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ORIGINAL ARTICLE

Relationship of endothelial nitric oxide synthase gene polymorphism with atherosclerotic coronary and carotid arterial disease in Egyptian population

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KEYWORDS

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Abstract *Introduction:* Atherosclerosis is partly a heritable disorder. Various genetic polymorphisms have been linked to the atherosclerotic process and its complications. Glu298Asp polymorphism of endothelial nitric oxide synthase gene is one such genetic marker for atherosclerosis.
Aim of the work: To study the relationship between endothelial nitric oxide synthase gene polymorphism and atherosclerotic coronary and carotid arterial disease in Egyptian population.
Patients and methods: Our study included 95 Egyptian patients with Egyptian father and mother, classified into two groups: Group 1; 63 patients with ischemic heart disease and Group 2; 32 control subjects and subjected to careful history taking, thorough clinical examination, standard twelve-lead surface electrocardiogram, routine laboratory investigations, echo Doppler study, carotid arterial duplex, invasive coronary angiography and analysis of the endothelial NOS3 gene polymorphism using PCR–RFLP for detection of different genotype variants (Glu/Glu (GG), Glu/Asp (GT) and Asp/Asp (TT) genotype).
Conclusion: Glu298Asp polymorphism in the endothelial nitric oxide synthase gene did not increase the susceptibility to coronary and carotid arteries disease in the studied patients.

1. Introduction

Atherosclerosis, a chronic inflammatory disease, underlies the pathogenesis of coronary artery disease (CAD). The molecular etiology of coronary atherosclerosis involves interaction of many genes and environmental factors.[22] Nitric oxide (NO)

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produced by the enzyme endothelial nitric oxide synthase (eNOS), has critical roles in the regulation of vascular homeostasis and prevention of atherogenesis by inhibiting leukocyte, platelet activation and smooth muscle cell proliferation.[17] Polymorphisms of eNOS gene were shown to be associated with CAD in some populations.[8] The guanine to thymine (Glu298Asp) polymorphism at position 894 of the eNOS gene (replacement of glutamate (G) by aspartate (T)) is under extensive study and T allele had been described as susceptibility allele for CAD in some studies.[28] There have been conflicting reports on the relationship between this polymorphism and CAD from studies done in various ethnic groups across the world.[11,8] Therefore, we undertook the current study to investigate whether the eNOS Glu298Asp polymorphism is related to coronary and carotid arterial disease.

2. Aim of the work

To study the relationship between endothelial nitric oxide synthase gene polymorphism and atherosclerotic coronary and carotid arterial disease in Egyptian population.

3. Patients and methods

Our study included 63 patients consecutively admitted to our Institute (Fayoum University Hospital) and had angiographically defined CAD (> 50% stenosis affecting at least one coronary vessel) and 32 individuals with normal coronary angiography. Inclusion criteria: All patients and controls were from the Egyptian population with Egyptian father and mother. Exclusion criteria: Acute renal failure, history of severe contrast reaction, uncontrolled infection, active bleeding, severe hypertension, coagulopathy (INR > 1.8) and patients of non Egyptian population or with non Egyptian father or mother.

3.1. Each patient included in the study was subjected to

1. Careful history taking and thorough physical examination.
2. Standard twelve-lead electrocardiogram (12-lead ECG): For the assessment of cardiac rhythm, features suggesting myocardial ischemia (ST segment and T wave abnormalities and pathological Q waves), and voltage criteria of left ventricular hypertrophy.
3. Routine laboratory testing: Complete blood picture, fasting plasma glucose, blood urea, serum creatinine, SGOT, SGPT, PT, INR and complete lipid profile.
4. Echocardiography study: Both patients and control subject were studied by trans-thoracic echocardiography using a commercially available echocardiographic machines (Siemens), equipped with 3.5 MHz phased array transducer. M-mode echocardiography was used to assess chamber dimensions and calculation of the ejection fraction. Two dimensional echocardiography for the assessment of the overall ventricular systolic function by eyeballing and biplane Simpson's rule, assessment of the LV end-diastolic and systolic dimensions of regional wall motion abnormalities, valvular thickening, calcification, pericardial effusion or thickening and to rule out intra-cardiac masses or thrombi. Trans-mitral pulsed wave Doppler to measure peak E velocity, peak A velocity and the E/A ratio.
5. Carotid arterial duplex: High resolution B mode, color Doppler and pulse Doppler ultrasonography of both carotid arteries were performed with an ultrasound machine: General Electric (GE) (Logic 7) equipped with 4–11 MHz linear array transducer and centralized reading was performed by one trained reader blinded to subject's characteristics and according to a standardized protocol. Patients were examined in the supine position with the head tilted backwards. After the carotid arteries were located by transverse scans the probe was rotated 90° to obtain and record a longitudinal image of the anterior and posterior walls. The maximum cIMT measured at the near and far walls of the right and left CCAs was scanned at least 1.5 cm proximal to the origin of the bulb (defined as the point of divergence of the walls of the CCAs). For each side, at least one optimal longitudinal image was frozen in end-diastole by electrocardiogram R-triggering. The IMT was measured only at a plaque-free site, along a 10 mm segment of the far wall of the CCA and measured as the distance between the lumen–intimal interface and the media–adventitia interface using an automated edge detection algorithm. The mean cutoff cIMT value was 0.89 mm. Image showing the maximum intimal-media thickness was stored and the average values were considered. For visualized plaques, the maximum diameter was determined and included in further analysis. The presence of plaques was defined as localized echo structures encroaching into the vessel lumen for which the wall thickening was at least 50% greater than that of the surrounding vessel wall. Furthermore, the grade of stenosis in the carotid and vertebral arteries was assessed through the increase in the peak systolic and end-diastolic velocities.[24]
6. Coronary Angiography was done to all patients via a retrograde transfemoral approach, using Seldinger's technique. The angiograms were done in all standard views using right and left coronary catheters with the assessment of the number of diseased vessels, sites of lesions and estimation of the severity of stenosis.
7. Analysis of the endothelial NOS3 gene polymorphism: Three milliliters (3 ml) venous blood samples were withdrawn from subjects under aseptic conditions into sterile EDTA vacutainer tubes for DNA extraction. The samples were stored at −200°C. The test was done in five main steps:
   - Extraction of genomic DNA from peripheral blood leucocytes of EDTA anti-coagulated blood.
   - Amplification of the extracted DNA.
   - Detection of PCR amplification products using agarose gel electrophoresis.
   - The amplified products were digested with (MboI) restriction enzyme.
   - The digested products were then analyzed by electrophoresis on agarose gel containing ethidium bromide and visualized by ultraviolet light transillumination for determination of Glu298 → Asp endothelial nitric oxide synthase genotypes.

4. Statistical analysis

Collected data were computerized and analyzed using the Statistical Package for Social Science (SPSS) version 16.