

ON HUMAN TISSUE KALLIKREIN ACTIVITY IN URINE OF BRAZILIAN WHITE AND BLACK PRIMARY HYPERTENSIVE PATIENTS

Objective: To evaluate the possible relationship between the human tissue kallikrein amidase activity with sex and ethnicity in Brazilian primary hypertensive patients.

Design: Population-based study.

Participants: One hundred men and women, Black and White primary hypertensive patients aged 20 years and older were selected. Eighty nine healthy individuals, paired according to age, sex, and ethnics were used as controls.

Methods: Early-morning midstream urine was used. Human tissue kallikrein amidase activity was estimated with D-Val-Leu-Arg 4-nitroanilide substrate. Creatinine was determined by a method based on Jaffe's reaction. hK1 amidase activity is expressed in $\mu\text{M}/(\text{min} \cdot \text{mg creatinine})$ to correct for differences in urine flow rate. Data are expressed as medians.

Results: Human tissue kallikrein amidase activity was significantly lower in the urine of hypertensive patients ($0.210 \mu\text{M}/(\text{min} \cdot \text{mg creatinine})$) than in the urine of control subjects ($0.260 \mu\text{M}/(\text{min} \cdot \text{mg creatinine})$) ($P=.010$). This result supports data from the literature. Contrasting to what was already reported, namely that human tissue kallikrein excretion is higher in females than in males, and especially higher in Caucasians than in African Americans, our results show that, in the urine of Brazilian hypertensive patients and control subjects, no significant effect of sex and ethnicity on human tissue kallikrein amidase activity was observed.

Conclusions: The lack of ethnicity effect supports what was already asserted, namely, that, in Brazil, at an individual level, color, as determined by physical evaluation, is a poor predictor of genomic African ancestry, estimated by molecular markers. (*Ethn Dis.* 2009;19: 265–270)

Key Words: Human Kallikrein, Human Tissue Kallikrein, Hypertension, Ethnic Groups, Brazilian

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INTRODUCTION

Primary (essential) hypertension is a multifactorial disease influenced by a combination of genetic and environmental factors.¹ Once hypertension develops, it tends to become self-perpetuating by amplifying mechanisms mediated by secondary structural changes in blood vessels, heart, and kidney. The long-term sequelae from hypertension are atherosclerotic vascular disease complications, cardiac hypertrophy and failure, stroke, and renal failure.² The primary cause of human essential hypertension is not yet known, despite intensive research on the various mechanisms that may be involved in its development.³

Abnormality of the tissue kallikrein-kinin system has long been documented in the pathogenesis of hypertension.⁴ Epidemiological studies have identified an inverse relationship between urinary or renal kallikrein levels and blood pressure in patients with primary (essential) hypertension.⁴ Reduced urinary kallikrein excretion has also been described in a number of genetically hypertensive rat models.⁴

Kallikreins (EC 3.4.21.8) are a subgroup of the serine protease family known to have several physiological functions.⁵ The kallikreins are divided into two main groups: plasma (EC

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3.4.21.34) and tissue (EC 3.4.21.35) kallikreins.⁴ The KLK1 gene, located on chromosome 19q13.4, expresses human tissue kallikrein (hK1), whose principal known biochemical function is to release the vasoactive and spasmogenic decapeptide kallidin (lysyl-bradykinin) (Lys-BK) from the plasma protein low molecular weight kininogen (LMWK).^{5,6} Lys-BK is a potent vasodilator and also promotes diuresis and natriuresis.⁷ Urinary excretion of renal (tissue) kallikrein has emerged as an intermediate phenotype for hypertension, because of the association of diminished kallikrein excretion with the disease state, bimodal frequency distribution in the population, familial aggregation, and early diminution even in the still-normotensive offspring of patients with hypertension.⁸

Kallikrein excretion is diminished in African Americans, especially those with hypertension, suggesting a potential pathophysiologic mechanism for the frequent development of hypertension in this population.⁸ Several studies have demonstrated ethnic differences in urinary kallikrein excretion, with African Americans consistently having lower urinary kallikrein excretion when compared to Caucasians, across both normal and high blood pressure strata.¹ Another study showed that the urinary tissue kallikrein levels were, in general, lower

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in Indian hypertensives when compared to the Black hypertensive patients.⁹ On the other hand, another study reported no difference in urinary kallikrein excretion between Caucasian and Asian.¹

According to the Brazilian Institute of Geography and Statistics (IBGE, in Portuguese),¹⁰ the Brazilian population in 2000, based on skin color, comprised: White Brazilians - 53.7%; 'Pardo' Brazilians (ie, non-White and non-Black) - 38.4%; African Brazilians - 7.2%; Asian Brazilians - 0.4% and Aboriginal Brazilians - 0.4%.

