Prevalence of IGFBP3, NOS3 and TCF7L2 Polymorphisms and Their Association With Hypertension: A Population-Based Study With Brazilian Women of African Descent

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Abstract

Background: African ancestry seems to be a risk factor for hypertension; however, few genetic studies have addressed this issue. This study aimed to investigate the prevalence of polymorphisms *NOS3; rs1799983*, *IGFBP3; rs11977526* and *TCF7L2*; *rs7903146* in Brazilian women of African descent and their association with hypertension.

Methods: This is a cross-sectional study with a sample of 1021 women (19–59 years old) from the quilombola communities of Alagoas (Brazil). Demographic, socioeconomic, lifestyle, anthropometric, biochemical, and blood pressure data were collected. DNA was extracted from mucosa epithelial cells of the participants' cheek. Genotyping was performed by PCR allelic discrimination. Prevalence ratio (PR) was the measure of association, calculated by Poisson regression, with a hierarchical selection of variables.

Results: The prevalences of the less frequent genotypes were 26.5% TT genotype of *NOS3*; *rs1799983*, 16.7% AA genotype of *IGFBP3; rs11977526*, and 18.3% TT genotype of *TCF7L2; rs7903146*. For these conditions, the prevalence of hypertension and PR (adjusted) relatively to the ancestral genotype were, respectively: 52.0% vs 24.5% (PR=1.54; $p<0.001$), 62.0% vs 24.1% (PR=1.59; $p<0.001$), and 38.9% vs 27.9% (PR=0.86; $p=0.166$). Associations with hypertension were statistically significant, except for the *TCF7L2*; *rs7903146* polymorphism, after adjusted analysis.

Conclusions: Brazilian Afro-descendant women with the TT genotype for the *NOS3* gene and the AA genotype for the *IGFBP3* gene are more susceptible to hypertension. The understanding of underlying mechanisms involving the pathogenesis of hypertension can motivate research for the development of new therapeutic targets related to nitric oxide metabolism and the management of oxidative stress.

Background

Arterial hypertension characterized by important structural changes the vascular system in response to changes in hemodynamic conditions. It is intimately associated with other morbidities, such as dyslipidemia and diabetes mellitus, and is one of the most prevalent chronic non-communicable diseases in the world [1]. This disease is often associated with metabolic disorders, functional and/or structural changes in target organs. It can be aggravated by the presence of other risk factors, such as dyslipidemia, abdominal obesity, glucose intolerance, and diabetes mellitus [2].

Considered a disease of multifactorial etiology, hypertension is more prevalent among people of African descent. Therefore, it is important to understand the impact of the genetic component on the genesis of this pathology, with emphasis on polymorphisms [3]. Although there is a higher prevalence of hypertension in Afro-descendant populations in comparison with other ethnicities, studies involving the association of single nucleotide polymorphism (SNP) with this pathology have mostly been conducted with people of European ancestry, and few studies are dealing with populations of African origin [4, 5].

Hypertension is a condition characterized by endothelial dysfunction, which is a phenomenon that, although discussed as being primary or secondary to hypertension, has a fundamental importance in its genesis and maintenance [6]. hypertension is also accompanied by structural changes in the vascular system in response to changes in hemodynamic conditions. The endothelial dysfunction of hypertension is mainly characterized by a non-relaxation of blood vessels caused by lower bioactivity of nitric oxide (NO) in the vascular wall, due to oxidative stress, causing an imbalance between the antioxidant and pro-oxidant systems, and leading to the prevalence of deleterious actions of reactive oxygen species on cells, tissues and organs [7, 8].

IGFBP3 is a protein with the function of regulating the bioavailability of IGF-1 [9]. In vitro experiments indicate that IGFBP3 regulates IGF-1 by reducing vascular resistance when stimulating the synthesis of nitric oxide in endothelial cells [10]. Therefore, IGFBP3 serum levels are closely related to the production of endothelial nitric oxide and, consequently, to oxidative stress and hypertension [11].

Sedentary lifestyle, visceral adiposity, and insulin resistance are important risk factors for both hypertension and diabetes mellitus (DM). However, although several studies have demonstrated a relationship between the *TCF7L2* gene and DM, its relationship with the prevalence of arterial hypertension and oxidative stress has not been investigated [12, 13].

The SNPs in the *NOS3 rs1799983*, *IGFBP3 rs11977526*, and *TCF7L2 rs7903146* genes can directly influence the protein expression in the respective genes, making them important biomarkers for the development of hypertension. However, no studies addressing this association in Afro-descendant populations were found [14, 15].

Given the above, this study aimed to verify the prevalence of SNPs in the *NOS3 rs1799983*, *IGFBP3 rs11977526*, and *TCF7L2 rs7903146* genes, as well as to investigate the possible association of SNPs occurrence with arterial hypertension in Afro-descendant women, in quilombola communities in the state of Alagoas, northeastern Brazil.

Methods

This is a household cross-sectional population-based survey, whose data were collected between April 2017 and January 2018 from women aged 19–59 years, living in remaining quilombola communities, in the state of Alagoas, Brazil.

