Hierarchical Analysis of Hypertension with the Polymorphic Variant of the ACE Gene and Other Risk Factors in the Elderly

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Abstract

Background: Hypertension is a clinical condition of multifactorial etiology that imposes serious harm to the health of elderly individuals. Despite the fact that cardiovascular disorders influence the development of hypertension in this age group, several other genetic and environmental factors have been described in the literature, including the polymorphic variant of the angiotensin-converting enzyme (ACE) gene and the distribution of body fat.

Objective: To assess the prevalence of hypertension among elderly individuals and its possible correlation with the I/D polymorphic variant of the ACE gene and other associated risk factors.

Methods: Population-based study using a hierarchical model and including 387 elderly individuals residing in the urban area of Ibiaí (Minas Gerais, Brazil).

Results: On statistical analysis, the polymorphic variant of the ACE gene was not associated with hypertension (p = 0.316). On the other hand, there was a significant association between hypertension and the variables female sex, absence of a partner, consumption of more than one portion of salt per day, and changes in body mass index and waist-to-hip ratio.

Conclusion: Although the polymorphic variant of the ACE gene showed no influence on the prevalence of hypertension in elderly individuals, some variables such as individual, socioeconomic, metabolic, and behavioral habits were associated with this condition. (Int J Cardiovasc Sci. 2017;30(1):52-60)

Keywords: Hypertension; Peptidyl-Dipeptidase A; Polymorphism, Genetic; Aged; Risk Factors; Prevalence.
(RAS), converts the decapeptide angiotensin I into the octapeptide angiotensin II, the most powerful circulating vasoconstrictor in the human body. This way, ACE plays a fundamental role in BP control.6 Intraindividual serological levels of ACE remain stable, but its interindividually variation is high and is attributed to polymorphic ACE gene variants (DD > DI > II).7 It should be pointed out the lack of recent studies evaluating the association of these polymorphic variants with hypertension exclusively in elderly individuals.8

In addition to genetic factors, other factors have been reported as being a risk for hypertension: age above 60 years, gender (postmenopausal women), ethnicity (non-white skin color), diet, sedentary lifestyle, obesity, alcoholism, and smoking, among others.9 The interaction of these various factors over time contributes to the development and worsening of hypertension, and the greater the number of risk factors to which individuals are exposed, the higher their chance of becoming hypertensive.10

Therefore, the objective of this study was to assess the prevalence of hypertension among elderly individuals, and its possible correlation with the polymorphic variant I/D of the ACE gene and with other associated risk factors.

Methods

Study design

This was an epidemiological, cross-sectional, and population study including elderly individuals aged 60 years or more, resident and domiciled in the municipality of Ibaiá, north of Minas Gerais (MG), Brazil.

Procedure and instrument for data collection

The data collection was carried out between 2011 and 2012. The elderly individuals were evaluated by health professionals who were trained and calibrated (kappa = 0.63) to the demographic, socioeconomic, behavioral, anthropometric, and laboratory characteristics, and genotypic variant of the polymorphic ACE. According to the 2010 census, a total of 505 elderly individuals aged 60 or more years were registered in the Primary Care Information System. Of the 479 names obtained from the Family Health Strategy (FHS) list, 449 (93.74%) were found. The registration also included 27 elderly people whose names were not listed, but who were nonetheless also added, yielding a total of 476 elderly individuals. Of these, 387 (84.31%) answered the questions and/or were assessed in regards to the main variable of interest in this study (hypertension).

The measurement of BP levels was conducted by the auscultatory technique with a calibrated sphygmomanometer. The BP measurements were performed with the individual seated and after a 5-minute rest. The individuals were questioned about being with an empty bladder, having avoided physical activity, food, smoking, ingestion of alcoholic beverages or coffee (at least 30 minutes before the measurement) and if they had taken drugs that could interfere with the mechanisms of BP regulation.11 A total of three BP measurements were obtained; the first was discarded and the mean of the second and third measurements was considered. The patients were instructed about the procedure beforehand, with an explanation of its steps and objectives. The diagnosis of hypertension was established in the presence of a mean diastolic BP ≥ 90 mmHg and/or mean systolic BP ≥ 140 mmHg and/or use of systemic antihypertensive medication.12

To achieve the objective of the study, the variables were grouped into individual, socioeconomic, behavioral, metabolic, and health-related, as described below:

Individual: age (60 to 69 years and ≥ 70 years), sex (male and female), self-declared skin color (White/Yellow and Brown/Black), I/D polymorphic variant (rs 4646994) of the ACE gene (II, DI, and DD).

Socioeconomic: marital status (with a partner [married and stable union] and without a partner [single, widowed/divorced]), education (≥ 4 years and 0 to 3 years), household crowding (less than one person/room and more than one person/room), material goods (at least one material goods [house and/or automotive] or lack of material goods), work status (yes [employed, self-employed, informal work, retired/employed] and no [retired/without work, does not work/never worked, and unemployed]), and income (assessed by the monthly individual income in Brazilian real).

