

A STUDY OF ANGIOTENSIN CONVERTING ENZYME GENE POLYMORPHISM IN THE PATIENTS WITH DIABETIC NEUROPATHY

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Keywords: ACE gene polymorphism, diabetic neuropathy, nerve dysfunction, microvascular injury

Abstract: Neuropathies are a common complication of diabetes mellitus. There are a few theories to explain diabetic neuropathy: the polyol pathway theory, the oxidative stress, protein kinase C activation, and the glycosylation end-product theory; all contributes to microvascular injury involving small blood vessels that supply nerves and nerve dysfunction. It has become obvious that several pathophysiological factors probably operate simultaneously and it may be too simplistic to try to explain the many clinical presentations and pathological findings of diabetic neuropathy by a single theory. A lot of studies suggest that diabetic neuropathy may involve genetic susceptibility. The aim of this study was to investigate the influence of angiotensin-converting enzyme (ACE) gene I (insertion)/D (deletion) polymorphism in the development of diabetic neuropathy. The study consists of 23 patients with diabetic neuropathy and 20 without diabetic neuropathy. ACE polymorphism was detected by the restriction fragment length polymorphism method after a polymerase chain reaction. A significantly higher frequency of ACE I allele was observed in the diabetic neuropathy patients compared with the healthy control group where there was a higher frequency of ACE D allele. The distribution of ACE genotypes and alleles frequency showed differences between the patients with diabetic neuropathy and without this complication ($p < 0.05$). Our results indicate that ACE D allele protects against diabetic neuropathy.

Cuvinte cheie: polimorfismul genei ACE, neuropatie diabetică, disfuncție neuronală, leziune microvasculară

Rezumat: Neuropatiile sunt o complicație obișnuită a diabetului zaharat. Există o serie de teorii care explică apariția neuropatiei diabetice: calea polioli, stresul oxidativ, activarea proteinei C și teorii glicozilării proteinelor; toate acestea determină leziuni microvasculare care afectează vasele mici de sânge care irigă nervii periferici și determină în final disfuncția acestora. Este evident că există o serie de factori care operează, probabil, simultan și ar părea prea simplist să încercăm să explicăm multiplele variante clinice de prezentare și modificările fiziopatologice din neuropatia diabetică prin intermediul unei simple teorii. O serie de studii sugerează că neuropatia diabetică poate să apară și ca urmare a unei susceptibilități genetice. Scopul acestui studiu este investigarea influenței polimorfismului I (inserție)/D (deleție) al genei enzimei de conversie a angiotensinei în dezvoltarea neuropatiei diabetice. Studiul a inclus 86 de pacienți cu neuropatie diabetică și 90 de pacienți fără neuropatie diabetică. Pentru analiza polimorfismului ACE I/D s-a folosit reacția polimerizării în lanț și polimorfismul fragmentelor de restricție. S-a observat o frecvență semnificativ mai mare a alelei D a ACE la pacienții cu neuropatie diabetică comparativ cu grupul de control format din pacienți sănătoși unde s-a observat o frecvență mai mare a alelei I. Distribuția genotipului ACE și frecvența alelelor a arătat diferențe între grupul pacienților cu neuropatie diabetică și grupul de control ($p < 0.05$). Rezultatele noastre indică faptul că alela I protejează împotriva neuropatiei diabetice.

INTRODUCTION

People with diabetes have an increased risk of developing microvascular complications, diabetic retinopathy, diabetic nephropathy and diabetic neuropathy, which, if late diagnosed and improperly treated, can have a negative effect on the quality of life and place additional costs on public health systems. In plus, diabetic microvascular complications are important factors that reduce life expectancy.(1)

Neuropathy is an important complication of diabetes mellitus type 1 and 2 and is observed in some studies in up to 30% - 50% of the patients.(1,2) The most common neuropathy in the developed countries is diabetic neuropathy and it is associated with a variety of sensory and motor symptoms and autonomic manifestations. Diabetes mellitus can affect both central, peripheral and autonomic nervous system but the most

common form of manifestation is the symmetric sensory-motor peripheral polyneuropathy of the lower limbs representing over 90% of neuropathies in the patients with diabetes. Diabetic polyneuropathy is a diagnosis of exclusion. Patients complain of pain, paresthesias, trophic changes of the lower limbs and autonomic disturbances.(3) These complications arise as a result of microvascular injury that affects vasa nervorum providing nerves nutrients and oxygen. Microvascular damage appears early in diabetes and evolves in parallel with neural dysfunction that is demonstrated by nerve conduction studies. In diabetes, the peripheral nervous system pathology and the capillaries pathology are closely interrelated through their functional interdependencies. Normal functionality of blood vessels depends on nervous regulation and the neurons and peripheral nervous system viability depends on nutrients and oxygen intake

