Absence d'association entre les polymorphismes du système rénine angiotensine (SRA) et l'hypertension artérielle chez les diabétiques de type 2 tunisiens

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Lack of association between renin-angiotensin system (RAS) polymorphisms and hypertension in tunisian type 2 diabetics

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RÉSUMÉ

Prérequis : Les gènes codant pour les composants du système rénine angiotensine (SRA) sont des gènes candidats potentiels pour le diabète de type 2 et l'hypertension artérielle. En effet, le gène ACE qui code pour l'enzyme de conversion de l'angiotensine I présente au niveau de son intron 16 un polymorphisme (I/D) associé avec les taux plasmatiques de l'enzyme. D'autre part, le gène AGT codant pour le précurseur du SRA présente un polymorphisme M235T qui est associé avec une élévation plasmatique d'angiotensinogène.
But : Nous avons testé l'implication de ces 2 polymorphismes dans la susceptibilité à l'hypertension artérielle chez les diabétiques de type 2 Tunisiens.
Méthodes : Une étude cas/témoins a été conduite chez 61 patients (39 hypertendus et 22 non hypertendus). Le polymorphisme (I/D) a été analysé par PCR nichée afin de bien identifier les individus hétérozygotes et le polymorphisme M235T par PCR suivie d'une digestion enzymatique.
Résultats: La distribution des génotypes DD/ID et II ne diffère pas de façon statistiquement significative entre les diabétiques type 2 avec ou sans hypertension (DD: 49%; ID: 41%; II: 10% vs DD: 36%; ID: 55 %; II: 9%, respectivement) (χ^2=1.06, p=0.58). Il n'existe également pas de différence entre ces deux groupes pour le polymorphisme M235T (TT: 20%; MT: 54%; MM: 26% vs TT: 27%; MT: 41 %; MM: 32%, respectivement) (χ^2=0.95, p=0.62)
Conclusion: Les résultats trouvés montrent que les polymorphismes I/D et M235T ne sont pas associés à un risque plus élevé d'hypertension artérielle chez les diabétiques de type 2 dans l'échantillon étudié de la population Tunisienne.

MOTS-CLÉS

Hypertension artérielle, diabète de type 2 (DT2), polymorphisme, système rénine angiotensine

SUMMARY

Background : The genes encoding renin-angiotensin system (RAS) components are potent candidate genes in both hypertension and diabetes namely ACE encoding the angiotensin converting enzyme and AGT encoding angiotensinogen. It has been suggested that the insertion/deletion (I/D) polymorphism in intron 16 of ACE gene is associated with ACE levels, and M235T gene polymorphism is associated with plasma AGT levels.
Aim : We examined in this report the association between ACE I/D and AGT M235T polymorphisms with hypertension status in Tunisian type 2 diabetic subjects.
Methods: Thirty nine hypertensive and 22 normotensive type 2 diabetic Tunisian patients were recruited for this study. The I/D polymorphism of ACE gene was analysed with nested PCR in order to avoid mistyping heterozygous individuals and the M235T polymorphism of AGT gene was analysed using PCR and allele specific restriction.
Results: The distribution of DD, ID and II genotypes did not significantly differ between type 2 diabetic patients with or without hypertension (DD: 49%; ID: 41%; II: 10% vs DD: 36%; ID: 55 %; II: 9%, respectively) (χ^2=1.06, p=0.58). There was also no significant statistical difference between these two groups for the M235T polymorphism (TT: 20%; MT: 54%; MM: 26% vs TT: 27%; MT: 41 %; MM: 32%, respectively) (χ^2=0.95, p=0.62)
Conclusion:RAS polymorphisms do not seem to play a role in the development of hypertension in the studied Tunisian type 2 diabetic subjects.

KEY-WORDS

Hypertension, type 2 diabetes (T2D), polymorphism, renin-angiotensin system (RAS).