On the other hand, studies have shown that Brazilians are one of the most heterogeneous populations in the world, resulting from five centuries of interethnic crosses among peoples from three continents: the European colonizers, mainly represented by the Portuguese, the African slaves, and the autochthonous Amerindians. Therefore, in Brazil, at an individual level, color, as determined by physical evaluation, is a poor predictor of genomic African ancestry, when the latter is estimated by molecular markers.¹¹

Sex was also reported to be a substantial influence on human tissue kallikrein excretion, with women excreting more kallikrein than men, regardless of ethnicity.¹

The following facts laid the groundwork for our research: 1) renal kallikrein excretion varies according to ethnicity, with especially low values in African-American individuals;⁸ 2) the special ethnic heterogeneity of Brazilian population; and 3) the influence of ethnicity and sex on the hK1 amidase activity both in normotensive subjects and in hypertensive patients has never been described for the Brazilian population. Thus, our work had the following aims: 1) to evaluate the behavior of urinary hK1 amidase activity among Brazilian primary hypertensive patients, considering healthy subjects as controls; 2) to evaluate whether urinary hK1 amidase activity is influenced by sex and ethnicity among Brazilian primary hyperten-

sive patients, considering healthy subjects as controls.

METHODS

This study was approved by the ethical committee of the Federal University of Minas Gerais (UFMG). All patients and control subjects gave written informed consent.

Patients of both sexes were eligible for inclusion in the study as long as they were older than 20 years of age and had primary hypertension. All subjects were studied as outpatients. Patients and control subjects were consecutively screened by the same physician (ELGM) for a two-year period, and underwent a thorough clinical interview as well as physical examination. All of their symptoms and signs indicative of either primary hypertension or any other cardiovascular disease were analyzed, as well as the patients' personal antecedents and the types of (cardiovascular or non-cardiovascular) medication they were making use of. Individuals were stratified according to ethnicity by the physician (ELGM).

Patient Criteria

To be included in the study, patients had primary hypertension, either diastolic blood pressure of ≥ 90 mm Hg or systolic blood pressure of ≥ 140 mm Hg, on three separate determinations. The criteria for patient exclusion included: non-agreement for participating in the study, secondary hypertension, heart failure, renal failure (serum creatinine level ≥ 1.5 mg/dL or $133 \mu\text{mol/L}$ in man and ≥ 1.4 mg/dL or $124 \mu\text{mol/L}$ in woman), hepatic alterations and diabetes mellitus. Premenopausal women were not studied during their menstruation phase of their menstrual cycle. Because hK1 amidase activity and creatinine determinations are spectrophotometric assays, the presence of blood in urine could interfere with the results. The population studied

was divided into two subgroups and were compared according to the following variables: systolic arterial pressure, diastolic arterial pressure and hK1 amidase activity. The hK1 amidase activity was expressed in $\mu\text{M}/(\text{min} \cdot \text{mg}$ creatinine).

Chemicals

D-valyl-L-leucyl-L-arginine 4-nitroanilide (D-Val-Leu-Arg-Nan) was purchased from Chromogenix AB (Italy). Trasyol[®] was provided by courtesy of Bayer (Brazil). Bovine serum albumin and soybean trypsin inhibitor (SBTI) were purchased from Sigma Chemical Company (St. Louis, MO, USA). Dipstick tests (Urofit 10U bioBRÁS Diagnósticos, Biobrás S.A., Belo Horizonte, MG, Brazil) were purchased from Biobrás. The diagnostic kit for creatinine determination was freely provided courtesy of KATAL Biotecnológica Indústria e Comércio Ltda (Belo Horizonte, MG, Brazil). All other reagents were from Sigma reagent grade.

Assessments

A random urine sample, the early-morning midstream specimen, was used. In the laboratory, the urine sample was visually and chemically examined with dipstick test. All the urine samples were negative for blood and for all chemical compounds evaluated. Hydrolysis of the chromogenic substrate D-Val-Leu-Arg-Nan was assayed spectrophotometrically at 410 nm, to allow the release of 4-nitroaniline (4-NAn) ($\epsilon_{410} = 8800/(\text{M} \cdot \text{cm})$) to be monitored as previously described.^{12,13} The hK1 amidase activity assay was carried out as already described.¹⁴ The reaction rate (v) was expressed in $\mu\text{M}/(\text{min} \cdot \text{mL}$ urine).

