombosis
Severe	Fistula failure reported > 5	Extensive

Fig. 1 Showing plasminogen activator inhibitor-1 (4G/5G) polymorphism genotyping, lane 1: marker-ladder 100 bp (ferments), all lanes next to lane 1: showed bands at 300 bp (representing amplification of the control primer), after lane 1: each two subsequent lanes represent insertion (4G) then deletion (5G) polymorphism genotyping for every case, lanes 2, 3 (case 1) and 6, 7 (case 3): show bands at 139 bp representing both 4G and 5G heterozygous genotype, lanes 4, 5 (case 2): show a 139 bp band only at lane 4 representing 4G genotype

electrolytes, lipid profile) were done. Conventional echocardiography for assessment of cardiac indices (systolic and diastolic function). Doppler study on the renal artery at the site of anastomosis in the transplanted cases and fistula site in the hemodialysis cases for diagnosis of vascular thrombosis. Echocardiography findings were variable in severity; patients were graded in a discretionary manner into mild, moderate, and severe. The severity of thrombosis was graded in a discretionary manner into mild, moderate, and severe (Tables 1 and 2).

This study investigated the detection of plasminogen activator inhibitor gene and Apolipoprotein E gene polymorphisms by allele-specific PCR (AS-PCR) and PCR-RFLP respectively. DNA extraction was done from whole blood using QIA amp DNA Kit (QIAGEN, USA, catalog no. 51104). This single allele deletion (4G)/insertion (5G) was situated at − 675 bp of the promoter. Extracted DNA was amplified using allele-specific primers [12] and Taq PCR Master Mix (QIAGEN, catalog no. 201443) containing Taq DNA Polymerase, QIAGEN PCR Buffer (with 3 mM MgCl2) and 400 μM of each dNT.

PCR-RFLP technique was used to determine Apo-E genotyping [13]. APO-E genotypes were determined as E2/E2 with 144 bp and 96 bp bands, E4/ E4 with 72 bp and 48 bp, E3/ E4 with144 bp, 72 bp, and 48 bp, E3/E3 with 144 bp and 48 bp fragments, E2/E3 with 144 bp, 96 bp, and 48 bp fragments and E2/E4 with 144 bp, 96 bp, 72 bp, and 48 bp fragments (Fig. 1) [14].

Statistical methodology
The data was coded and entered using the statistical package SPSS version 15. The data was summarized using descriptive statistics. Number and percentage for qualitative values, statistical difference between groups were tested using chi-square test or Fisher's exact test for qualitative variables. *P* values ≤ 0.05 were considered statistically significant.

Results
The study sample mean age was 11.5 (0.6 year − 17 years), with male/female ratio 65%/35%.

Clinical data
Hypertension was present in all cases of groups I and II and only two cases of group III. Echocardiography findings were classified according to our discretionary grading as shown in Table 3. Frequency for echocardiography findings in each patient group is evident in (Fig. 2).

Thrombosis was absent in group I, present at fistula site in all cases of group II and present in three cases of group III at site of renal artery anastomosis and near segment. The severity of thrombosis in groups II and III was classified according to our discretionary grading as shown in Table 4. Frequency and severity of vascular thrombosis among patients of groups II and III are evident in (Fig. 3). Triglycerides showed significant difference between studied groups (*P* = 0.034) (Table 5), whereas other lipid markers showed no significant difference between groups. This may be explained as TG is an

Table 3 Frequency of echocardiography findings

	Group I conservative (20 cases)		Group II hemodialysis (20 cases)		Group III transplant (10 cases)	
	Case	%	Case	%	Case	%
Absence of changes	0	0	0	0	8	80
Mild	10	50	7	35	0	0
Moderate	10	50	12	60	0	0
Severe	0	0	1	5	2	20