In the Tunisian population like most developing countries, hypertension is common and fast emerging as a major health problem. It is a principal risk factor for many diseases such as myocardial infarction, end stage renal disease, and peripheral vascular disease [1] particularly when associated with diabetes. Both diseases are strongly associated but the mechanism underlying the high incidence of vascular diseases in diabetic patients is still not fully understood. Essential hypertension and type 2 diabetes are under an interplay between genetic and environmental factors [2-3] and their genetic basis may overlap. The investigation of genetic risk factors for hypertension in diabetic patients is a useful approach for the identification of genes that contribute to the complex form of this disease. Although, the nature of the genetic factors is still unfolding, the genes encoding components of the renin-angiotensin system (RAS) are potential candidate genes in both hypertension and diabetes. In fact, inhibition of the activity of this system may reduce the risk of vascular diseases and delay onset of diabetes [4-5]. Two RAS gene polymorphisms: the angiotensin converting enzyme (ACE) insertion/deletion (I/D) and the angiotensinogen (AGT) M235T were shown to be associated with RAS activity. Experimental and clinical studies demonstrated that the insertion–deletion (I/D) polymorphism is associated with plasma and tissue ACE levels [6]. The highest levels have been observed in homozygotes DD [7]. The M235T polymorphism has been reported associated with a plasma angiotensinogen concentration. Moreover this polymorphism has been found to be in complete linkage desequilibrium with a polymorphism in AGT promoter. The latter affects basal transcription of AGT in vitro with the highest tissue levels in T235/T235 individuals [8]. Consequently, the involvement of these two polymorphisms in the development of hypertension in diabetic patients has been studied in different populations. However, conflicting findings were reported in different ethnicities [9-12]. In the present study we examined whether these two polymorphisms increase the risk of hypertension in the Tunisian type 2 diabetics.

PATIENTS AND METHODS

Patients

The patients recruited for this study were attending the National Institute of Nutrition (Tunis, Tunisia). A total of 61 (25 men and 36 women) unrelated Tunisian type 2 diabetic patients were randomly selected: 22 patients without hypertension and 39 patients had hypertension. Type 2 diabetes was diagnosed according to the WHO criteria [13]. Individuals were considered hypertensive if their systolic blood pressure was ? 140mmHg or if their diastolic blood pressure was ? 90 mmHg regardless of treatment status (mean of at least two readings). In addition, subjects who were on antihypertensive therapy but who had systolic blood pressure <140 mmHg and diastolic blood pressure <90 mmHg were enrolled in this study. Subjects with secondary forms of hypertension such as: hyperthyroidism, tumours, glomerular nephritis were excluded by routine investigation. The mean age of patients is 59.8 years ± 10.5 with a mean duration of diabetes 16.5 years ± 6.9. The patients recruited present or no a diabetic nephropathy (urinary albumin excretion>30mg/24h on two consecutive urine samples). Approval for the study was obtained from the local ethical committee, all patients gave their fully informed consent and were examined for the following variables: body mass index (poids/taille^2), diabetes duration, total cholesterol and triglycerides. Clinical and biochemical measurements of patients with T2D were performed after at least 8 hours of fasting. Blood pressure was measured in a sitting position after at last 5 minutes of rest. Plasma levels of total cholesterol and triglycerides were analyzed by enzymatic methods using Beckman reagents on a synchron C?9 Beckman analyser.

DNA analysis

After informed consent, samples of whole blood were collected in EDTA tubes from each patient. DNA was extracted from leukocytes using phenol/ chloroform standard method. The I/D polymorphism in intron 16 of ACE gene was amplified by nested PCR using three primers as described by Marre et al., 1997 [14] with minor modifications. The polymorphism M235T in AGT gene was determined by RFLP according to Niu T et al., 1998 [15].

Statistical analysis

All data for continuous variables are expressed as means± SD. The Mann-whitney and the student's t test were used as appropriate when comparing metabolic parameters between hypertensive and normotensive diabetics. The distribution of genotypes and alleles frequencies of each polymorphism were compared between case and control subjects using ¯2 test. All statistical calculations were performed using the Statistical Package for Social Sciences (SPSS). A value of $p < 0.05$ was considered statistically significant for all statistical tests.