Creatinine concentration was determined spectrophotometrically using a kit of reagents based on the Jaffé's reaction and was expressed in mg/mL urine.

Kallikrein specific amidase activity was calculated dividing the reaction rate (v) [$\mu\text{M}/(\text{min} \cdot \text{mL}$ urine)] by creatinine concentration (mg/mL urine). The re-

sult was expressed as $\mu\text{M}/(\text{min} \cdot \text{mg}$ creatinine) to correct for differences in urine flow rate.

Statistical Analysis

Descriptive statistics are reported as medians from the irregular distribution of the variables studied. Differences between the groups were evaluated by the non-parametric Kruskal-Wallis test, since the population studied had a non-Gaussian distribution with non-homogeneous variance. A *P* value of $<.05$ was considered statistically significant.

RESULTS

Patients and Control Subjects Characteristics

One hundred patients (26 White, 74 Black, stratified with basis on their identification by physician) fulfilled the study criteria. Patients' continued their diets of customary intake of fluid, electrolytes, calories, or other macronutrient/micronutrient composition. The serum sodium, chloride, potassium, blood urea and serum creatinine values were normal in all the patients. Twenty-nine (29%) patients were using a diuretic, 25% an ACE inhibitor, 10% a calcium channel blocker, 9% a β -blocker, 7% a selective α_2 -agonist, whereas 20 patients (20%) were using no medication. Patients in this study were otherwise healthy, but for their hypertension. Eighty-nine healthy normotensive (diastolic blood pressure consistently < 90 mm Hg or systolic blood pressure < 140 mmHg on three separate determinations) individuals (31 White and 58 Black, stratified with basis on their identification by physician), paired according to ethnicity, sex and age (± 5), were used as normal controls, thereby constituting the control subgroup.

The baseline characteristics of the two groups are shown in Table 1. The age medians of the patients and controls were 52 and 48 years, respectively. Fifty hypertensive men and 50 hypertensive

Table 1. Baseline characteristics of the study population

Parameter	Patients	Controls	P
Number	100	89	-
<i>Demographic</i>			
Age, y*	52 (39-61)	48 (35-57)	-
Sex (male/female)	50/50	48/41	-
Race (White/Black)	26/74	31/58	-
<i>Physiologic</i>			
SBP, mm Hg*	160 (150-170)	120 (120-130)	$<.001$
DBP, mm Hg*	90 (85-100)	80 (80-80)	$<.001$
<i>Biochemical</i>			
hK1 am act***	0.210 (0.100-0.395)	0.260 (0.180-0.445)	.010

Abbreviations: DBP, diastolic blood pressure; hK1 am act, hK1 amidase activity; SBP, systolic blood pressure; *median value; numbers in parentheses are the interquartile ranges 25% to 75%; ** $\mu\text{M}/(\text{min} \cdot \text{mg}$ creatinine).

women constituted the patient group. On the other hand, 48 normotensive men and 41 normotensive women constituted the control group. Twenty six hypertensive Whites and 74 hypertensive Blacks constituted the patient group. On the other hand, 31 normotensive Whites and 58 normotensive Blacks constituted the control group. All 100 patients had primary hypertension. The systolic blood pressure (SBP) and diastolic blood pressure (DBP) were significantly higher in hypertensive patients than in controls. The hK1 amidase activity was significantly lower in primary hypertensive patients than in controls (Table 1, Figure 1).

Effect of Gender on Human Tissue Kallikrein Amidase Activity

We did not observe a statistically significant difference in hK1 amidase activity between men and women, either with hypertension ($0.185 \mu\text{M}/(\text{min} \cdot \text{mg}$ creatinine and $0.210 \mu\text{M}/(\text{min} \cdot \text{mg}$ creatinine, respectively) ($P=.992$), or without hypertension ($0.206 \mu\text{M}/(\text{min} \cdot \text{mg}$ creatinine and $0.260 \mu\text{M}/(\text{min} \cdot \text{mg}$ creatinine, respectively) ($P=.795$).

Effect of Ethnicity on Human Tissue Kallikrein Amidase Activity

We did not observe a statistically significant difference in hK1 amidase

activity between White and Black patients, either with hypertension ($0.220 \mu\text{M}/(\text{min} \cdot \text{mg}$ creatinine and $0.180 \mu\text{M}/(\text{min} \cdot \text{mg}$ creatinine, respectively) ($P=.931$), or without hypertension ($0.280 \mu\text{M}/(\text{min} \cdot \text{mg}$ creatinine and $0.260 \mu\text{M}/(\text{min} \cdot \text{mg}$ creatinine, respectively) ($P=.914$).

