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# Low Ankle Brachial Index in Acute Ischemic Stroke: Does ApoE Gene Polymorphism Have a Role?

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## Abstract

**Background: The existence of asymptomatic peripheral arterial disease among patients with acute ischemic stroke has been studied and proved. Low ankle brachial index (ABI) is considered as a marker of atherosclerosis, and its relation to stroke severity was documented in some studies. The effect of different alleles of ApoE gene on acute ischemic stroke presentation in patients with low ABI is not known. Objective: To study the effect of ApoE gene polymorphism on stroke severity, outcome and recurrence in patients with asymptomatic peripheral arterial disease identified by low ABI. Methods: Patients with acute ischemic stroke were screened for the presence of asymptomatic peripheral arterial disease by estimating the ABI using a pocket Doppler ultrasound device. Assay of ApoE gene was done using the real-time PCR technique. Results: Low ABI was present in 31% of patients with acute ischemic stroke. There was no significant difference among patients with different ApoE alleles regarding the severity of their symptoms. Also, there was no significant difference among patients with normal ABI and those with abnormal ABI regarding the ApoE gene polymorphism. Conclusion: The current study showed that there was no significant relation between ApoE gene polymorphism and low ABI in ischemic stroke patients who had asymptomatic peripheral arterial disease.**

## Keywords

**Peripheral Arterial Disease, ABI, Acute Ischemic Stroke, ApoE Gene Polymorphism**

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## 1. Background

Peripheral arterial disease (PAD) proved to be a marker for atherosclerosis in different vascular beds of the body [1]. Ankle brachial index (ABI) is a non-invasive and effective screening tool for diagnosis of PAD [2]. The prevalence of asymptomatic PAD among patients with atherothrombotic cerebral infarction and TIA was found to be high (about 40%) [3]. Ankle brachial index (ABI) was shown to be lower in patients with ischemic stroke and was considered as a predictor for stroke recurrence [4].

There were conflicting results about ApoE gene polymorphism and its role in ischemic stroke [5] [6]. Possible link between ApoE4 allele and presence of PAD has been shown earlier in diabetic non-smokers [7], though this association required further confirmation, as a more recent study failed to prove this later association [8].

ApoE polymorphism as a risk factor for both ischemic stroke and PAD has not been studied adequately. The patient harboring the triad of vascular pathology in brain, peripheral vascular system and a specific ApoE allele will be able to give a clue for the true effect of this genetic variability on body vasculature and hence ischemic strokes.

The aim of our study is to find an association between low ABI (indicator of peripheral arterial disease) and ApoE polymorphism in ischemic strokes, and their role, severity, outcome and recurrence.

## 2. Methodology

We studied prospectively one hundred consecutive patients with first ever acute ischemic stroke referred to the Neurovascular ultrasound diagnostic laboratory, Cairo University hospital from January 1$^{st}$ 2012 to September 1$^{st}$ 2012. Ischemic stroke patients with different vascular risk factors and severity were recruited for the study.

**Initial evaluation:** Patients of the study group were initially evaluated thorough clinical history taking, national institution of health stroke scale (NIHSS) [9], screening for presence of PAD (clinically and by calculating ABI), blood samples were withdrawn for ApoE gene assay, only 72 patients accepted genetic screening.

**Follow up:** Seventy five (75%) of the study group were successfully evaluated after one year from initial assessment. The remaining twenty five (25%) dropped out because of changing contact information. The follow up parameters included the following:

(a) Modified Rankin scale (MRS) [10], (b) occurrence of new cerebrovascular events (CVS) in the form of TIAs, new stroke or vascular death.