RESULTS

Clinical characteristics of hypertensive and non hypertensive type 2 diabetic Tunisian patients are given in table I. Hypertensive patients were older than non hypertensive diabetics but there were no statistical differences with regard to diabetes duration, body mass index, total cholesterol and triglyceride levels between the two groups. The genotype distribution did not significantly differ between type 2 diabetic patients with or without hypertension neither for ACE I/D (DD: 49%; ID: 41%; II: 10% vs DD: 36%; ID: 55 %; II: 9%, respectively) (¯2=1.06, p=0.58) nor for AGT M235T (TT: 20%; MT: 54%; MM: 26% vs TT: 27%; MT: 41 %; MM: 32%, respectively) (¯2=0.95, p=0.62) gene polymorphisms (Table II).There is also no statistical difference in the frequency of D (0.69 vs 0.64, respectively) nor T (0.47 vs 0.48, respectively) risk alleles between the two groups. Furthermore, a possible additive effect of M235T polymorphism on highest risk of hypertension in diabetics with the ACE DD genotype was

examined (10% TT/DD for hypertensive vs 14% TT/DD for normotensive, ¯2=0.15, p=0.69), results show that an additive effect is unlikely.

Table 1: Characteristics and clinical data of Tunisian type 2 diabetics with or without hypertension.

	HTA	N	Mean ± SD
Age (years)*/	Yes	39	62,8 ± 9,9
	No	22	54,4 ± 9,5
Diabetes Duration	Yes	38	17,1 ± 6,1
(years)	No	21	15,4 ± 8,1
BMI (kg/m²)	Yes	32	28,8 ± 5,9
	No	18	25,8 ± 3,6
Chol (mmol/l)	Yes	29	5,08 ± 0,6
	No	18	4,7 ± 1
TG (mmol/l)	Yes	29	1,3 ± 0,6
	No	18	1,5 ± 0,6

* p : 0,002

Table 2: Distributions of I/D polymorphism in ACE and M235T in AGT genes according to patients' status of hypertension.

	61 type 2 diabetic patients	
Genotypes	**Hypertensive (N=39)**	**Normotensive (n=22)**
I/D polymorphism		
	N (%)	N (%)
DD	19 (49)	8 (36)
ID	16 (41)	12 (55)
II	4 (10)	2 (9)
M235T polymorphism		
MM	10 (26)	7 (32)
MT	21 (54)	9 (41)
TT	8 (20)	6 (27)

DISCUSSION

Many studies in Caucasians support the involvement of hyperinsulinemia and/or insulin resistance in the pathogenesis of hypertension [16-17]. However, a clear relationship between insulin resistance and hypertension is not consistently found in all ethnic groups. The association between RAS polymorphisms and the combination of hypertension and type 2 diabetes is still not sufficiently studied. Association studies of these two polymorphisms and hypertension in diabetics have been tested in Caucasians [9-11] and Asians [12] but not in Africans. To our knowledge, this is the first study in North Africans in which the associations between ACE (I/D) and AGT M235T gene polymorphisms and hypertension among type 2 diabetics were investigated. Contrary to some studies, but in accordance with others, we report no association between the ACE (I/D) or AGT M235T polymorphisms and hypertension in the studied sample. Association studies of these two polymorphisms and hypertension in diabetic population have yielded conflicting results. Digermenci et al [9] did not show a significant association between ACE gene polymorphism and hypertension in Turkish type 2 diabetic patients. However, Nakhjavani et al [12] showed that the DD genotype is strongly associated with increased risk of hypertension in Iranian patients with type 2 diabetes. The same result was also reported by Bengtsson et al [11] in Sweden. Moreover, other studies support the association of the T allele of the AGT M253T polymorphism with hypertension [18]. This discrepancy may be due to several factors mainly ethnicity and selection criteria of the patients. Although, recent reverse approaches showing a relationship between the ACE polymorphism and insulin resistance in hypertensive patients [19], our study did not show a positive association between ACE (I/D) polymorphisms and hypertension among type 2 diabetics. Furthermore, since both hypertension and diabetes are both complex disorders with many interacting parameters, we tested the implication of several known risk factors, no significant difference was found for BMI, triglyceride and cholesterol concentrations. The present study provides no evidence that ACE (I/D) nor AGT M253T polymorphisms may increase the risk of hypertension in type 2 diabetic patients in the Tunisian studied population. Further studies are needed to confirm the effect of RAS system in the pathogenesis of hypertension in diabetics.

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FOOTNOTES
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Conflicts of interest: None.

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