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CLINICAL ASPECTS

through capillaries. Most authors refer to diabetic neuropathy as a neurovascular disease or as an impairment of the microvascular system due to diabetes.(4)

The first pathological change that appears in the microvasculature is vasoconstriction. Although it is clear that diabetes complications result from chronic hyperglycemia (influenced by factors such as age, age of onset and disease duration) (5), the actual development of these complications in any individual depends on the genetic susceptibility to damage in that particular individual. One study demonstrated the familial factors influence on the development of microvascular complications.(6) A lot of studies suggest that diabetic neuropathy may involve genetic factors and the renin-angiotensin system (RAS) has been proposed as an important genetic factor for diabetic complications.

Angiotensin Converting Enzyme (ACE) is a key component of the Renin-Angiotensin System (RAS): it converts Angiotensin I to Angiotensin II (a vasopressor) and degrades bradykinin (a vasodilator) and other active oligopeptides. Recent studies have shown that ACE has a role in diverse cellular processes, including cell growth and survival of nonvascular tissues.(7)

PURPOSE

The purpose of this study was to investigate the frequency of angiotensin-converting enzyme (ACE) gene I (insertion)/D (deletion) polymorphism in diabetic neuropathy. Another objective of this work was to investigate the relation between ACE gene polymorphism and diabetic neuropathy in the population of our area.

METHODS

Patients: We performed a case-control study on 86 patients with diabetic neuropathy and 90 healthy controls. Informed consent was obtained from all patients. The patients were recruited between January and July 2011 at the Medical Centre Mediab from Tîrgu Mureş.

Inclusion criteria: Patients with diabetes mellitus type 1 and 2 who presented for neurological consult and were diagnosed with sensorimotor neuropathy by clinical examination and by nerve conduction studies.

Exclusion criteria: Patients diagnosed with other possible causes of neuropathy (thyroid malfunction, vitamin B12 deficiency, viral hepatitis etc).

ACE Gene Insertion/Deletion Polymorphism

Genotyping: Genomic DNA was extracted from whole blood using ZymoBead Genomic DNA Kit (ZymoResearch). Analyses of ACE I/D polymorphism were performed using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). PCR was carried out according to the method of Rigat B et al. 1992.(8) The sequences of the forward and reverse primers were: 5'-CTGGAGACCACTCCCATCCTTCT-3' and 5'-GATGTGGCCATCACATTCGTGAT-3', respectively. The PCR products were visualized by electrophoresis in a 2% agarose gel with ethidium bromide and documented with a gel documentation system.

Statistical analysis: We used the analysis of a contingency table with Fisher's exact test to calculate p-value. A p value less than 0.05 is considered significant. Allele frequencies were estimated by the gene counting method.

RESULTS

The study group included 86 patients with diabetes mellitus, the male/female ratio was 1.3:1 and the mean age was 59.6 years old. ACE genotypes (figure no. 1) in the two groups compared was 32.55% DD homozygotes, 51.16% ID

homozygotes and 16.27% II homozygotes in the diabetes mellitus group and 25.55% DD homozygotes, 36.66% ID homozygotes and 37.77% II homozygotes in the control group.

Figure no. 1. Insertion (I)/deletion (D) polymorphism of ACE gene. M: marker, lanes 1, 3, 5, 6 heterozygous ID and lane 2, 4, 7: homozygous DD case.

