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Association of Apoe4 Genotypes, Peripheral Arterial Disease And Cardiovascular Complications Among A Cohort of Elderly Diabetic Egyptian Patients: A Case-Control Study

KEYWORDS

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ABSTRACT *Background Peripheral arterial disease (PAD) in the lower extremities is a marker of widespread vascular disease particularly in the coronary and cerebral circulation and is the leading cause of mortality especially in diabetic population. Coronary heart diseases (CHD) may be related to genetic mutations in the production of apolipoprotein E (ApoE), partly via alterations to the metabolism of CHD-related lipids as LDL and TG, in addition to other unexplained and interacting factors. In this work, we studied the association of apoE4 genotypes with PAD and CHD in a cohort of elderly diabetic Egyptian patients compared to controls.*

Methods Our study was a case-control study, conducted on 90 elderly subjects categorized into 30 patients with type2 diabetes mellitus (T2DM) with atherosclerotic cardiovascular (CV) complications, 30 patients with type T2DM without atherosclerotic CV complications and 30 normal healthy elderly subjects without DM or evidence of atherosclerotic CV disease. Lipid profile was assessed for each subject in the study, ankle-brachial index (ABI) and arterial duplex ultrasound (DUS) were performed to detect those with poorly compressible peripheral arteries (PCA) in this older age diabetic group and to confirm or exclude the presence of PAD among the study subjects. ApoE genotyping was done by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) to identify the genetic characteristics of the study population with the subsequent study of the variables among ApoE-ε4 allele carriers and non-carriers.

Results The frequencies of E3/E3and E3/E4 genotypes as well as the prevalence of PAD and PCA, were significantly increased among the diabetic patients with CVD compared to those with T2DM without CVD and controls. Moreover, it was found that, among the diabetic group with CHD and PAD, the frequency of the ε4 allele carrier state was significantly higher than those without this allele. However, this significant difference was not found among those diabetic patients with CHD and PCA.

Conclusions The Apo ε4 allele may be associated with clustering of cardiovascular complications in DM in the form of its association with CHD in elderly diabetic patients with PAD. Further genetic studies to add information beyond the traditional cardiovascular risk factors in T2DM and to identify risk genotypes that might be involved in the occurrence of diabetic complications, will help in early prediction and identification of at risk patients.

Background

Cardiovascular diseases (CVD), which include CHD, cerebrovascular disease, and peripheral vascular disease (PVD), is the leading cause of mortality in populations, particularly in the diabetic one. DM and PAD are both CAD equivalents ("coronary equivalents"). PAD is more common among those aged 50 years and older, with approximately 1 in 20 Americans in this age group affected **[1**].

ABI thresholds of less than 0.9 and more than 1.3 [denoting poorly compressible arteries (PCA) due to medial arterial calcification (MAC)] are highly suggestive of PAD in diabetic patients **[2].** It has been used in most studies on PAD, because of its simplicity and non-invasiveness **[3].** However, in patients with DM, patients with distal lesions in elderly patients, and patients with mild stenosis, diagnostic value of ABI was decreased **[4].** Either abnormally low or high ABI can predict CV morbidity and mortality in patients with DM **[5][6].**

Genetic epidemiologic studies have suggested that certain genetic variant including polymorphisms in the gene encoding APOE is associated with an increased prevalence of CAD in T2DM patients **[7].** An association between Apo E genotype and PAD may be present and this may be modified by DM, which is also associated with dyslipidemia that predisposes to macrovascular disease **[8].** However at older ages, the deleterious effects of apo ε4 allele may not be exclusively mediated by dyslipidemia but also by other mechanisms **[9].**

The ApoE gene is polymorphic with three alleles ε2, ε3, and ε4, coding for isoforms E2, E3, and E4 having different binding inclination for corresponding receptors. ApoE isoforms differ in amino acid sequence at positions 112 and 158. From these alleles arise six phenotypes; their ranking from most to least common is generally 3/3, 4/3, 3/2, 4/4, 4/2, and 2/2 **[10][11].**

There are few large studies examining the role of ApoE4 in relation to atherosclerosis in the population of elderly diabetics. Differences features such as ethnicity, sources of

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controls among studies have led to discrepancy in estimating the true effect of apoE genotypes on CHD risk **[12]**.

