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Association between APO E4 and Abdominal Aortic Atherosclerosis among Elderly Diabetic Patients

KEYWORDS: APO E4, abdominal aortic plaque, diabetic elderly

Dr. Moatasem Salah Amer
Professor of Geriatric Medicine and Internal Medicine, Faculty of medicine, Ain Shams University, Cairo, Egypt.

Dr. Randa Reda Mabrouk
Professor of Clinical Pathology, Faculty of medicine, Ain Shams University, Cairo, Egypt.

Dr. Hala Samir Sweed
Professor of Geriatric and Gerontology, Faculty of Medicine, Ain Shams University, Cairo, Egypt.

Dr. Nesrine Aly Mohamed
Lecturer of Clinical Pathology, Faculty of Medicine, Ain Shams University, Cairo, Egypt.

Dr. Sherine Mohamed Ibrahim Sharara
Lecturer of Radiology, Faculty of Medicine, Ain Shams University, Cairo, Egypt.

Hanaa Farag Bekhet Awad
Assistant lecturer of Geriatric Medicine and Gerontology, Faculty of medicine, Ain Shams University, Cairo, Egypt. (Corresponding author)

ABSTRACT

Objective: *This study assessed the association between APO E4 gene and abdominal aortic atherosclerosis among Elderly Diabetic Patients.*

Method: *A Case control study, Ninety elderly patients were recruited for the study. All participants were subjected to laboratory measurements of Lipid profile and Apo E genotyping tested by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and abdominal ultrasound to assess abdominal aortic diameter and plaques.*

Results: *the aortic plaques were significantly more frequent in the group of diabetics with cardiovascular complications compared to other groups (p= 0.026). And these plaques were significantly more frequent among E4 allele carriers in this group compared to E3 allele carriers in the same group (P=0.003). After pooling the overall subjects, there was significantly higher mean LDL among APO E4 carriers compared to E3 and E2 (P=0.02).*

Conclusion: *APO E4 allele is associated with significantly higher plasma LDL levels in elderly while aortic plaque were significantly more frequent among E4 allele carriers among diabetics with cardiovascular complications.*

Introduction

Diabetes mellitus is an important public health problem worldwide because of its high prevalence and complications **[1].** Diabetes is associated with high mortality and morbidity as a result of coronary artery disease (CAD) and other atherosclerotic diseases **[2].** Among the vascular changes associated with diabetes, abdominal aorta in diabetic patients might be affected **[3].** Aortic atherosclerosis has a long subclinical phase and can be assessed by measuring aortic plaque **[4].**

There is a relation between the presence of aortic plaque and the development of cardiovascular disease as the atherosclerotic process that results in coronary artery disease (CAD) is a generalized process that may involve the entire vasculature **[5].**

Multiple factors contribute to the accelerated atherosclerosis in diabetes. One of these factors is dyslipidemia along with modifications of lipoproteins **[6].**

APO E modulates lipoprotein transport and metabolism, and its polymorphism explains 4 to 15% of the variation in serum LDL-*C* **[7].**

An autopsy study done by **Ilveskoski et al., (1999)** found that apoE4 was associated with atherosclerotic lesions in the abdominal aorta **[8].**

The aim of this work was to assess the association between APO E4 gene and abdominal aortic atherosclerosis among Elderly Diabetic Patients As a part of our ongoing study to evaluate the effect of APO E4 on abdominal aortic atherosclerosis and CIMT in diabetic elderly Egyptian patients, the data represented in the current research article concerned with the association between APO E4 and abdominal aortic atherosclerosis, while the other part which investigate the relation between APO E polymorphism and CIMT in diabetic elderly patients will be discussed in another research article.

Methodology

A case control study was conducted. The subjects of this study were recruited from Ain Shams University Hospitals in Egypt and were subdivided into:

Group A: thirty elderly diabetic subjects with cardiovascular complications. **Group B:** thirty elderly diabetic subjects without cardiovascular complications. **Group C:** thirty elderly subjects without DM as the control group matched for age and sex.

The cardiovascular complication in this study included ischemic heart disease and stroke.

All groups were subjected to:

Comprehensive geriatric assessment.

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Laboratory investigations were done including: Venous blood (6 ml) was withdrawn aseptically into a sterile disposable syringe from each patient and control where, 5 ml were placed in EDTA tube for APOE genotyping and 2 ml were collected plain vacutainers to be clotted and centrifuged for measurement of total cholesterol, HDL-C, LDL-C ,TG and fasting glucose. On Synchron CX-9 autoanalyzer (Beckman Instruments Inc.; Scientific Instruments Division, Fullerton, CA 92634, 3100, USA). The laboratory work was conducted at Clinical Pathology Department, Ain shams university .