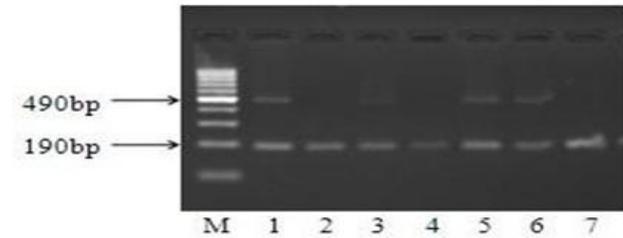


Table no. 1. Allele and genotype frequency of ACE insertion/deletion polymorphism in the patients with diabetic neuropathy and in the control subjects

Variable	Patients with diabetic neuropathy n=86 (%)	Normal control n=90 (%)
Genotypes		
II	14 (16.27)	34 (37.77)
ID	44 (51.16)	33 (36.66)
DD	28 (32.55)	23 (22.55)
ID + DD	72 (83.71)	56 (59.21)
Alleles		
I	72 (41.86)	101 (56.11)
D	100 (58.14)	79 (43.89)

Table no. 1 shows the ACE genotype distributions and allele frequency in the control patients and in those with diabetic neuropathy. A significantly higher frequency of ACE D allele was observed in the diabetic neuropathy patients compared with the healthy control group, where there was a higher frequency of ACE I allele. P value was calculated with the Fisher test = 0.0079, being considered statistically very significant, OR = 0.5632; 95% CI = 0.3691-0.8593. The genotype ID was more frequent in the patients with diabetic neuropathy compared with the healthy control group; p = 0.0031 being considered statistically very significant, OR = 0.3088; 95% CI = 0.1431-0.663. The genotype DD was more frequent in the patients with diabetic neuropathy compared with the healthy control group; p = 0.0143 being considered statistically significant, OR = 0.3382; 95% CI = 0.1472-0.7771. The genotype ID+DD was more frequent in the patients with diabetic neuropathy compared with the healthy control group; p = 0.0021 being considered statistically very significant, OR = 0.3203; 95% CI = 0.1569-0.6539.

DISCUSSIONS

The human ACE gene is located on the long arm of chromosome 17 (17q23). The gene has 26 exons and 25 introns. The polymorphism based on the presence (insertion I) or absence (deletion D), resulting in three genotypes (DD and II homozygotes and ID heterozygotes).(8)

The DD genotype or D allele of this polymorphism proved to be associated with an increased risk of hypertension (9), diabetic nephropathy and diabetic cardiovascular complications (10,11), as well as with elevated circulating and tissue ACE activity.(12) There are many association studies showing the influence of ACE I/D polymorphism on the onset of diabetic mellitus.(13) However, some studies did not find any association between ACE gene polymorphisms and the risk of diabetes mellitus.(14)

CLINICAL ASPECTS

Some studies found frequent occurrence of the D allele in the patients with proliferative diabetic retinopathy.(15) No relation of the ACE polymorphism with diabetic retinopathy was observed by Wiwanitkit et al.(16) The insertion/deletion (I/D) polymorphism of the ACE gene has been reported to be associated with diabetic microvascular or macrovascular complications. Some reports showed an association between ACE genotype and hypertension.(17) A beneficial effect of angiotensin converting enzyme (ACE) inhibitors on the treatment of diabetic neuropathy has been demonstrated in several clinical trials. The vasodilator agents (ACE inhibitors) can improve neuronal blood flow with improvements in nerve conduction velocities. The microvascular dysfunction that appears in the early stages of diabetes and evolve in parallel with neural dysfunction may be enough to support the magnitude of functional, structural, and clinical symptoms observed in the patients with diabetic neuropathy. The pathophysiological mechanisms underlying the relation between the ACE polymorphism and diabetic neuropathy are not completely understood, and appear to be multifactorial. To our knowledge, this is the first study that examines the relation between the ACE gene polymorphism and diabetic neuropathy in a Romanian population. We have to mention that we found in literature only one study about diabetic polyneuropathy and ACE gene polymorphism; this study is in Japanese and no abstract is available.

CONCLUSIONS

In our study, the genotype ID was more frequent in the patients with diabetic neuropathy compared with the healthy control group where the II genotype was more frequently found. ACE D allele was more frequently found in the patients with diabetic neuropathy. The observation made in the present study revealed the protective role of I allele in our cohort. According to Katsuya et al., D allele had a protective role in the patients with diabetes mellitus.(18) Our results indicate that ACE I allele protects against diabetic neuropathy and ACE D allele may play a significant role in individual's susceptibility to the disease. In their study, Bhavani et al. observed that D allele of ACE gene protects against diabetes, however it increases susceptibility to hypertension, particularly when associated with type II diabetes.(19) In conclusion, our preliminary findings revealed the protective role of ACE I allele in diabetic polyneuropathy. However, the relative small number of subjects studied here remains a limitation to be conclusive regarding the results obtained.