Fig. 2 Frequency distribution of cases according to echocardiography changes
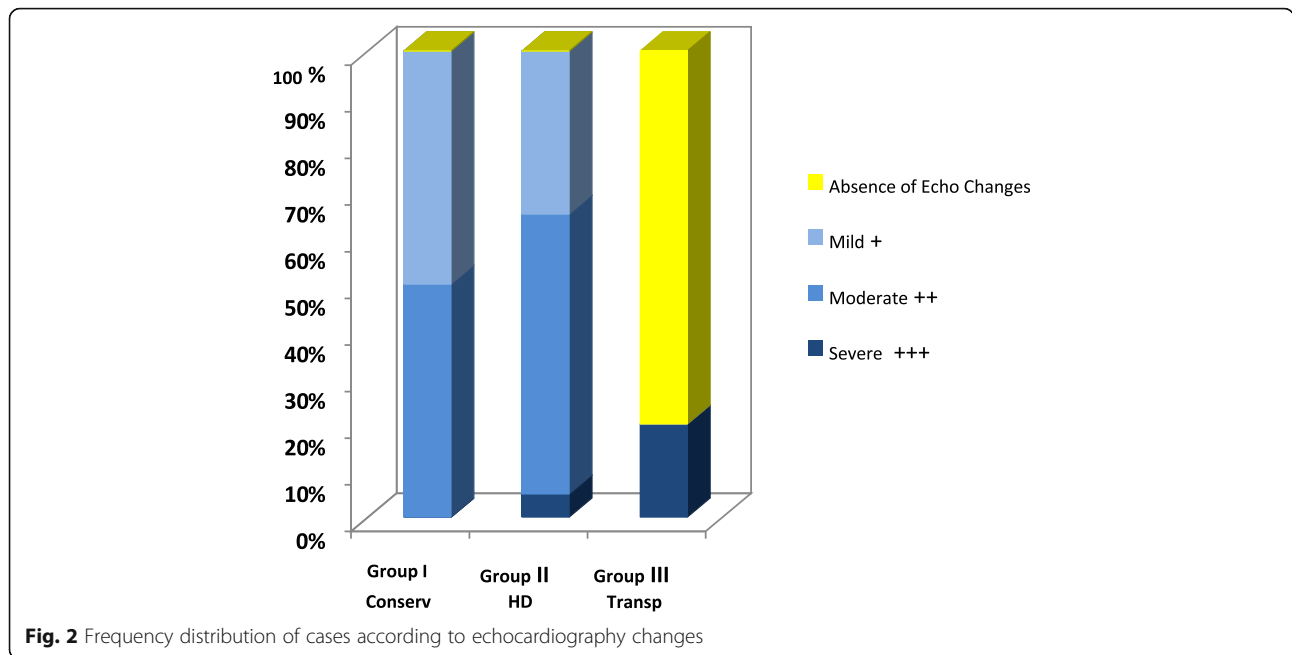
early marker to be normalized after transplantation. Dyslipidemia showed high triglycerides (TG) and high low-density lipoproteins (LDL) as the most frequent pattern recording incidence of 46% and 44%, respectively, next followed by low HDL, high cholesterol, high CHO/HDL recording incidence of 28%, 20%, and 16%.

Plasminogen activator inhibitor-1 gene polymorphism results

There was a significant difference between groups regarding frequency distribution for PAI-1gene polymorphism; 4G/4G and 4G/5G showed high frequency within each patient group ($P = 0.004$) (Table 6). Also, there was significant high-frequency for the presence of 4G allele (in its homozygous and heterozygous) among total patient sample versus control ($P = 0.000$) (Table 7) and (Fig. 4).

Apolipoprotein-E gene polymorphism results

Frequency distribution for Apo-E; E3E3 showed a significant difference between four groups ($P = 0.000$) (Table 8

and Fig. 5) and also significant high frequency among total patients versus control ($P = 0.000$) (Table 9, Fig. 5). Patient distribution according to the presence of E3E3 polymorphism showed a significant difference between groups ($P = 0.000$) as well as a significant difference between total patients and control ($P = 0.000$) (Table 7).

Correlation between prevalent gene polymorphisms and pattern of dyslipidemia in four groups

There was a non-significant association between the presence of 4G allele and hypertriglyceridemia, hypercholesterolemia, low HDL, high LDL, and high CHO/HDL in each of the four groups. There was a non-significant association between presences of E3E3 polymorphism with altered any of the lipid markers in any of the four groups (Table 10). Therefore, dyslipidemia in the studied sample was not related to the most prevalent studied gene polymorphism it could be related to other Apo isomers or to renal insufficiency.

Correlations between cardiovascular changes versus prevalent gene polymorphism (Tables 11 and 12) and dyslipidemia (Tables 13 and 14)

There was a non-significant association between echocardiography severity and presence of PAI-1 4G allele among each group and in total patients. But, there was a significant association between echocardiography severity and presence of APO-E (E3E3) polymorphism in the total patient sample but not significant in each group (Table 11). There was a non-significant association between thrombotic severity and PAI-1(4G) and APO-E (E3E3) genotyping in any group or total cases (Table 12).