In the sample size calculation, hypertension was the variable of interest, whose prevalence in women of African descent was estimated at 35.8% [16]. Due to the lack of accurate demographic data categorized by sex, it was used the number of families (6,465) estimated by the State Department of Planning, Management, and Heritage of Alagoas State (2015). Thus, it was considered that there would be a woman in each family, totaling 6,465 people. The calculations were performed using the StatCalc software (Epi Info, version 3.5.4). For a sampling error of 3.0%, a 95% confidence interval, and adding 10% (852 + 85), to compensate for possible sample losses, 937 women would be needed.

Using the systematic sampling strategy, 50% of the 68 quilombola communities of Alagoas were selected, which are distributed in 27 of the 102 municipalities of the State; the majority located between the 'Agreste' and 'Sertão' regions. In each residence, a woman aged 19–59 was eligible for the study. When there were two or more women, one of them was selected by lot. Being pregnant or in the postpartum period, as well as having drunk alcohol on the day of the interview were exclusion criteria.

The data were collected through interviews using structured questionnaires. During the interviews, blood pressure was measured and anthropometric data were obtained. Then, the women were referred to take biochemical tests in a predetermined place in each community (health service, school, community center, or any other place deemed more appropriate in the context of the respective community).

Hypertension was the dependent variable, defined by systolic blood pressure (SBP) ≥140 mmHg and/or diastolic blood pressure (DBP) ≥90 mmHg and/or when the participant reported regular use of antihypertensive drugs. The blood pressure was measured in duplicate, with the participant seated and after 15 minutes of rest, using Omron® digital devices (model HEM-7113). When there was a difference greater than 20 mmHg between the two measurements, a third one was performed. To calculate the mean, for the analyses, the most discrepant value was disregarded [17].

The *NOS3; rs1799983*, *IGFBP3; rs11977526* and *TCF7L2*; *rs7903146* polymorphisms were the independent variables. For DNA extraction and polymorphisms testing, cell samples were collected from the women's oral mucosa. They were previously asked to rinse their mouth with 100 ml of distilled water and the collection was performed by scraping the cheek mucosa with small sterile cytological brushes, making circular movements (approximately 30 times). Subsequently, the bristle part of the brushes was removed and placed into 2 ml microtubes. The samples were stored in a refrigerator for subsequent DNA extraction using the salting-out method [18].

The gene encoding the endothelial nitric-oxide synthase *NOS3*, responsible for the production of NO, is located on chromosome 7 (gene locus 7q36). The *NOS3*; rs1799983 SNP is responsible for replacing a residue of glutamic acid with another of aspartic acid (Glu298Asp) [19]. The *IGFBP3* gene is responsible for encoding the insulin-like growth factor binding protein and is located on chromosome 7 (7q12.3); rs11977526, SNP, an intron variant, is in a gene locus associated with *IGFBP3* concentrations [20]. The *TCF7L2* gene is responsible for encoding the TCF7L2 protein, which acts as a transcription factor. In humans, it is located on chromosome 10 (10q25.2). The *746 C>T* (rs7903146) SNP is an intron variant [21].

The *NOS3,* rs1799983, *IGFBP3,* rs11977526, and *TCF7L2*, rs7903146 SNPs were chosen for this study after bibliographic research in a complete genome database (Genome-Wide Association Studies - GWAS) [22, 23]. Genotyping was performed using the Step One Plus™ Real-Time PCR System (Applied Biosystems, Foster City, CA, USA), based on a previously standardized protocol [18].

The following covariables were used to control possible confounding factors and characterize the sample:

Demographic variables: age (19–30, 30.1–40, and 40.1–59 years).

Socioeconomic variables: waste disposal (public garbage collection and others); participation in the "Bolsa Família" Program, which is a social assistance intervention of the federal government based on income supplementation (yes or no); employment situation (formal, informal, unemployed, retired, pensioner); per capita family income (≥1 minimum wage and <1 minimum wage); single register for social programs (yes or no); schooling level (≤4 years, >4 years); self-reported skin color (caucasian, hispanic, african, asian, indigenous).

Regarding race/color, only two categories were considered in the statistical analysis: african / hispanic and others (caucasian, asian, indigenous). This categorization was necessary because of the small number of participants who declared themselves to be asian or indigenous (n=9).

The food and nutrition security (FNS) or food and nutrition insecurity (FNI) was measured based on the Brazilian Food Insecurity Scale (EBIA), which classifies the home situation as security and mild, moderate, and severe insecurity. The validity of this instrument for quilombola communities was evidenced from the observation that families of such ethnicity were included in the sample used during the validation process [24, 25].

Variables related to health and lifestyle: Alcoholism (yes or no); smoking (yes or no); physical activity level (PAL) measured based on the results obtained by applying the International Physical Activity Questionnaire (IPAQ), short version 8, classifying women as sedentary or active, according to the obtained scores [26].