Genotyping of Apo E was performed using polymerase chain reaction and restriction fragment length polymorphism. Genotyping of three common alleles (E2, E3, and E4) of the Apo E gene was performed. Genomic DNA was extracted from human blood cells using QIAamp DNA Mini Kit (Qiagen, USA) the extracted DNA was stored at -20°C until processed. The primers used were 5'-ACAGAATTCGCCCCGGCCTGGTACAC-3' and 5'-TAAGCTTGGCACGGCTGTCCAAGGA-3' as described by Emi et al. (1988) (9) and prepared by Promega (Madison,USA). PCR product were carried out in volume of 50 µl containing 5 µl genomic DNA, 25 µl of the ready to use master mix supplied by Qiagen, 2.5µl (25 pmol) of forward primer, 2.5 µl (25 pmol) of reverse primer and 15 µl of deionized water. The PCR conditions were an initial denaturation at 95°C for 3 minutes and 40 cycles consisting of denaturation at 95°C for 15 seconds, annealing at 62°C for 15 seconds, extension at 72°C for 45 seconds before a final extension at 72°C for 4 minutes. Restriction digestion of the amplified product was performed using 0.5 µl (10 units/ µl)Hha1 restriction enzyme supplied by promega. Site of restriction: (5´GCG¯C 3´) (3´ C¯GCG 5´). The enzyme was added to 5 µl of PCR amplified product, 12.3 µl deionized water, 2 µl of buffer (10 x of 60mM tris-Hcl) and 0.2of µl acetylated bovine serum albumin (BSA)(10ug/ µl), followed by gentle mix. Then the tubes were placed on a heat block for 3 hours at 37°C. The enzyme was inactivated at 65°C for 15 minutes. As experimental control: no-enzyme "mock" digest was used

Amplified product of DNA samples and restriction fragments were run on 8% polyacrylamide gel and stained with ethidium bromide. DNA molecular weight marker was also run to identify the site of bands (20 bp DNA ladder supplied by(Promega, USA). Digestion of the amplified product (270 bp) by the restriction enzyme Hha1 resulted in the following different individual genotypes that were categorized based on the following band length criteria:E2/2=91 ,83,38;E3/3:91,48,38,35;E4/4:72,48,38,35;E2/3:91,83,48,38, 35;E2/4:91,83,72,48,38,35;E3/4:91,72,48,38,35 (figure 1)

Abdominal ultrasound to assess aortic plaques and aortic diameter at the level of iliac bifurcation.

Results

The sample of this study included 90 participants; 30 diabetics with cardiovascular complications (group A) and 30 diabetics without cardiovascular complications (group B) together with 30 non diabetics matched for age and sex as their controls.

The mean age of group A was 65.4±6.6 and 65.3±6.6 for group B compared to 67.2±6.4 for control group.

Aortic plaques were significantly highest in Group A (p= 0.026) compared to Group B and C. While there was no statistically significant difference between the three studied groups as regard abdominal aortic diameter (mean= 17.9, 16.1, 17.2 in group A, B and C respectively).

As regard APO E4 allele frequency there was no statistically significant difference between the three studied groups (40%, 36.7% and 30% respectively in group A, B and C).

There was no statistically significant difference between APO E alleles as regard mean abdominal Aortic diameter in the three studied groups while aortic plaque were significantly higher among E4 allele carriers in the group (A) compared to E3 allele carriers in the same group (P=0.003).

After pooling the overall subjects, the results showed statistically significant higher mean LDL among APO E4 carriers compared to E3 and E2 (P=0.02).while total cholesterol, HDL and triglycerides had no significant association with different APO E alleles .

Discussion

The coronary and cerebrovascular atherosclerotic disease has been closely linked to adverse cardiovascular outcomes, but less is known about asymptomatic aortic atherosclerosis. Aortic atherosclerosis has a long subclinical phase and can be assessed by measuring aortic plaque **[4].**

The current study showed that there was no statistically significant difference between the 3 groups as regard mean abdominal Aortic diameter (mean= 17.9, 16.1, 17.2 in group A, B and C respectively).

Our result agreed with **Astrand et al., (2007)** who examined 39 patients with diabetes mellitus and 46 age and sex matched healthy subjects with B-mode ultrasound to determine the diameter of the abdominal aorta and found no significantly difference in the diabetic patients compared to controls **[3].**

Asbun et al., (2005) reported that down-regulation of metalloproteinase activity in arteries of diabetic patients, and high glucose levels accelerating synthesis of collagen might be the mechanism behind increased wall thickness, reduced aortic wall stress, decreased aneurysm prevalence and reduced frequency of aortic dilatation **[10].**

As regard aortic plaques, they were significantly higher in the group of diabetics with cardiovascular complications compared to other two groups (p= 0.026).

This result agreed with **Khoury et al., (1997)** who conducted a prospective study to correlate the presence of angiographically significant coronary artery disease (CAD) and atherosclerotic disease in the aorta, reported that aortic plaques were more strongly associated with CAD than carotid plaques **[11].**

Also **Momiyama et al., (2008)** examined 146 patients, 108 had CAD (>50% luminar diameter stenosis) on angiograms. The prevalence of plaques in both thoracic and abdominal aorta was high in patients with CAD than without CAD (73% and 94% vs. 32% and 79%, P < 0.025) **[12].**

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There was no statistically significant difference between APO E alleles as regard mean abdominal Aortic diameter in the three studied groups while aortic plaque were significantly higher among those having E4 alleles in the group (A) diabetics with cardiovascular complications compared to E3 allele carriers in the same group (P=0.003).