Table 4 Frequency of different grades of thrombosis among three groups

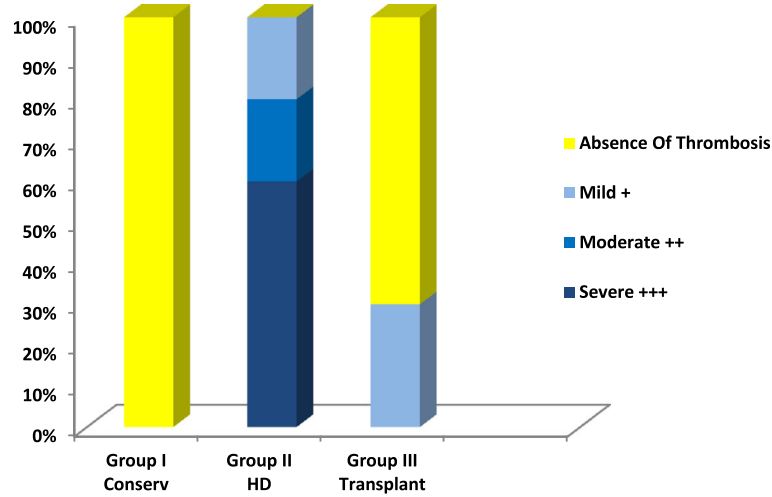|	Group I conservative (20 cases)		Group II hemodialysis (20 cases)		Group III transplant (10 cases)	
	Cases	%	Cases	%	Cases	%
Absent	20	100	0	0	7	70
Mild	0	0	4	20	3	30
Moderate	0	0	4	20	0	0
Severe	0	0	12	60	0	0

Fig. 3 Frequency distribution of cases according to thrombosis score

There was a significant association between echocardiography severity and dyslipidemia only in group I, such relation was non-significant in other groups and total sample (Table 13). Statistical relation between thrombotic severity with dyslipidemia shows significance in group II and total cases and non-significant in group III (Table 14).

Previous results may be summarized as echocardiography findings that were related to E3E3, and not 4G in total cases, where also dyslipidemia showed its role but only in predialytic group, possibly, reduction of TG present after HD or transplantation may explain such finding. Thrombosis was prevalent in group II, was not related to prevalent 4G or E3E3, but related to dyslipidemia and HD.

Discussion

Cardiovascular disease was recognized as a major cause of death in children with chronic kidney disease CKD. Similar to adults, children with CKD have an extremely high prevalence of traditional and uremia-related CVD risk factors as hypertension, dyslipidemia, hyperglycemia, uremia, hypoalbuminemia, anemia, and hyperparathyroidism. Early markers of cardiomyopathy include left ventricular hypertrophy, systolic and diastolic dysfunction to be followed with cardiac dilatation and valvular regurge [15]. Subclinical forms of atherosclerosis are reported in children and its early markers include increased carotid arterial intima-media thickness and coronary

Table 5 Mean values and frequency of altered lipid profile

Group	Parameter	Mean	ST.D	Min	Max	P value	Significant	Frequency (%)
GR1	Triglycerides (T.G)	200.5	112.03	59.0	366.0	0.034	Sig	46
GR2		177.75	84.46	66.0	428.0			
GR3		107.40	51.12	45.0	203.0			
GR1	Total cholesterol (CHO)	177.6	41.07	135.5	273.0	0.569	N.S	28
GR2		167.0	36.96	112.0	267.0			
GR3		183.6	57.21	114.0	282.0			
GR1	High-density lipoprotein (HDL)	42.40	8.03	29.00	52.0	0.247	N.S	20
GR2		46.35	5.48	31.00	56.0			
GR3		48.00	16.56	34.00	85.0			
GR1	Low-density lipoprotein (LDL)	96.80	3.05	71.0	198.0	0.496	N.S	44
GR2		87.15	42.65	28.0	179.0			
GR3		114.10	42.90	69.0	204.0			
GR1	CHO/HDL	4.54	1.87	2.9	8.3	0.625	N.S	16
GR2		3.61	0.95	2.3	6.4			
GR3		4.00	1.40	2.7	7.6			