Anthropometric indicators: Body mass index (BMI; kg/m^2) and waist circumference (WC) were used. To calculate the BMI of the participants, their weight and height were measured. For body mass measurement, portable digital scales (Seca®, model 813), with a capacity of 200 kg and subdivisions of 100 g and calibrated weekly against standard weight, were used. Height was measured using a Seca® stadiometer 213, equipped with a 205 cm inextensible tape measure subdivided in millimeters. The cut-off point proposed by the World Health Organization were used, obtaining the following categories: eutrophy (18.5–24.9 kg/m^2), overweight (25.0–29.9 kg/m^2), and obesity (≥30.0 kg/m^2). Women with BMI<18.5 (n=18), which defines low body weight (thinness), were analyzed together with those classified as eutrophic. The WC was measured with the woman standing, using a non-extensible tape measure, which was positioned at the midpoint between the last rib and the iliac crest. A cut-off point ≥80 cm was used to identify high cardiovascular risk or metabolic complications associated with obesity [27].

Biochemical variables: Total cholesterol and fractions, triglycerides, and glycated hemoglobin (HbA1c) were determined without mandatory fasting. These analyses were performed using capillary blood obtained by puncturing the fingertip with disposable lancets. The lipid profile was determined in an Alere Cholestech LDX® System (Abbott, USA). Dyslipidemia was classified according to the cut-off points recommended by the Brazilian Guideline for Dyslipidemia and Atherosclerosis Prevention for samples

obtained without previous fasting, as follow: cholesterol ≥190 mg/dl, low-density lipoprotein (LDL) ≥160 mg/dl, high density lipoprotein (HDL) of 50 mg/dl, and triglycerides ≥175 mg/dl [28]. Diabetes mellitus was diagnosed based on HbA1c level of ≥ 6.5% and/or the use of oral hypoglycemic agents. HbA1c was determined using a NycoCard Reader II® device (Abbott, USA) [29].

Data processing and analysis

Double independent data entry was performed using the Epi-Info^{tm} 3.5.4 software, identifying and correcting possible typing errors, after comparison. The obtained database was exported to the Stata/SE 12.1 software for Windows (StataCorp LP, College Station, TX, USA), through which all analyses were performed.The Hardy-Weinberg equilibrium (HWE) was analyzed using the χ^2 test (p>0.05). This procedure is necessary to certify that the study population obeys the principles of population genetics and, additionally, as a measure to control the accuracy obtained in the analysis of the genotypes [30].

To investigate differences in systolic blood pressure based on the genotypes of each polymorphism, distribution adherence to parametric assumptions was verified using the Kolmogorov-Smirnov test, complemented by graphical methods. Since this assumption was not clearly evidenced, it was decided to use both forms simultaneously. Thus, the means were subjected to analysis of variance (ANOVA), and the medians were tested using the Kruskal-Wallis test. Bonferroni and Dunnet post-hoc tests were used, respectively.

Multiple analysis was performed according to an adapted hierarchical theoretical model (NETO et al., 2019) (Figure 1), composed of four levels, as follows: (1) included demographic and socioeconomic variables, (2) variables related to lifestyle, (3) included anthropometric and biochemical indicators, and (4) associations with *NOS3*, rs1799983, *IGFBP3,* rs11977526, and *TCF7L2*, rs7903146, polymorphisms were analyzed. Level 2 was adjusted by the variables of level 1 (p<0.05), level 3 was adjusted by the variables of levels 1 and 2 (p<0.05), and level 4 was adjusted by all variables (p<0.05) in the previous levels.

To identify an association between hypertension and the polymorphism genotypes, prevalence ratio (PR), and respective confidence interval (95% CI) were used, which were calculated using Poisson regression with robust variance. All covariables associated with hypertension (p<0.2) in the crude analysis were eligible for hierarchical analysis. In each of the four levels of analysis, there was a successive exclusion of non-significant variables (backward stepwise elimination), remaining only those with p<0.05. From this definition, all these variables remained in the final adjusted model, even though they have lost statistical significance in the levels following their original level, in which they were inserted as control variables.

Two models were developed to verify the association between the oxidative stress model and the polymorphisms with a value of p<0.05 in the hierarchical analysis: (1) dominant, composed of ancestral genotypes vs heterozygous genotypes + less frequent genotypes, and (2) recessive model, composed of less frequent genotypes vs ancestral genotypes + heterozygous genotypes [31].

Results

A total of 1,091 women were eligible for this research. Those who had neither anthropometric (n = 8; 0.73%) nor blood pressure (n = 4; 0.36%) data and those whose DNA was not extracted (n = 58; 5.31%) were excluded. Therefore, the analyzed final sample was composed of 1021 women (37.9 ± 10.9 years old), most of them self-declared as african / hispanic (91.1%), with per capita family income below one minimum wage (66.0%) and beneficiaries of the “Bolsa Família” Program (74.1%). Half of them had up to four years of schooling (50.1%) and 73.7% belonged to families in a situation of food insecurity.