Methods

The study sample comprised 90 elderly subjects recruited from the inpatient wards and outpatient clinics of Ain Shams University Hospitals in Cairo. The studied sample was divided into 3 groups;

Group A (cases): (30) elderly patients diagnosed to have DM with atherosclerotic CV complications diagnosed by history taking and clinical data. Group B (cases): (30) elderly patients diagnosed to have DM without atherosclerotic CV complications as evidenced by history taking and clinical data. Group C (controls): (30) apparently healthy elderly without DM or any history or clinical data suggestive of atherosclerotic diseases. Before enrollment in this study, each participant gave a written informed consent, and the study protocol was approved by the Ethics Committee of the University. Critically ill patients were excluded from the study.

A detailed medical history was taken about other systemic and/ or CVD. Macrovascular disease was defined as a history of MI, presence of angina, revascularization procedures or stenosis >50% of the coronary artery, a history of cerebrovascular diseases or PAD based on a previous L.L Doppler U/S examination **[13].** DM was defined according to American Diabetes Association (ADA) criteria **[14].** Hypertension was defined as blood pressure above 140/90 mmHg or taking antihypertensive drugs.

Dyslipidemia was defined as level of total cholesterol (TC) >200 mg/dL, triglycerides (TG) >150 mg/dL, LDL-C >130 mg/dL, HDL-C <40 mg/dL, TC/HDL-C ratio >4.0 or under medication of lipid lowering drugs **[15].**

The laboratory work and lipid analysis were conducted. at the Clinical Pathology Department, Ain Shams University,using Synchron CX-9 autoanalyzer (Beckman Instruments Inc.; Scientific Instruments Division, Fullerton, CA 92634, 3100, USA).APOE Genotyping was tested by PCR-RFLP resulting in the three genotype groups that have been analysed in this study: APOE2 (carriers of the ε 2/2 and the ε 2/3 genotype), APOE3 (carriers of the ε 3/3genotype), and APOE4 (carriers of the ε 3/4 and the ε 4/4 genotype).

The measurement of the ABI was done using a handheld Doppler device; the Diaped Flux-200 with 8-MHz Doppler probe device. Variables were categorized according to ABI values to: <0.90, the grouping that reflects PAD; 0.90 to 1.40, the referent group; and ≥1.40 to define subjects having stiffened non-compressible peripheral vessels **[16].** A high ABI > 1.4 was included in the ABI risk category for CVD in the current study **[17]**.Duplex ultrasound study (DUS) of the lower extremities was done in the Interventional and Vascular Radiology Unit in Ain Shams University Hospital, using Hitachi EUB-565A. B mode Doppler with colour imaging. Categorization of the results was done according to wave form pattern; whether triphasic, biphasic or monophasic wave forms. Diagnosis of PAD was based on an ABI of 0.90 or less **[18]** or the onscreen loss of reverse flow (loss of triphasic signal) or a combination of both. Analysis of data was done by IBM computer using SPSS (statistical program for social science version16) where P value >0.05 insignificant**,** P<0.05 significant and P<0.01 highly significant.

Results

The characteristics of the study population were described in table (1).There were no statistically significant differences between groups as regards gender;mean age and other lipid variables except mean HDL values which were higher among control group compared to the diabetic groups A and B. The prevalence of dyslipidemia was significantly higher among group A subjects compared to groups B and C (60%, 32%, 8% respectively). Group A subjects had more frequent ABI risk than those in groups B and C in the form of higher frequencies of PAD and PCA, showing high statistically significant differences. These results were reinforced by the statistically significant findings of DUS; where abnormal monophasic and biphasic wave forms were more prevalent among group A than groups B and C.

As regards APOE genotyping, genotypes with ε4 allele showed a tendency towards a higher frequency among group A followed by groups B then C (37.5%, 34.4%, 28.1% respectively), but without a statistically significant difference. Phenotype 4/4 was equally prevalent among groups B and C and higher than A as demonstrated in table (2) of the genotyping characteristics of the study groups. Mean SBP and mean serum LDL values were higher among ApoE-ε4 carriers than non-carriers and these showed statistically significant differences (table 3).

There were no statistically significant differences between apoE-ε4 carriers and non-carriers as regards ABI risk categories and abnormal DUS wave pattern although there was a tendency for abnormal (monophasic and biphasic) wave forms to predominate among ApoE-ε4 carriers than non-carriers.