Table 6 Frequency distribution of PAI-1 gene polymorphism

PAI gene polymorphism	Group I (20 cases)		Group II (20 cases)		Group III (10 cases)		Group IV (30 cases)		P value 1 significance	Total patients sample		Healthy control (30 cases)		P value 2 significance
	Case	%	Case	%	Case	%	Case	%		Case	%	Case	%	
4G/4G	8	40	9	45	1	10	4	13.3	0.004 Signif	18	36	4	13.3	0.059 Non Signif
4G/5G	4	20	4	20	8	80	10	33.3		16	32	10	33.3	
5G/5G	8	40	7	35	1	10	16	53.9		16	32	16	53.3	

artery calcification. Cardiovascular disease is particularly evident in those on maintenance hemodialysis as they are very much exposed to altered hemodynamics.

Genetic factors

Contributing to CKD and/or CVD has been recently on focus. Apolipoprotein E and plasminogen activator inhibitor 1-related genes are common examples. The first one is highly involved in lipid metabolism and the latter blocks fibrinolytic pathway and thus promotes vascular thrombosis. The aim of this work is to study the two mentioned gene polymorphisms in children with CKD associated with CVD, with special consideration to those on hemodialysis, looking for special allele patterns. As dyslipidemia is a common sequence for CKD, a major risk factor for CVD and genetically controlled by the Apolipoprotein E gene; therefore, it has been also extensively studied. Links between gene results with dyslipidemia and between each of them with echocardiography findings and thrombosis will be discussed.

Dyslipidemia

Altered serum lipid profile (dyslipidemia) secondary to renal insufficiency is well known since early time. In this study, it was reported in each group and in the total patient sample with different patterns and frequency. Total patient sample with CKD showed that high triglycerides (TG) and high low-density lipoproteins (LDL) as the most frequent pattern recording incidence of 46% and 44% respectively, next followed by low HDL, high cholesterol, and high CHO/HDL recording incidence of (28%, 20%, 16%) (Table 3). Cases et al. reported high TG, normal to high total CHO, low HDL, normal to high LDL, and high VLDL dyslipidemic patterns to associate CRI and described it as atherogenic dyslipidemia
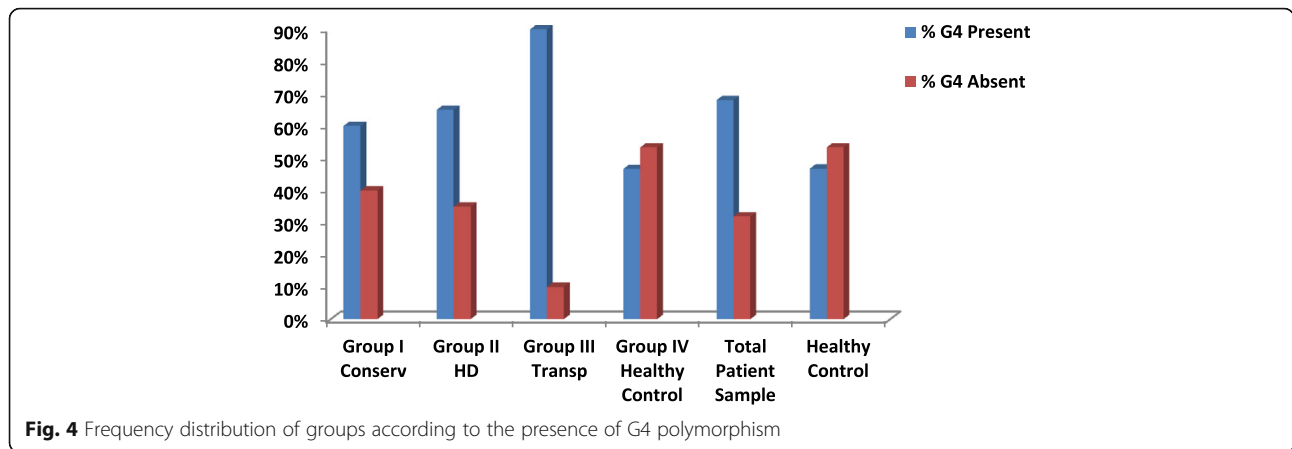
[16]. The mean level of TG in this study showed the non-significant difference between predialytic compared to those on hemodialysis. Group III showed a lower mean of triglycerides after renal transplantation (Table 3). Aser also reported no particular effect of hemodialysis on TG as compared to predialytic patients [17]. Pattern and frequency of dyslipidemia in CKD, as well as its relation with hemodialysis and peritoneal dialysis have been extensively discussed in the literature. Normal to high total cholesterol, normal LDL, low HDL, high VLDL in predialytic, and hemodialysis patients were reported by Bregman et al. [18]. Possible mechanisms include postprandial dyslipidemia as the intestine is the site of synthesis of APO-A1 and A2, APO-B48, and APO-E [19]. Also, Charlesworth JA related the greater and prolonged postprandial rise of TG in CRI to be due to impaired clearance of chylomicron remnants [20]. Hepatic role for dyslipidemia in CRI is related to the role of the liver in the synthesis of APO-B100. AP0-C11, APO-C111, AP0–1, and AP0–11, and the role of the liver in expression of receptors for all lipoprotein classes and so its ability to synthesize HDL and VLDL [21]. Other mechanisms for dyslipidemia in CRI include insulin resistance [22], proteinuria [23], and increased oxidative stress with increase LDL [24]. LDL has a high atherogenic effect.