Alcoholism and smoking were reported by 5.4% and 17.1% of women, respectively. Regarding physical activity, 61.2% of the women were classified as sedentary. The prevalence of hypertension among them was 31.4%, and 26.6% were affected by diabetes. Changes corresponding to hypertriglyceridemia and low HDL were recorded in 32.3% and 40.3% of women, respectively. High total and LDL cholesterol were recorded in 44.4% and 25.9% of the participants, respectively. The sample characterization according to socioeconomic, demographic, lifestyle, anthropometric, biochemical, and genetic variables are shown in Table 1.

Table 1
Distribution of systemic arterial hypertension the according to socioeconomic, demographic, lifestyle, anthropometric, and biochemical variables in women of African descent in the state of Alagoas (n = 1021).

Variables	No. (%)	Hypertension (%)	*P-value
Age group (years)		-	-
19–30	293 (28.7)	6.5	-
30,1–40	325 (31.8)	25.5	< 0.001
40,1–59	403(39.5)	54.3	< 0.001
Public Garbage Collection		-	-
Yes	498 (48.9)	29.6	-
No	521 (51.1)	33.3	0.194
“Bolsa Família” Program		-	-
Yes	757 (74.1)	27.2	-
No	264 (25.9)	43.6	< 0.001
Unemployed		-	-
No	551 (54.3)	34.6	0.017
Yes	463 (45.7)	27.7	-
Per capita income		-	-
≥ Minimum wage	276 (34.0)	31.5	-
< Minimum wage	536 (66.0)	33.3	0.602
Single register for social programs		-	-
Yes	801 (78.4)	28.3	-
No	220 (21.6)	42.7	< 0.001
Schooling level		-	-
> 4 anos	509 (49.9)	18.9	-
≤ 4 anos	511 (50.1)	43.8	< 0.001
Skin color (self-reported)		-	-
Others	91 (8.9)	28.6	-
African/Hispanic	928 (91.1)	31.8	0.528
Food Insecurity		-	-

Variables	No. (%)	Hypertension (%)	*P-value
No (0)	266 (26.3)	32.1	-
Yes (≥ 1)	*746* (73.7)	31.2	0.657
Alcoholism		-	-
No (0)	961 (94.6)	31.4	-
Yes (≥ 1)	55 (5.4)	32.7	0.840
Smoking (last three months)		-	-
No (0)	842 (82.9)	29.5	-
Yes (≥ 1)	174 (17.1)	41.4	< 0.002
Physical activity level		-	-
Active	394 (38.8)	31.5	-
Sedentary	622 (61.2)	31.9	0.83
BMI classification (kg/m^2)		-	-
Eutrophy (18,5 a < 25)	315 (31.3)	19.6	-
Overweight (≥ 25 a < 30)	377 (37.5)	32.6	< 0.001
Obesity (≥ 30)	313 (31.1)	42.5	< 0.001
Waist circumference		-	-
Normal (< 0.80)	325 (32.5)	17.2	-
Increased (≥ 0.80)	676 (67.5)	38.8	*< 0.001*
Diabetes Mellitus		-	-
No (0)	749 (73.4)	26.0	-
Yes (≥ 1)	272 (26.6)	46.3	< 0.001
Triglycerides (mg/dL)		-	-
Normal (< 175)	691 (67.8)	24.8	-
High (≥ 175)	329 (32.3)	45.6	< 0.001
Total cholesterol (mg/dL)		-	-
Normal (< 190)	568 (55.6)	23.9	-
High (≥ 190)	453 (44.4)	40.8	< 0.001
*LDL-C (mg/dL)		-	-

Variables	No. (%)	Hypertension (%)	*P-value
Normal (< 130)	703 (74.2)	29.5	-
High (≥ 130)	245 (25.9)	38.8	< 0.007
*HDL-C (mg/dL)		-	-
Normal (≥ 40)	609 (59.7)	26.6	-
Low (< 40)	411 (40.3)	38.4	< 0.001

LDL: low-density lipoprotein; HDL: high density lipoprotein; hypertension: systemic arterial hypertension

P-value determined by the chi-square test

The prevalences of genotypes for *NOS3*; rs1799983 SNP were GG = 47.9%, GT = 25.6% and TT = 26.5%, which was the least frequent genotype. The prevalences for *TCF7L2;* rs7903146 SNP was CC = 51.6%, CT = 29.8% and TT = 18.6%. The *IGFBP3;* rs11977526 SNP had the following prevalences: GG = 39.5%, GA = 43.8% and AA = 16.7%. There was a statistically significant difference between blood pressure levels and the *NOS3*, rs1799983 and *IGFBP3* rs11977526, SNPs, with emphasis on the genotypes: TT *NOS3*; rs1799983, SNP and AA *IGFBP3;* rs11977526 SNP.

The less frequent genotypes of the *NOS3*, rs1799983, *TCF7L2* rs7903146, and *IGFBP3* rs11977526, SNPs were associated with a higher prevalence of hypertension in comparison with the ancestral and heterozygous genotypes. The distribution of polymorphisms in accordance with the Hardy-Weinberg equilibrium (Table 2).