There were no statistically significant differences between ApoE-ε4 carriers and non-carriers as regards CV complications (CAD and stroke) among group A, although there was a statistically significant difference between ApoE-ε4 allele carriers and non-carriers as regards prevalence of CAD among PAD cases in this group, where it was found that CAD was more prevalent among PAD cases with the ε4 allele than those without it (71.4%,28.6% respectively). No one without CAD and among PAD cases had the ApoE-ε4 allele. This significant relation was not found among those with PCA (table 4).

Discussion

In the current study, the prevalence of ApoE-ε4 allele among the study population was (35.6%), it was more prevalent among group A (37.5%) compared to C (28.1%) but without a statistically significant difference, agreeing with the French study **[19]** but inconsistent with Chaudhary and colleagues, who demonstrated in their study that ε4 allele frequency manifested itself as significantly higher in both T2DM and CAD patients as compared to controls **[20].** It is to be noted that in this study, there was a higher frequency of E3/E4 genotype only in group A (40%) compared to B and C with a statistically significant difference suggesting its relation to atherosclerosis, inconsistent to the results reported by Chaudhary and colleagues, who found a significant higher frequency of the ε4 allele among both diabetic groups with and without complications compared to controls, suggesting in their study, that among those selected ApoE alleles (ε2, ε3, and ε4), the ε4 allele may be one of the predictors of both DM and its complications in the studied subjects **[20].**

The current study shows that there was no statistically significant difference between carriers and non-carriers of

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APOE-ε4 allele as regards ABI risk for CVD by using X2 test despite a tendency for non-APOE4 carriers to have normal ABI than APOE-ε4 carriers. Consistently with these results were those of El-Jaafary and colleagues, where there was no significant difference among patients with normal ABI and those with abnormal ABI regarding the APOE gene polymorphism **[21].**

The cause of non-significance may be explained by the still little knowledge about genetic factors influencing inter-individual variation in ABI as a complex trait; influenced by a large array of genetic, environmental, behavioral, and social factors and their interactions **[22]**.As regards CV comorbidities present in group A subjects in the current study, it was found that the frequency of CAD cases carrying the ε4 allele was lower than that of CAD cases without the allele, inconsistently with the study of Djan and colleagues where the frequency of ε4 allele was significantly higher among CHD patients **[23].** This inconsistency with the current results may be explained by the fact that ApoE gene polymorphism confering susceptibility to or protection from CAD in patients with T2DM and ND may be quite different in different ethnic populations. There may also be gene-by-environmental interaction involving dietary composition, antioxidant intake, smoking habits, etc. **[24-29].** In the largest prospective cohort study to date, CHD risk was not associated with ApoE4 genotype after controlling for a variety of CV risk factors, particularly the ratio of LDL to HDL-C **[30].**

Highr risk of CAD was found among PCA cases (66.7%) compared to cases with PAD (58.3%), not agreeing with the study of Sarangi and colleagues where none of the values were >1.5 and who reported that the occurrence of CAD in PAD-positive cases was 46.88% **[31].** On the other hand, and consistent with the current study, no significant association was found between CV conditions and low ABI (PAD) in the Pakistanian study carried by Akram and colleagues **[32].**

Consistent with the current study as regards the possible link between ApoE4 and incidence of CHD in PAD cases, are the results from previous studies suggesting that apoE ε4 allele has a variable significance in terms of predicting the risk of vascular events in different populations **[33].** A recent meta-analysis showed that subgroup analysis based on clinical phenotypes of atherosclerosis (clinical and subclinical atherosclerosis) showed that ε4 allele associated with the incidence of clinical atherosclerosis **[34].**

Conclusion and recommendations

Elderly diabetic subjects with CV complications have significantly higher prevalence of dyslipidemia, PAD and PCA, defined by ABI and higher prevalence of abnormal (monophasic and biphasic) wave forms defined by arterial duplex study of the L.L, when compared to non-diabetic controls and to elderly diabetic without complications. Although the ε4 allele was scarcely represented in this elderly cohort, it was found that it is associated with higher coronary risk factors including higher mean SBP pressure and lower mean LDL-c, with a tendency to be associated with macrovascular complications and to be associated with CHD and PAD in the elderly diabetic patients with CV complications.

If clinicians possessed ApoE genotype information, they could provide more patient-specific preventive advice that would particularly help ApoE4 carriers reduce their risk of CVD. Therefore, further researches in the same issue are needed to be done on a larger scale on elderly diabetic subjects to improve the statistical power of the results and to study the interactions of ethnic and environmental factors with the genetic susceptibility.