Relation between dyslipidemia and CVD

The relation between dyslipidemia and CVD in this study showed non-significant relation with echocardiography changes in total cases, HD, and post-transplantation. It was only significant in the predialytic group as high TG was most evident in this group (Table 10). Dyslipidemia by itself accelerates glomerular injury to mesangium, endothelium, and podocytes through lipid mediators of oxidative stress, this contributes to the development of

Table 7 Frequency distribution for presence and absence of G4 allele

G4	Group I (20 cases)		Group II (20 cases)		Group III (10 cases)		Group IV (30 cases)		P value 1 significance	Total patient sample		Healthy control (30 cases)		P value 2 significance
	Case	%	Case	%		%	Case	%		Case	%	Case	%	
G4 (+)	12	60	13	65	9	90	14	46.6	0.0103 Non	34	68	14	46.7	0.0000 Signf
G4 (−)	8	40	7	35	1	10	16	53.3						

Fig. 4 Frequency distribution of groups according to the presence of G4 polymorphism

hypertension, uremic toxins, hypervolemia, and anemia with their impact on cardiac changes [16]. In adults, dyslipidemia is a high-risk factor for atherosclerosis. Such finding is milder or lacking in children and young adults [25]. Subclinical atherosclerosis begins in childhood and is likely accelerated in children with CKD [26]. As for the association between dyslipidemia and thrombosis (Table 12), there was a significant association between dyslipidemia and the severity of thrombosis among patients on HD. High VLDL triglyceredemia, in particular, is a very important stimulant to express a high level of PAI-1 that promotes thrombosis through its antifibrinolytic effect. HD by itself is a major contributor to vascular thrombosis through vascular access trauma and the release of endothelin, thrombin, and Tpa. It may also be related to the inflammatory response that may happen on the contact of blood with the dialyzer and dialysate through activation of kallikrein-kinin system and generation of cytokines, expression of PAI-1 in high plasma levels that promotes thrombosis [27].

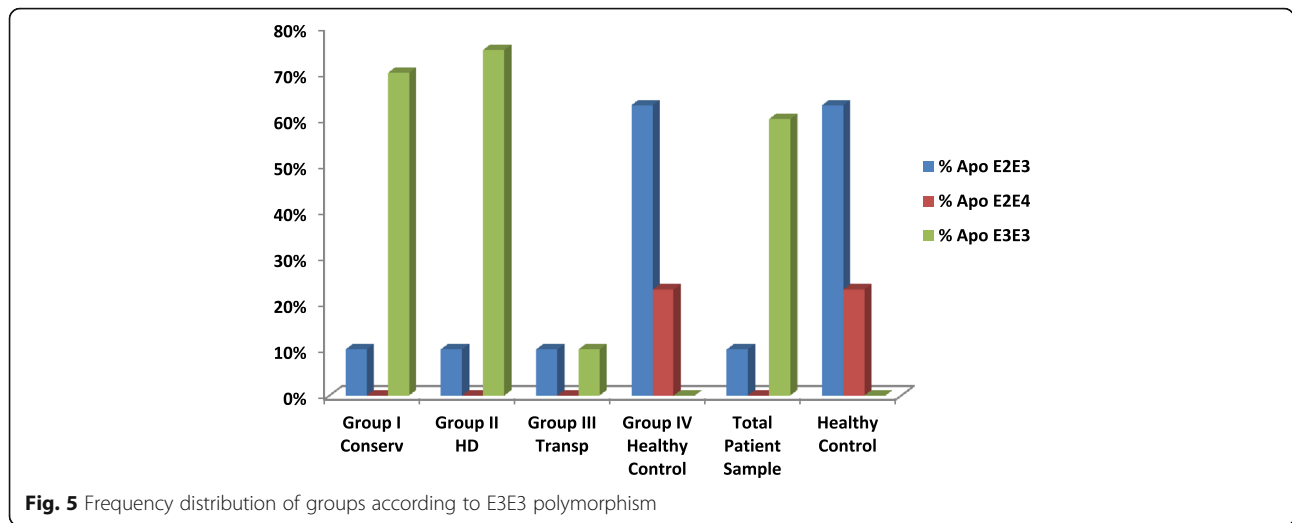### Plasminogen activator inhibitor-1 results
4G in its homozygous 4G/4G and its heterozygous 4G/5G forms constitute the most common within the total patient sample (Table 6 and 7, Fig. 4). The statistical comparison regarding PAI-1 gene polymorphism between the total patient sample and healthy control showed a significant difference, as 4G in its homozygous and heterozygous