Table 2
– Prevalence of arterial hypertension (%) and systolic blood pressure (mean ± SD; median and interquartile range), according to the genotypic frequencies for *NOS3 rs1799983, TCF7L2 rs7903146*, and *IGFBP3* rs11977526 genes. Brazilian women descended from African descent, 2018.

Genotype	Genotype frequency (%)	Hypertension (%)	Systolic blood pressure[x]	
			Mean ± SD	Median (P25 – P75)
NOS3 **rs1799983**[a]				
GG	489 (47.9)	24.5	123.8 ± 17.9	120.5 (112.5–131.0)
GT	261 (25.6)	23.0	124.7 ± 18.8	121.0 (112.5–132.0)
TT	271 (26.5)	52.0	132.2 ± 21.9*	129.0 (114.5–147.5)**
			p < 0.001 (Anova)	p = 0.0001 (K-W)
TCF7L2 **rs7903146**[b]				
CC	527 (51.6)	27.9	125.44 ± 19.21	122.0 (113.0–133.0)
CT	304 (29.8)	32.9	125.86 ± 19.13	121.0 (113.5–134.8)
TT	190 (18.6)	38.9	129.13 ± 21.15	124.5 (113.5–140.0)
			p = 0.0775 (Anova)	p = 0.1118 (K-W)
IGFBP3 **rs11977526**[c]				
GG	403 (39.5)	24.1	123.49 ± 18.04	120.0 (111.5–129.5)
AG	447 (43.8)	26.4	125.49 ± 19.04	121.0 (112.5–134.0)
AA	171 (16.7)	62.0	134.78 ± 22.13*	131.0 (118.5–147.5)**
			p < 0.001 (Anova)	p = 0.0001 (K-W)

[a, b, c] Hardy Weinberg Equilibrium:

NOS3, rs1799983: chi² =220.1159, *p* = 0.606

TCF7L2, rs7903146: chi²=112.3333, *p* = 0.665

IGFBP3; rs11977526: chi² =6.009 *p* = 0.613

 One-sample Kolmogorov-Smirnov test against normal theoretical distribution: p < 0.001 (systolic blood pressure distribution differs significantly from Gaussian distribution).

* Differs significantly from the others (*p* < 0.01 according to the Bonferroni test).

K-W = Kruskal-Wallis test.

** Differs significantly from the others (*p* < 0.01 according to the Dunnet test with Bonferroni correction). Results similar to those recorded for distribution of prevalence were found when analyzing the measures of central tendency (mean and median) related to systolic blood pressure (Table 2). The values found for genotypes of *NOS3*, rs1799983 and *IGFBP3* rs11977526 were significantly higher than those obtained for the other genes, both in the ANOVA, in the Kruskal-Wallis nonparametric test. For the *TCF7L2*, rs7903146 SNP, PAS levels were considered statistically similar (*p* > 0.05 in both analyses).

For the *NOS3*, rs1799983 SNP, the prevalences of hypertension among women with GG, GT and TT genotypes were 24.5%, 23.0% and 52.0%, respectively, with a statistically significant difference between TT and GG (PR = 1.54; 95% CI = 1.27–1.86; *p* < 0.001). Regarding *IGFBP3;* rs11977526 SNP, the prevalences of hypertension among women with GG, GA and AA genotypes were 24.1%, 26.4% and 62.0%, respectively, with a statistically significant difference between AA and GG (PR = 1.59; 95% CI = 1.27–1.98; *p* < 0.001). For *TCF7L2;* rs7903146 SNP, hypertension among women with CC, CT and TT genotypes had prevalences of 27.9%, 32.9% and 38.9%, respectively, with no statistical difference between TT and CC (PR = 0.86; 95% CI = 0.69–1.06; *p* = 0.166) (Table 3).

Table 3
– Prevalence ratios (PR) and respective 95% confidence intervals (95% CI) obtained by multivariable Poisson regression, according to the hierarchical theoretical model for determining arterial hypertension.

Variables	Level 1 PR (95% CI)	P value	Level 2 *PR (95% CI)	P value	Level 3 *PR (95% CI)	P value	Level 4 PR (95% CI)	P-value
Level 1								
Age group: *30.1–40*	3.95 (2.46–6.34)	< 0.001	3.95 (2.46–6.34)	< 0.001	3.54 (2.20–5.69)	< 0.01	3.36 (2.10–5.36)	< 0.01
Age group: *40.1–50*	8.06 (5.16–12.60)	< 0.001	8.06 (5.16–12.60)	< 0.001	7.33 (4.69–11.48)	< 0.01	6.78 (4.35–10.59)	< 0.01
Insertion in the formal labor market	1.18 (0.99–1.41)	0.050	1.18 (0.99–1.41)	0.051	1.17 (0.99–1.40)	0.06	*	*
Schooling level: ≤ 4 years	1.21 (0.99–1.48)	0.060	1.21 (0.99–1.48)	0.056	*	*	*	*
“Bolsa Família” Program: yes	1.20 (1.01–1.41)	0.037	1.20 (1.01–1.41)	0.037	1.15 (0.98–1.36)	0.094	1.12 (0.96–1.31)	0.156
Single register for social programs: yes	1.06 (0.78–1.44)	0.680	*	*	*	*	*	*
Level 2								
Smoking	-	-	0.99 (0.77–1.13)	0.48	*	*	*	*
Level 3								
BMI Overweight (≥ 25–<30)					1.26 (0.98–1.61)	0.067	1.29 (1.02–1.64)	0.033
BMI Obesity (≥ 30)	-	-	-	-	1.63 (1.29–2.06)	< 0.001	1.63 (1.29–2.06)	< 0.001
Waist circumference ≥ 80 cm	-	-	-	-	1.02 (0.72–1.43)	0.091		*