DISCUSSION

To our knowledge, this is the first study concerning the evaluation of the effects of sex and ethnicity on the activity of human tissue kallikrein (hK1) in the urine of Brazilian normotensive subjects and primary hypertensive patients. We have shown that hK1 amidase activity is significantly lower in the urine of primary hypertensive patients than in the urine of control subjects. This result is in agreement with data previously published. Indeed

We have shown that hK1 amidase activity is significantly lower in the urine of primary hypertensive patients than in the urine of control subjects.

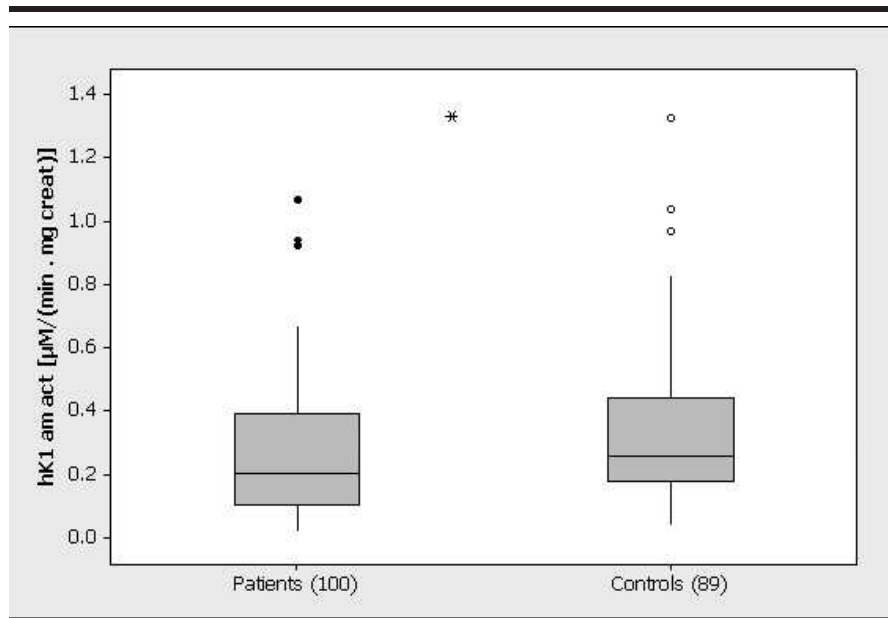


Fig 1. Comparison of hK1 amidase activity values between in the urine of hypertensive patients and in the urine of normotensive subjects. hK1 am act indicates hK1 amidase activity, expressed as $\mu\text{M}/(\text{min} \cdot \text{mg creatinine})$; the symbol (*) indicates statistically significant difference between hypertensive and normotensive groups

Elliot and Nuzum¹⁵ had already noticed that hypertensive patients, with no clinically apparent renal disease, have significantly lower levels of urinary kallikrein than normotensive subjects. Margolius et al¹⁶ reported lower levels of urinary kallikrein in patients with essential hypertension than in a control population. O'Connor¹⁷ reported that kallikrein excretion is diminished in hypertension (especially hypertension with reduced renal function), suggesting the enzyme involvement in the pathogenesis of the disease. However, in 1995, Margolius¹⁸ reported that, although it is clear that patients with essential hypertension excrete less kallikrein than normotensive subjects, there is much overlap in the population studies carried out over the past 25 years. According to the author, many hypertensive subjects show normal kallikrein excretion. In our study, three hypertensive patients (6%) had normal hK1 amidase activity. Black people, adults and children excrete markedly less kallikrein than Whites, regardless of

blood pressure, with Black hypertensive subjects generally showing the lowest measured levels.¹⁸ Wong et al⁸ reported that renal kallikrein excretion is diminished in patients with hypertension, and perhaps even in the early, pre-hypertensive phases of the syndrome.

In our study, patients were submitted to a normal sodium diet (140 mmol/L). It was already reported that hK1 amidase activity is inhibited, *in vitro*, by sodium and other uni and divalent cations and, also, that bovine serum albumin (0.2%) protected the inhibition of hK1 amidase activity by sodium and potassium.^{19,20} On the other hand, it is known that only a small amount of protein is excreted normally in the human urine, most of which being albumin.²¹ Thus, it is possible that urinary albumin can protect hK1 from sodium and potassium inhibition. If this is correct, the decrease in hK1 amidase activity observed in our study is unlikely to be due to its inhibition by urinary sodium or potassium.