forms represent the most prevalent pattern (Tables 6 and 7). PAI-1 plasma level is highly determined by PAI-1 genotype in addition to other factors. Higher plasma levels are correlated with polymorphic variance in the number of guanine bases 4G/5G in the promoter at position – 675. 4G is associated with higher plasma levels. 4G/4G shows the highest level, 5G/5G shows the lowest level, and 4G/5G is intermediate [28]. Other factors that determine its level include the balance between agonists and inhibitors. Suppressors include interferon-γ, nitric oxide, natriuretic factors, and lipid-lowering drugs [29]. Factors that increase its level include growth factors; coagulation factors as fibrin fragments, thrombin, and Tpa; metabolic factors as glucose, LDL, and APO-A; hormones as aldosterone, angiotensin, renin, and erythropoietin; and environmental factors as endothelial stretch, hypoxia, and endothelin [30].

PAI-1 contributes much to many acute and chronic kidney diseases such as acute thrombotic microangiopathy with fibrin deposition in glomeruli and arterioles as reported in hemolytic uremic syndrome (HUS), scleroderma, and antiphospholipid renal vasculitis [31]. This is also reported in crescentic glomerulonephritis, and antiglomerular basement membrane disease (antiGBM) [32]. Chronic kidney disease associated with high PAI-1 includes focal sclerosis glomerulonephritis (FSGS), diabetic nephropathy, focal necrotizing glomerulonephritis, membranous nephropathy, cyclosporine-induced glomerulosclerosis, and

Table 8 Frequency distribution for Apolipoprotein E gene polymorphism

Apo gene polymorphism	Group I (20 cases)		Group II (20 cases)		Group III (10 cases)		Group IV (30 cases)		P value 1 significance	Total patients sample		Healthy controls (30 cases)		P value 2 significance
	Case	%	Case	%		%	Case			Case	%	Case	%	
E2E3	2	10	2	10	1	10	19	63	0.000 Signi	5	10	19	63	0.000 Signi
E2E4	0	0	0	0	0	0	7	23		0	0	7	23	
E3E3	14	70	15	75	1	10	0	0		30	60	0	0	
E3E4	2	10	1	5	0	0	0	0		3	6	0	0	
E4E4	2	10	2	10	8	80	4	13		12	24	4	13	

Fig. 5 Frequency distribution of groups according to E3E3 polymorphism

chronic allograft nephropathy [33]. 4G polymorphism was reported with a high incidence among CKD and also in those with high risk of posttransplant rejection [34]. PAI-1 expression is stimulated in CKD through transforming growth factor-beta (TGF-B), angiotensin II, tissue growth factors, cytokines, endothelin – 1, glucose-insulin resistance, LDL, hormones, acute phase response, oxidative stress, endotoxins. PAI-1, as it reduces plasmin activity it promotes thrombotic and necrotizing glomerular lesions that end with sclerosis and it also can directly induce migration of macrophages, trans-differentiated tubular epithelium, and myofibroblasts that end with interstitial fibrosis [34].

Association between presence of G4 and dyslipidemia (Table 10)
There was non-significant association between each form of dyslipidemia with the presence of 4G mutation within each of the patient groups and within total patient sample. The literature reported that PAI-1 plasma level correlates positively with very low-density lipoprotein triglycerides [35], such combination is well evident in CKD with renal dysfunction and is independent on the presence of 4G polymorphism as evident in this study.