Variables	Level 1 PR (95% CI)	P value	Level 2 *PR (95% CI)	P value	Level 3 *PR (95% CI)	P value	Level 4 PR (95% CI)	P-value
Triglycerides ≥ 175 mg/dL	-	-	-	-	1.05 (0.88–1.26)	0.551	*	*
Total cholesterol ≥ 190 mg/dL	-	-	-	-	1.20 (0.96–1.49)	0.101	*	*
**LDL-C (mg/dL)* ≥ 160 mg/dL	-	-	-	-	0.90 (0.73–1.12)	0.384	*	*
**HDL-C (mg/dL) > 50 mg/dL*	-	-	-	-	1.27 (1.08–1.50)	0.003	1.24 (1.06–1.45)	0.007
Diabetes mellitus	-	-	-	-	1.28 (1.09–1.51)	0.003	1.16 (0.99–1.37)	0.060
Level 4								
TCF7L2								
CC	-	-	-	-	-	-	1	-
CT	-	-	-	-	-	-	1.04 (0.78–1.19)	0.623
TT	-	-	-	-	-	-	0.86 (0.69–1.06)	0.166
NOS3								
GG	-	-	-	-	-	-	1	-
GT	-	-	-	-	-	-	0.95 (0.74–1.23)	0.755
TT	-	-	-	-	-	-	1.54 (1.27–1.86)	< 0.001
IGFBP3								
GG	-	-	-	-	-	-	1	-

Variables	Level 1 PR (95% CI)	P value	Level 2 *PR (95% CI)	P value	Level 3 *PR (95% CI)	P value	Level 4 PR (95% CI)	P-value
AG	-	-	-	-	-	-	0.96 (0.78–1.19)	0.751
AA	-	-	-	-	-	-	1.59 (1.27–1.98)	< 0.001

LDL: low-density lipoprotein; HDL: high density lipoprotein; Prevalence ratios and respective 95% confidence intervals (95% CI)

After the adjusted analysis, older age group, family beneficiary of the “Bolsa Família” Program, obesity (BMI ≥ 30 kg/m^2), low HDL-C level, and diabetes mellitus were significantly associated with hypertension.

The dominant and recessive model analysis was performed using the following risk factors: age group (30.1–40.0), age group (40.1–59.0), “Bolsa Família” Program, overweight (BMI ≥ 25), HDL-C, and glycated hemoglobin. In the dominant model of oxidative stress (*NOS3*: GG + *IGFBP3*: GG vs *NOS3* GT + TT; *IGFBP3*: AG + AA), women had a prevalence of 34.7% hypertension (PR = 1.42; 95% CI: 1.12–1.79; p = 0.003). However, in the recessive model of oxidative stress (*NOS3*: TT + *IGFBP3*: AA vs *NOS3*: GG + GT *IGFBP3*: GG + AG), they had a prevalence of 77.0% hypertension (PR = 2.07; 95% CI: 1.78–2.42 p < 0.001). There was a statistical significance even after adjusting for all risk factors, in the dominant and recessive models, referring to the *NOS3* and *IGFBP3* genes (Table 4).

Table 4
– Dominant and recessive models for determining systemic arterial hypertension in Brazilian women of African descent.

Models	Hypertension (%)	*PR (95% CI)	P-value
Dominant			
(NOS3 GG) + (IGFBP3 GG)	21.3	1	-
(NOS3 TT + GT) + (IGFBP3 AG + AA)	34.7	1.42 (1.12–1.79)	0.003
Recessive			
(NOS3 GG + GT) +(IGFBP3 GG + AG)	26.5	1	-
(NOS3 TT) + (IGFBP3 AA)	77.0	2.07 (1.78–2.42)	< 0.001

Prevalence ratios (PR) and respective 95% confidence intervals (95% CI) adjusted by Poisson regression using the risk factors: age group (30.1–40.0), age group (40.1–59, 0), income supplementation program, overweight (BMI ≥ 25–<30), obesity (BMI ≥ 30), HDL-C and diabetes mellitus; * hypertension: systemic arterial hypertension

Discussion

This study provides evidence that, in Afro-descendant women from northeastern Brazil, the *NOS3*, rs1799983 and *IGFBP3*, rs11977526, SNPs gene are associated with higher blood pressure levels and, consequently, with a higher prevalence of arterial hypertension. This same relationship had already been observed for the *TCF7L2* rs7903146, SNP only in the crude analysis because after adjusted analysis there was no significance.