**Ankle-brachial index (ABI) measurements:** Screening for the presence of asymptomatic peripheral arterial disease (PAD) was performed during the hospital stay and ankle brachial index (ABI) was calculated according to the internationally published data [11] by measuring blood pressure from both the right and left brachial arteries and right and left posterior and/or anterior tibial arteries, while the patient was supine. Systolic pressure was detected with a handheld 5-MHz Doppler probe (Nicolet Elitevascular Doppler model no. 100R manufactured by Nicolet™ USA). The lower ABI values were used to classify the participant into an ABI category. We defined three ABI categories: ABI ≤ 0.90 (low ABI) established a diagnosis of PAD; ABI ≥ 0.90 and ≤1.40 was considered normal; ABI ≥ 1.40 was considered high [11].

**ApoE Gene polymorphism:** Genomic DNA was extracted from whole blood using High Pure PCR Template Preparation kits (Roche Diagnostics, GmbH, Germany). Light Cycler-ApoE Mutation Detection Kit (codon 112 and 158; Roche Diagnostics, GmbH Mannheim, Germany) was used to determine ApoE polymorphism.

**Statistical methods:** Data were described as mean ± standard deviation (SD), range, frequencies (number of cases) and relative frequencies (percentages). Categorical variables were expressed as percentages and numerical data were expressed as median and range. Measurements of the strength of the association between categorical variables, the odd ratio and with a 95% confidence interval (CI) were calculated. Comparative statistics were performed with Student's test, Mann-Whitney U or ×2 test as appropriate.

A probability value (p value) less than 0.05 was considered statistically significant. All statistical calculations were performed using Microsoft Excel version 7 and SPSS version 17 for MS windows (Statistical Package for the Social Science, SPSS Inc., Chicago, Ill., USA).

## 3. Results

We initially recruited 100 patients (56 males and 44 females) with documented acute ischemic stroke with mean

duration from onset was 8 ± 3.8 day. Out of 100 patients, only 75 patients (75%) could be followed for one year and 25% dropped during follow up period. The initial NIHS scale, frequency of ApoE and follow up MRS for our cohort summarized in **Table 1**.

Comparison of patients with normal and abnormal ABI showed a statistically significant increase in NIHSS in abnormal ABI group (P = 0.026). Patients with an abnormal ABI had a higher risk of CVS recurrence compared to those with normal ABI [OR = 8.7 with 95% CI (2.05 - 37.04)]. Comparison of patients with and without abnormal ABI showed no statistically significant difference regarding Modified Rankin Scale (MRS) (P = 0.126). There were no statistically significant difference between patients with normal ABI and those with abnormal ABI regarding the ApoE gene polymorphism, **Table 2**.

There was no significant difference between patients with different ApoE alleles and the stroke severity, outcome or recurrence (P = 0.61, 0.28 and 0.404 respectively), **Table 3** & **Table 4**.

**Table 1.** Baseline characteristics and one year follow up results of the study group.

| Initial evaluation | | | |
|---|---|---|---|
| No. of subjects | 100 | 56 M | 44 F |
| Age | Mean 61.1 ± 8.2y | Median 60 | Range (46 - 85) |
| Onset | Mean 3.8 ± d | 7 | (2 - 20) |
| NIHSS* | | 6 | (2 - 13) |
| ABI* | Normal (69%) 36% M, 33% F | Low (31%) 20% M, 11% F | |
| ApoE alleles frequencies | | | |
| E3E3 | n = 33 (45.8%) | | |
| E4E4 | n = 15 (20.8%) | | |
| E3E4 E2E2 E2E4 E2E3 | n = 12 (6.6%) n = 5 (7%) n = 5 (7%) n = 2 (2.7%) | | |
| Follow up | | | |
| No. of subjects MRS* | 75 | Median 2 | Range (2 - 6) |

*NIHSS: national institution of health stroke scale, ABI: ankle brachial index, MRS: modified Rankin scale.