Association between G4 polymorphism and severity of echocardiography changes
There was a non-significant association between severity of echocardiography changes with the presence of 4G polymorphism within each of the patient groups and

within total patient sample (Table 11). CKD and renal dysfunction per se contribute to the development of CVD through many mechanisms. Primary traditional factors include arterial hypertension, hyperuricemia, diabetes, dyslipidemia particularly high TG, and low HDL-CHO. Non-traditional risk factors include hyperparathyroidism, anemia, hyper homocysteinemia, increased oxidative stress, endothelial dysfunction, apolipoprotein A, inflammatory procoagulant activity [36]. Left ventricular hypertrophy is common in the early stages of CKD greater than would be expected for the degree of hypertension. Left diastolic and systolic dysfunction, cardiac dilatation soon follow and are highly related to uremic myopathy. Some degree of coronary atherosclerosis was found in 80% of a series of autopsies for children on HD [37]. 4G/5G polymorphism in PAI-1 gene in some studies showed association with arterial ischemic strokes, and others showed a significant high level for plasma PAI-1 that was not influenced by the 4G/5G polymorphism [38]. The homozygous or heterozygous carriage of 4G allele had been associated with higher PAI-1 levels and increased risk for CVD [38].

Association between G4 polymorphism and thrombotic changes (Table 12)
There was a non-significant association between the severity of thrombosis with the presence of 4G polymorphism within each of the patient groups and within the total patient sample. Kidney dysfunction itself may

Table 9 Frequency distribution for the presence and absence of E3E3 polymorphism

E 3 E 3 mutation	Group I		Group II		Group III		Group IV		P value 1 significance	Total patients sample		Health controls		P value 2 significance
	Case	%	Case	%		%	Case			Case	%	Case	%	
E3E3 (+)	14	70	15	75	1	10	0	0	0.000 Sign	30	60	0	0	0.000 Sign
E3E3 (−)	6	30	5	25	9	90	30	100		20	40	30	100	

Table 10 Association between plasminogen activator inhibitor G4 polymorphism and APO-E (E3E3) polymorphism with each pattern of dyslipidemia

Groups		Group I		Group II		Group III		Group IV	
Gene polymorphism		G4 +	E3E3+	G4 +	E3E3+	G4 +	E3E3+	G4 +	E 3E3+
↑ Triglyceride	P value	1.000	0.628	1.000	1.000	1.000	1.000	0.697	0.908
	Significance	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S
↑ CHO cholesterol	P value	1.000	0.267	1.000	0.720	0.400	0.400	0.707	0.720
	Significance	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S
↓ HDL high-density lipoprotein	P value	0.373	1.000	0.521	0.447	1.000	1.000	0.175	0.368
	Significance	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S
↑ LDL low-density lipoprotein	P value	0.495	1.000	1.000	1.000	0.400	0.400	1.000	1.000
	Significance	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S
↑ CHO/HDL	P value	1.000	1.000	0.350	1.000	1.000	1.000	0.699	1.000
	Significance	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S

generate a thrombotic milieu indirectly through electrolytes, acid-base imbalance, and uremic toxins through their actions on enzymes involved in coagulation [39]. Patients on hemodialysis show leucocyte activation and generation of cytokines that affect the fibrinolytic system. Many studies reported rise in PAI-1 in HD patients and attributed this to endothelial dysfunction and proinflammatory effect of bradykinines; however, the rise may be transient as it drops after dialysis due to bradykinine B2 receptor blockade [40].

Apolipoprotein E results

E3E3 was the most common pattern among HD and predialytic groups and among total sample reporting 75%, 70%, and 60% incidence for each. It was not reported in the control group (Tables 8 and 9 - Fig. 5). E3E3 showed a significant difference among the total patient group (50 cases) as compared to healthy control (60%, 0%) (Tables 8 and 9). APO-E polymorphism associating with renal insufficiency, in multiple studies, showed different data. Prevalence of E2 was reported among Japanese with renal insufficiency [41], E3/E4 among Sweden patients [42], and E3/4 and E4/4 in endstage renal disease secondary to IgA nephropathy [43]. Guz et al. coincides with this study as no difference in E pattern was reported between hemodialysis and predialytic stage [44]. The study of the relation between dyslipidemia