A study in an African population investigated whether biomarkers of endothelial function were related to the bioavailability of IGF-1 (IGF-1, IGFBP3, or IGF-1/IGFBP3M ratio) and showed that the bioavailable IGF-1, measured by the IGF1/IGFBP3 ratio, is beneficially associated with CAV-1, which is a biomarker of endothelial activation [32]. Also, bioavailable IGF-1 tended to be inversely associated with ICAM-1, another marker of endothelial activation, thereby increasing the expression of CAV-1 and ICAM-1 [11, 33].

Previous studies indicated that some SNPs in the *IGFBP3* and *NOS3* genes are associated with decreased serum levels of these proteins [34–36]. Meta-analysis data from 11,700 pregnant women with gestational-induced hypertension, in African and Latin American populations, showed a relationship between G*894*T polymorphism and susceptibility to this type of hypertension in the dominant model [37]. Also, several studies showed a strong association between *894 G > T* polymorphism of *NOS3* gene and hypertension [18, 38, 39].

Research on SNPs of the *IGFBP3* rs11977526 gene and hypertension indicated an association of these factors, as found in a study involving East African people, was associated with the risk of such disease [40] [41].

In this study, there was a higher predisposition for hypertension in the presence of TT and AA genotypes for the *NOS3* and *IGFBP3* genes, respectively. In the case of heterozygous Afro-descendant women (GT of the *NOS3* gene and AG of the *IGFBP3* gene), no statistically significant difference was found between the prevalence of hypertension. However, when analyzing the dominant and recessive models for oxidative stress, even after adjusting the risk factors, a significance was found for *NOS3* and *IGFBP3* models. Therefore, these data show that the presence of the TT genotype of the *NOS3* gene and the AA genotype of the *IGFBP3* gene constitutes an important risk factor for arterial hypertension.

The SNP rs1799983, variant of the *NOS3* gene causes a change in which the amino acid Asp is replaced by Glu at position 298. This substitution is associated with a decrease in protein stability [15, 42]. The AA genotype of the *IGFBP3* gene has been shown to regulate protein expression through miRNAs by destabilizing the mRNA, which is associated with a decrease in IGFBP3 serum levels [20, 41, 43].

In our analysis, the dominant and recessive showed a strong association of T and A alleles of the *NOS3* and *IGFBP3* genes, respectively, with hypertension. CAV-1 is the main link between the *NOS3* gene and *IGFBP3* because it physically interacts with these gene regions, making possible a co-expression between the two proteins [44–46]. Recently, some studies have shown that CAV-1, which is a protein responsible

for regulating eNOS function, is closely linked to IGF-1 and IGFBP3, regulating endothelial cell proliferation, vascular development, and oxidative stress [47–52].

The SNPs of *NOS3*, rs1799983, *TCF7L2* rs7903146, and *IGFBP3* rs11977526, had genotypic frequencies similar to those observed in studies performed in African populations [40, 53–55]. This study was part of a broader project, which aimed to diagnose nutritional and health conditions of the maternal and child population of quilombola communities in the state of Alagoas. Thus, it did not provide for the inclusion of men and elderly people. Therefore, the absence of men is a limitation of this research. Due to the differences in the occurrence of hypertension by gender, further studies including male participants should be conducted.

Conclusion

The TT genotype of the *NOS3* rs1799983 gene and the AA genotype of the *IGFBP3* rs11977526 gene are associated with a higher prevalence of arterial hypertension and are important risk factors for this condition, especially when associated with higher age groups, excess of body weight, diabetes, and dyslipidemia. Considering that the mechanism of action, responsible for higher blood pressure levels in women with the TT (*NOS3*) and AA (*IGFBP3*) genotypes, involves less metabolic production of nitric oxide and, consequently, an increase in oxidative stress, the results presented here suggest that these SNPs are directly related to blood pressure regulation.

Future molecular studies are needed to reveal the important roles of eNOS and IGFBP3 when they are related to hypertension. Association studies such as the one presented here are of great relevance for motivating research aimed to elucidate the molecular pathways involved in the etiology of hypertension and, consequently, in the development of new drugs related to these pathways.