To our knowledge, no previous studies exist on the association between abdominal Aortic diameter and plaque with different APO E genotypes apart from an autopsy study done by **Ilveskoski et al., (1999)** who enrolled 700 male autopsy cases (average age 53 years, range 33 to 70 years) and found that apoE4 was associated with atherosclerotic lesions in the abdominal aorta (P=0.014) **[8].**

When studying the relation between the different APOE alleles and lipid profile after pooling the overall subjects, the results showed statistically significant higher mean LDL among APO E4 carriers compared to E3 and E2 (P=0.02).

Our results agreed with **Jemaa et al. (2006), Morbois-Trabut et al. (2006) and Boemi et al. (2003)** studies that reportd that the E4 allele was usually associated with a high level of plasma LDL cholesterol compared to E3 **[13,14,15].**

Conclusions and Recommendations

We can concluded that atherosclerosis is multifactorial both genetic and environmental factors and their interaction all together play a role in the development and progression of atherosclerosis. Further studies are needed to determine if APO E4 can be considered an independent risk for aortic atherosclerosis after controlling environmental risk factors like HTN and smoking.

Noninvasive screening tests for subclinical aortic atherosclerosis may offer new insights into atherogenesis within different vascular beds and may help refine traditional cardiovascular risk stratification guidelines. As Aortic atherosclerosis has a long subclinical phase and can be assessed by measuring aortic plaque by abdominal ultrasonography.

Table (1): Characteristics of the studied groups

Variable	Group A (N=30)	Group B (N=30)	Control (N=30)	$P_{A/B}$	$P_{A/C}$	$P_{B/C}$
Age Mean±SD Range	65.4±6.6 60.0–85.0	65.3±6.6 60.0–86.0	67.2±6.4 60.0–82.0	0.969^	0.285^	0.268^
Sex (n, %) Female Male	 21 (70.0%) 9 (30.0%)	 21 (70.0%) 9 (30.0%)	 17 (56.7%) 13 (43.3%)	1.000#	0.284#	0.284#
Aortic diam. Mean±SD Range	 17.9±4.9 9.0–28.0	 16.1±2.8 10.0–24.0	 17.2±2.9 11.0–24.0	0.103	0.517	0.161
Aortic plaques	8 (26.7%)	1 (3.3%)	1 (3.3%)	0.026*	0.026*	1.000
APOE4 allele	12(40.0%)	11 (36.7%)	9 (30.0%)	0.791	0.417	0.584

^Independent t-test, #Chi square test, &Fisher's Exact test

Table (2): Comparison between group A alleles regarding abdominal aorta (diameter and plaques).

Variable	E3(e33) (N=18)	E4(e34, e44) (N=12)	P
Aortic diameter	17.3±5.1	18.8±4.8	^0.429
Aortic plaques	1 (5.6%)	7 (58.3%)	&0.003*

Table (3): Comparison between APOE alleles regarding lipid profile.

Variable	E2(e22,e2) (N=30)	E3(e33) (N=28)	E4(e34,e44) (N=32)	F	P
Cholesterol	172.6±60.2	178.2±67.0	**191.7**±50.0	0.8	0.4
TG	158.4±78.0	145.9±85.3	130.3±57.4	1.1	0.3
LDL	97.4±45.1	93.8±34.3	**120.8±**44.7	3.7	0.02*
HDL	40.3±14.1	35.4±13.8	42.1±13.3	1.8	0.1

*P<0.05 significant

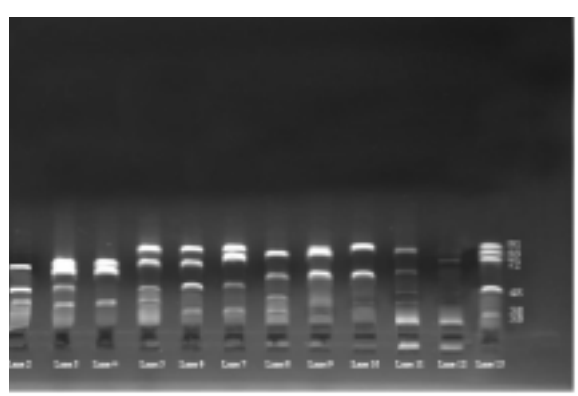

Figure (1): APO E genotypes by polyacrylamide gel electrophoresis: lane 1 shows ladder 20 bp , lane 5,9,10 shows 2/2 genotype , lane 6,7,11 shows 3/4 genotype ,lane 2,4,3,12 shows 4/4 genotype and finally lane 8,13 shows 3/2 genotype

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