**Table 2.** Relation between different ApoE gene alleles and ankle brachial index.

| Alleles | ABI | | Total | P value |
|---|---|---|---|---|
| | Normal | Abnormal | | |
| E2E2, E2E3, E2E4 | 9 (75%) | 3 (25%) | 12 (100%) | |
| E3E3 | 22 (66.7%) | 11 (33.3%) | 33 (100%) | 0.769 |
| E3E4, E4E4 | 17 (63%) | 10 (37%) | 27 (100%) | |

**Table 3.** Relation between different ApoE gene alleles stroke severity & outcome.

| Scale | E2E2, E2E3, E2E4 | E3E3 | E3E4, E4E4 | P value |
|---|---|---|---|---|
| | Median (range) | Median (range) | Median (range) | |
| NIHSS* | 5 (2 - 13) | 7 (3 - 13) | 6 (2 - 13) | 0.61 |
| Modified Ranking Scale | 3.5 (1 - 6) | 3 (1 - 6) | 2 (1 - 6) | 0.27 |

*NIHSS: national institution of health stroke scale.

**Table 4.** Relation between different ApoE gene alleles and stroke recurrence.

| Alleles | Recurrence | | Total | P value |
| --- | --- | --- | --- | --- |
| | Yes | No | | |
| E2E2, E2E3, E2E4 | 2(20%) | 8 (80%) | 10 (100%) | |
| E3E3 | 2 (7.4%) | 25 (92.6%) | 27 (100%) | 0.404 |
| E3E4, E4E4 | 4 (23.5%) | 13 (76.5%) | 17 (100%) | |

## 4. Discussion and Conclusions

Thirty-one percent of our cohort showed low ABI at initial baseline assessment. This highlighted the role of ABI as a predictor for stroke occurrence and was in concordance with some European studies that reported similar proportions among noncardioembolic ischemic stroke patients which reflected the presence of asymptomatic peripheral arterial diseases (PAD) [4] [12]-[16]. This finding was not proved in Asian studies particularly in Japan and Thailand who reported low prevalence of asymptomatic PAD in acute ischemic stroke patients [17]-[19]. We attributed this variability to ethnic and racial factors as well as different methodological aspects including sample size between different cohorts.

In our study, abnormal ABI was significantly related to higher NIHSS at stroke onset (P = 0.026) and a risk factor for stroke recurrence after one year of the first event [OR = 8.7 with 95% CI (2.05 - 37.04)] (P value = 0.001). In some reports, ABI has been shown to be an independent risk factor for stroke recurrence [3] [4] [14] [20] [21], yet others failed to prove this relation [22] which could be explained by different methods to measure and estimate ABI.

Analysis of ApoE polymorphism among our patients showed that the prevalent allele was E3E3 representing about 45.8% of patients, coming next to it is the E4E4 representing 20.8%, E3E4 representing 16.6%, E2E2 & E2E4 representing 7% each and E2E3 representing 2.7%. This distribution comes very different from other studies analyzing different populations like European or Asian descent [23] [24]. This finding confirms that Egyptian population carries a different genotype variability that may affect differently disease presentation and outcome.

No relation was found between ApoE polymorphism and stroke severity, outcome and recurrence (P = 0.61, P = 0.27 and P = 4.04 respectively). These results in concordance with previous studies showed no relation of ApoE polymorphism to ischemic stroke event [6] [25]-[28]. This may emphasize that the relation between ApoE gene and its alleles, stroke severity, outcome or recurrence is not clear.

To the best of our knowledge, the combined effect of ApoE gene polymorphism and abnormal ABI on ischemic stroke patients has not been studied adequately. Results from this study showed that there was no significant relation between ApoE gene polymorphism and abnormal ABI in stroke patients. That is to say Egyptian stroke patients with asymptomatic peripheral vascular disease didn't show specific ApoE allele prevalence that could be linked to occurrence, recurrence or outcome of ischemic stroke. However, our study results should be taken with caution due to several limitations. Firstly, it was a small case series and the statistical power to detect the associations was limited. Secondly due to the relatively short follow-up period, further studies are recommended to clarify the relation between presence of asymptomatic peripheral arterial disease and ApoE gene polymorphism in patients with ischemic stroke.

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