and E pattern in this work (Table 8) showed nonsignificant association between E3E3 pattern and hypertriglyceridemia, hypercholesterolemia, high LDL, low HDL, and high CHO/HDL among each group and among total sample. This was also reported by Guz G [50]. However, Keane W F et al., reported an association between APO-E and its different patterns with renal disease and progress of renal failure. He attributed this to the role of APO-E in altered lipids, lupoid glomerulosclerosis, dialysis-related amyloidosis, and autocrine role of APO-E as a modulator of glomerular and mesangial proliferation [45]. Eto confirmed the role of E2 in particular in renal disease and renal failure and reported high APO-E polymorphism among patients with ESRD with the secondary rise to APO-A [46]. The relation between E3E3 and echocardiography changes (Table 9) reflects a significant association between E3E3 and severity score for echocardiography changes among total cases. E3E3 pattern represents about 70% incidence in groups 1 and 2 who constitute the major part of the total sample and also that patients who show high incidence and severity of high-risk factors contributing to CVD and echocardiography changes, in other way CV changes is multifactorial and not only associated with APO-E3E3 pattern.

The literature reported the prevalence of APO-A among those with coronary heart disease as they show atherogenic lipid profile and even independently on

Table 11 Statistical significance for relation between echocardiography findings severity versus gene polymorphism (G4 and E3E3) in each group and total cases

Gene polymorphism	Group I		Group II		Group III		Total cases	
	G4 (+)	E3E3 (+)	G4 (+)	E3E3 (+)	G4 (+)	E3E3 (+)	G4 (+)	E3E3 (+)
P value	0.06	0.329	0.638	0.180	1.000	1.000	0.205	0.001
Significance	N.S	N.S	N.S	N.S	N.S	N.S	N.S	Significa

Table 12 Statistical significance for relation between thrombotic severity versus gene polymorphism (G4 and E3E3) in each group and total cases

Group	Group I		Group II		Group III		Total cases	
Gene polymorphism	G 4 (+)	E3E3 (+)	G 4 (+)	E3E3 (+)	G 4 (+)	E3E3 (+)	G 4 (+)	E3E3 (+)
P value	No thrombosis		0.249	1.000	1.000	1.000	0.219	0.461
Significance	No thrombosis		N.S	N.S	N.S	N.S	N.S	N.S

Table 13 Statistical significance for relation between echocardiography findings severity versus dyslipidemia in the three groups and total cases

Group	Group I	Group II	Group III	Total cases
Significance	Sig.	N.S	Non Sig.	Non Sig.
P value	0.007	0.289	1.000	0.149

dyslipidemia. AP0-E enhances APO-A which contributes to CVD and directly contributes to the development of atherosclerosis [47]. Association between apolipoprotein E3E3 and thrombotic grading as evident in (Table 10) shows that thrombotic grade severity was not associated with APO-E3E3 pattern. As thrombosis showed association with dyslipidemia, whereas dyslipidemia did not show significant association with APO-E3E3 presence, we can say that renal insufficiency (RI)-induced dyslipidemia is more influential in promoting thrombosis than APO-E role. The role of dyslipidemia in promoting thrombosis is well known.

Conclusion
Although PAI 4G Genotyping (the most frequent in this study) did not show significant association with echocardiography severity or thrombosis severity, yet genetic expression for high levels of PAI in plasma is expected in response to chronic renal insufficiency (CRI) factors known to trigger its release, in addition to those related to dialysis as previously mentioned. APO-E3E3 Genotyping (the most frequent in this study) showed a significant association with echocardiography severity score as it enhances APO-A which contributes to CVD. The study confirmed the significant association between dyslipidemia and CVD (echocardiography and vascular); however, the two prevalent patterns 4G and E3E3 did not show a significant association with dyslipidemia. The genetic role for other APO-A, B, O, or even other isomers for APO-E should be studied as well. Therefore, the presence of dyslipidemia in such patients, irrespective of being secondary to CRI and or genetic predisposition, should be early managed to slow decline of renal function, to prevent post-transplant interstitial fibrosis and graft loss and to prevent the development of accelerated CVD. Extended study to allocate other patterns of gene polymorphism of high-risk value will be of great help.

Table 14 Statistical significance for relation between thrombotic severity versus dyslipidemia in the three groups and total cases

	Group I	Group II	Group III	Total cases
Significance	–	Sig.	Sig.	N.S
P value	–	0.057	0.057	0.475

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