Abbreviations

Asp: Aspartic acid

BMI: Body mass index

CI: Confidence interval

CAV-1: Caveolin-1

DNA: Deoxyribonucleic acid

DM: Diabetes mellitus

DBP: diastolic blood pressure

EBIA: Brazilian Food Insecurity Scale

eNOS: Nitric oxide synthase

FNS: Food and nutrition security

FNI: Food and nutrition insecurity

Glu: Glutamic acid

GWAS: Genome-Wide Association Studies

HbA1c: Glycated hemoglobin

HDL: High density lipoprotein

HWE: Hardy-Weinberg equilibrium

ICAM-1: Intercellular Adhesion Molecule 1

IGF-1: Insulin-like growth factor-1

IGFBP3: Insulin-like growth factor binding protein -3

IPAQ: Physical Activity Questionnaire

LDL: Low-density lipoprotein

NO: Nitric oxide

PAL: Physical activity level

PR: Prevalence ratio

SBP: systolic blood pressure

SNP: Single nucleotide polymorphism

WC: waist circumference

Declarations

Ethics approval and consent to participate

Ethics approval for this study was obtained from the Ethics Committee of Federal University of Alagoas, Brazil (No: 33527214.9.0000.5013). The study conformed to the principles of the Declaration of Helsinki. Written informed consent was obtained from all individual participants included in the study.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

A.B.L.N. participated in designing the study, analysis and interpretation of data and drafting the article. N.B.R.V, T.R.S, L.E.C.D, C.S.M and M.L.A. took part in the acquisition of data, data entry, analysis and interpretation and writing. H.S.F. took part in the project's conception and obtained the respective financial support, coordinating all implementation steps and realized the final review of the article. All authors approved the final version to be submitted.

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Figures

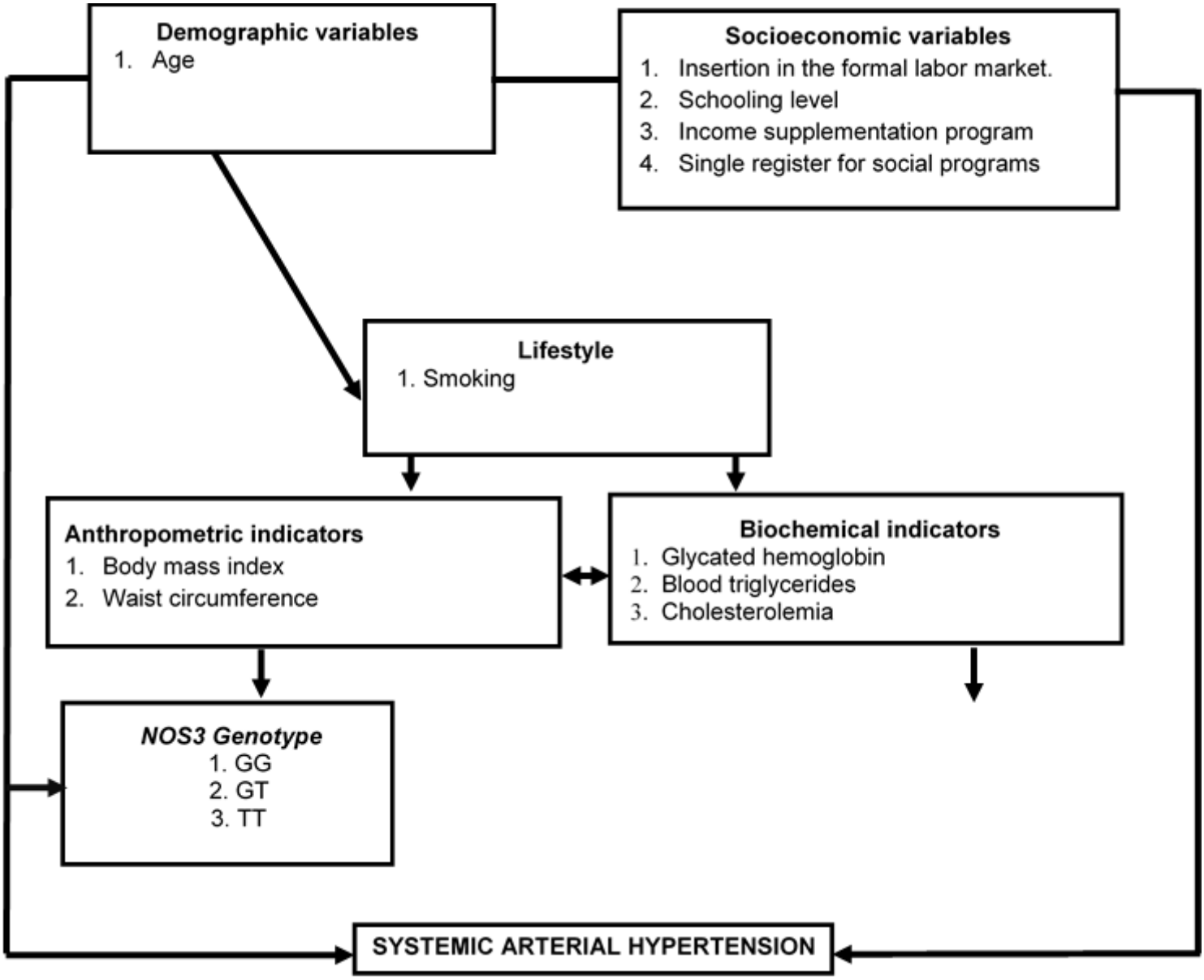

Figure 1

Hierarchical conceptual model explaining systemic arterial hypertension (Adapted from Neto et al., 2019).