Table (1): Comparison between the study groups as regards demographic, clinical, laboratory data and vascular findings

Variables		Group A n=30	Group B n=30	Controls n=30	X^2	P
Gender						
Male		9 (30%)	10(33.3%)	12(40%)	0.69	0.71^
Female		21 (70%)	20(66.7%)	18(60%)		
Age (mean+SD)		65.5±5	66±6.5	65.8+5	11	0.16^
Mean SBP+SD		137+22.6	135+19.96	124.7+17.4	-	0.041*
Mean DBP+SD		85±14.3	81±9.8	77±11.5	-	0.041*
Mean FBS (mg/dL)		142+38	152+67	90+22	11.69	0.000**
Mean 2HPP (mg/dL)		214+50	235+88	128+26	15.87	0.000**
Mean Cholesterol (mg/dL)		161+45	182+45	181+45	1.4	0.25^
Mean TG (mg/dL)		135+30	140+26	144+75	0.22	0.8^
Mean HDL (mg/dL)		33+10	33+11	44+12	5.08	0.008*
Mean LDL (mg/dL)		92+40	132+50	109+45	1.98	0.14^
Dyslipidemia		15 (60%)	8 (32%)	2 (8%)	14.02	0.001**
ABI						
PAD		8(26.7%)	3(10%)	1(3.3%)	24	0.000**
Referent		11(36.7%)	25(83.3%)	27(90%)		
(PCA)		11(36.7%)	2(6.7%)	2(6.7%)		
Duplex wave form	Biphasic	6 (85.7%)	1(14.3%)	0(0%)	16.68	0.002**
	Monophasic	3 (100%)	0(0%)	0(0%)		
	Triphasic	21 (26.3%)	29 (36.3%)	30 (37.5%)		
ApoE-ε4 allele		12(37.5%)	11(34.4%)	9(28.1%)	0.68	0.71^

Table (2): Characteristics of the study population as regards APOE genotyping

Variables	Group A n=30	Group B n=30	Controls n=30	X2	P
APO genotyping					
E2/E2	0	5(16.7%)	5(16.7%)		
E2/E3	0	11(36.7%)	9(30%)	35	0.000**
E3/E3	18(60%)	3(10%)	7(23.3%)		
E3/E4	12(40%)	7(23.3%)	5(16.7%)		
E4/E4	0	4(13.3%)	4(13.3%)		

**Highly significant

Table (3): Comparison between ApoE-ε4 carriers and non-carriers as regards clinical, laboratory parameters and vascular findings among the study population

Means		Apo-e (4) Absent	Present	P
Age (years)		66.0±6.6	66.3±6.2	0.88 ^
Systolic BP(mmHg)		128.3±18.4	139.4±22.7	0.014*
Diastolic BP(mmHg)		80.4±11.3	82.0±13.9	0.55 ^
Fasting(mg/dL)		131.1±56.9	124.3±61.8	0.60 ^
2HPP (mg/dL)		197.8±94.2	185.1±85.2	0. 53 ^
Cholesterol (mg/dL)		169.1±60.1	187.7±48.4	0.14 ^
TG (mg/dL)		145.7±79.9	130.4±57.4	0.23 ^
HDL (mg/dL)		36.9±13.9	42.3±12.8	0.76 ^
LDL (mg/dL)		96.8±40.5	118.7±45.5	0.022*
ABI risk category for CVD		16 (59.3%)	11 (40.7%)	0.50^
Duplex wave pattern	Bi	3(42.9%)	4(57.1%)	0.22^
	Mono	1(33.3%)	2(66.7%)	
	Tri	54(67.5%)	26(32.5%)	
CAD		14(53.9%)	12(46.1%)	0.079^
Stroke		7(70%)	3(30%)	0.429^

Table (4): Relation of abnormal ABI values, CAD and the ApoE-ε4 allele carrier state among the study population

ABI values			Phenotypes with e4 Absent	Present	Total	X2	P-value
PAD	CAD	no	5 (100%)	0 (0%)	5	6.122	0.013*
		yes	2 (28.6%)	5 (71.4%)	7		
		Total	7 (58.3%)	5 (41.7%)	12		
PCA	CAD	no	3 (60%)	2 (40%)	5	0.000	1.000^
		yes	6 (60%)	4 (40%)	10		
		Total	9 (60%)	6 (40%)	15		

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