Sex Effect

The influence of sex on hK1 amidase activity is not clear. Ash et al²² reported that the human urinary kallikrein (hK1) output values among young men and women were similar. On the other hand, Hughes Jr. et al²³ found sex differences in tissue kallikrein levels in urine in groups of young, White subjects without hypertension and patients with normal renin essential hypertension. According to these authors, urinary kallikrein excretion was higher in women than in men regardless of sodium intake or level of blood pressure. Lieberthal et al²⁴ found larger urinary kallikrein excretion in White men with hypertension than in White women with hypertension. On the other hand, Song et al¹ reported that sex has a substantial influence on human kallikrein excretion, with women excreting consistently higher activities than men, regardless of ethnicity. According to these authors, the sex difference in kallikrein excretion has been attributed to an effect of the female's menstrual cycle, wherein kallikrein rises ~50% from the mid-follicular to the mid-luteal phase of the cycle. Black women notably lack the cycle effect on kallikrein. Although their female subjects were not studied at a particular phase during the menstrual cycle, they still observed ~50% higher kallikrein excretion in women than men, and sex effect was independent of ethnicity.

In our study, we did not observe a statistically significant difference in hK1 amidase activity between male and female, either with hypertension or without hypertension. Thus, our results agree with those reported by Ash et al²² and disagree with those reported by Song et al¹, Huges Jr et al²³ and Lieberthal et al.²⁴

ETHNICITY EFFECT

Katori and Majima²⁵ reported that kallikrein excretion in White hyperten-

sive men was lower than that in White normotensives during normal sodium intake, but was not different from that in Black hypertensives and Black control subjects under the same conditions. The kallikrein levels in the urine of normotensive Black subjects were significantly lower than those in normotensive White subjects.

Seedat et al,⁹ in South Africa, evaluated the excretion values for urinary tissue kallikrein in four groups: Black normotensive, Black hypertensive, Indian normotensive and Indian hypertensive. This research confirmed that the renal excretion of tissue kallikrein was reduced in Black hypertensives. According to the authors, the specific activity of tissue kallikrein (ng/ μ g protein) was in general higher in the Black hypertensives than in the Indian hypertensives, whereas the normotensives in the two groups showed variable trends that were not significantly different. In conclusion, the authors reported that their study showed that urinary tissue kallikrein levels were, in general, lower in Indian hypertensives compared to Black hypertensive patients.

Song et al¹ reported that several studies have demonstrated substantial ethnic differences in urinary kallikrein excretion, African Americans consistently have lower urinary kallikrein excretion when compared to Caucasians, across both normal and high blood pressure strata. Song et al found a substantial diminution in urinary kallikrein excretion in African Americans, but no difference between Caucasian and Asian values.

Wong et al⁸ evaluated the excretion of hK1, K⁺ and aldosterone in the urine of healthy young normotensive African American (sub-Saharan African ancestry) men and concluded that normotensive African Americans have diminished urinary excretion of kallikrein, K⁺ and aldosterone. According to the authors, kallikrein excretion was diminished in African Americans, especially in those with hypertension, suggesting a

potential pathophysiologic mechanism for the frequent development of hypertension in this population.

In our study, we did not observe a statistically significant difference in hK1 amidase activity between White and Black, either with hypertension or without hypertension. Thus, our study does not support those previously reported.^{1,8,25} A similar result was observed by Figueiredo et al,¹⁴ who evaluated the hK1 amidase activity in the urine of patients with systolic heart failure (SHF), and in the urine of healthy individuals, paired according to sex, ethnicity and age, as controls. The authors observed that the hK1 amidase activity was significantly lower in the urine of SHF patients. In that study, the majority of patients and controls were White and there was no significant effect of ethnicity on hK1 amidase activity. The lack of ethnicity effect on hK1 amidase activity reported by Figueiredo et al,¹⁴ which is also observed in this study, supports the data reported by Parra et al.¹¹ The authors concluded that their data suggest that in Brazil, at an individual level, color, as determined by physical evaluation, is a poor predictor of genomic African ancestry, estimated by molecular markers.

In conclusion, the evidence presented here shows: 1) in Brazilian patients with primary hypertension, the hK1 amidase activity was significantly lower than in healthy controls, supporting data previously published involving studies with other populations; 2) in Brazilian subjects, the hK1 amidase activity was not influenced by sex; 3) in Brazilian subjects, the hK1 amidase activity was not influenced by skin color.

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