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BARYCENTRE POSTUROGRAPHIC ANALYSIS IN THE PATIENTS WITH COXARTHROSIS

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Keywords: barycentre, stabilometric platform, testing

Abstract: The present study analyzes the projection of barycentre within the support polygon and the distribution of the body weight on the lower limbs, comparatively for two batches of subjects. For the batch A, the assessment was unique, while for the batch B, there was a preoperative assessment. This way, we will analyse the loading and straining degree at the level of preoperative hip joints, with a subsequent identical postoperative analysis for the study batch. In this sense, we have used the GPS400 stabilometric platform that through the pressure sensors tests both the repartition of the body weight on the lower limbs and analyses at the same time the oscillations that occur during the orthostatic position. The principle of the stabilometric method is to analyse the barycentre variations in the orthostatic position. Postural assessment has been accomplished with a view to diagnose the deviations of the mechanical loading charges at the level of hips, the efficiency of the surgical intervention and of the recovery programmes.

Cuvinte cheie: centrul de greutate, platforma stabilometrică, testare

Rezumat: Studiul de față analizează proiecția centrului de greutate în cadrul poligonului de susținere și distribuția greutății corporale pe membrele inferioare comparativ pentru cele 2 loturi de subiecți. Pentru lotul de A evaluarea a fost unică iar pentru lotul B evaluarea s-a realizat preoperator. Astfel, se va analiza gradul de încărcare și de solicitare de la nivelul articulațiilor șoldului preoperator urmând a se realiza o analiză identică postoperator în cadrul lotului de studiu. În acest sens s-a utilizat platforma stabilometrică GPS400 care prin senzorii de presiune testează atât repartiția greutății corporale pe membrele inferioare analizând în același timp oscilațiile ce apar în timpul poziției ortostatice. Principiul metodei stabilometrice este analiza variației centrului de greutate în poziție ortostatică. Evaluarea posturală se realizează în scopul diagnosticării abaterilor sarcinilor de încărcare mecanică de la nivelul șoldurilor, a eficienței intervenției chirurgicale și al programelor de recuperare.

INTRODUCTION

In orthostatic position, the torso tends to bend forward because of the overall weight of the upper body, its common barycentre projecting on the ground before the vertical. The paravertebral muscles go against this falling tendency through their permanent contraction.

Besides this, pelvis plays an essential part in the balance of the human body. It makes the connection between the torso and the lower limbs, being situated at the intersection of the three planes. In fact, the point where the three planes intersect determines the barycentre of the body – generally at the level of the second sacral vertebra.

The more stable the balance of the human body is the closer the barycentre to the support basis and the closer the projection from the centre of the support polygon. For a stable balance, another essential element is the atmospheric pressure, "as passive stabilizing factor," as the Weber brothers demonstrated. Some conditions at the level of the coxofemoral joints may influence the distribution of the weight at the level of the lower limbs, as well as the projection of the barycentre in the support polygon.

In the vision of the specialists, in case of the normal walking, the total weight of the hip joint approximately equals 2½ compared to the body weight. As the body line approaches the centre of the hip joint, the force component due to the action of the abductor mechanism decreases, with a reduction

corresponding to the force applied to the hip acetabulus, head, and bone.

According to ME Zeman, there are three tasks associated to the three critical cases within a walking cycle:

- support on the heel (I)
- total support on the limb (II)
- opening the toes (III)

PURPOSE

The present study analyzes the projection of barycentre within the support polygon and the distribution of the body weight on the lower limbs, comparatively for two batches of subjects.

METHODS

We have conducted a prospective study on a number of 21 healthy persons with no previous problems at the level of hip joints or lower limbs and 17 patients diagnosed with coxarthrosis with an indication for total hip arthroplasty, between January 2010 and December 2010. The study took place within the Orthopaedics Clinic of the Emergency Hospital "Prof. dr. D. Gerota", Bucharest.

The study comprised two batches of subjects: batch A – 21 healthy persons with no previous problems at the level of hip joints or lower limbs; batch B – 17 patients diagnosed with coxarthrosis with an indication for total hip arthroplasty.

We present below the inclusion and exclusion criteria

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