Behavioral disorders: dietary factors (consumption of fruits and vegetables [more than 3 servings/day and less than 3 servings/day] and salt intake [one portion per day and more than one portion per day]),12 smoking (non-smokers and smokers), alcoholism (never consumed versus consumption of alcohol), physical activity (active [moderately active, active, and very active] and not active [inactive and little active]).
**Metabolic and health-related:** optimal total cholesterol (<200 mg/dL), optimal high-density lipoprotein (HDL; women ≥ 50 mg/dL and men ≥ 40 mg/dL), optimal low-density lipoprotein (LDL; <130 mg/dL), optimal triglycerides (<150 mg/dL), and optimal waist-to-hip ratio (WHR; women < 0.85 and men < 0.95). We evaluated the glomerular filtration rate (GFR) using the Cockcroft-Gault formula: 

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\text{GFR (mL/min)} = \frac{(140 - \text{age}) \times \text{weight (kg)}}{72 \times \text{serum creatinine}} \times 0.85 \quad \text{(for female individuals)}
\]

In which values < 60 mL/min/1.73 m² are considered abnormal and ≥ 60 mL/min/1.73 m² are considered normal.13

The anthropometric variables weight and height were used to calculate the body mass index (BMI), with results < 25 kg/m² considered as optimal.

**ACE polymorphism evaluation – polymerase chain reaction**

In order to investigate the genotypic frequencies of the polymorphic variant I/D (rs 4646994) of the ACE gene (II, DI, and DD), saliva samples were obtained. Gene samples were obtained with a swab from buccal mucosa smears, which was stored at a temperature of -20 ºC in test tubes with Krebs solution. The samples were isolated with silica particles, which absorb the DNA. Subsequently, the DNA was washed to remove impurities and suspended with TE buffer. The genomic DNA was amplified with the polymerase chain reaction (PCR) technique.

**Statistical analysis**

All data were tabulated and analyzed using the program Statistical Package for the Social Sciences for Windows, version 20.0. The variables investigated were described as distributions of frequencies. In the univariate analysis, we estimated the crude odds ratios values with 95% confidence intervals. The variables with descriptive level p ≤ 0.20 were selected for the multivariate analysis. In the multivariate analysis, we adopted the model of hierarchical logistic regression, and the order of entry of the blocks was determined from a theoretical model and involved known factors associated with hypertension.11

For the hierarchical analysis in this study, we followed the diagram shown in Figure 1, which guided the order of entry of the variables blocks in the model. The block “individual factors” was the first to be included in the model and remained as a factor of adjustment for the other variables. We then included “socioeconomic factors”, and only those presenting a descriptive level p < 0.05 remained in the model after adjustment for the variables in the “individual factors” block. After that, we included the “behavioral habits” block and maintained in the model only those variables with descriptive level p < 0.05 after adjustment for “individual factors” and “socioeconomic factors”. For inclusion of the remaining blocks, we adopted the same procedure of the previous blocks.

**Ethical aspects**

The project was conducted in accordance with the Resolution 196/96 of the National Health Council of the Ministry of Health and presented a favorable opinion of the Research Ethics Committee at the Universidade Estadual de Montes Claros (CEP/Unimontes) with the unified opinion number 2903/11. The elderly subjects signed a free and informed consent form agreeing to participate in the study.

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**Figure 1** – Hierarchical model of variables analyzed in elderly individuals in Ibiaí municipality, MG.

ACE: angiotensin-converting enzyme; HDL: high-density lipoprotein; LDL: low-density lipoprotein.
Results

The prevalence of hypertension among the 387 elderly individuals evaluated was 76%. As for the individual characteristics, the following ones prevailed: age range of 60-69 years (n = 131, 78.7%), females (n = 184, 80.7%), skin color brown/black (n = 252, 77.3%), and genotype II (n = 67, 82.7%). Most subjects lived without a partner (n = 155, 82%), had a low education level (n = 231, 76.2%), lived in households with little crowding (n = 251, 75.8%), had at least one material goods (n = 269, 75.8%), did not work (n = 212, 77.4%), and had an average income of R$ 531.97 ± 219.86 (Table 1).

As for behavioral habits, low consumption of vegetables (n = 225, 76.5%) stood out, whereas the consumption of salt was within the level considered as ideal (n = 206, 80.2%). Most participants did not consume alcohol (n = 173, 77.6%) and were not smokers (n = 178, 78.8%). Among metabolic and health-related conditions, most subjects showed change in BMI (n = 134, 81.2%), WHR (n = 209, 80.1%), and GFR (n = 143, 76.9%) (Table 2).

In the univariate analysis, the age range of 60-69 years showed a positive association with hypertension, with women having a greater chance of hypertension when compared with men. Elderly individuals without a partner also showed this association, as well as those who had altered BMI and WHR (Tables 1 and 2). In this study, the distribution of the polymorphic variant I/D of the \textit{ACE} gene showed no association with hypertension (p = 0.316).

In the multiple logistic regression analysis after hierarchical adjustment, the following variables were maintained in the model after presenting statistically significant differences: gender, marital status, and WHR. The remaining variables lost the effect after this adjustment (Table 3).

Discussion

The hierarchical approach is an alternative applicable to epidemiological studies with a large number of variables. This study attempted to establish an interrelationship between several risk factors and at various levels, evaluating the importance of each block on hypertension. The main findings of this study indicate a prevalence of 76% of hypertensive elderly patients in the population studied, with a significant association with greater involvement of the disease in elderly individuals who were female, had no partner, consumed more than one portion of salt per day, and with changes in the WHR and BMI indices.

The present study found no association between hypertension and the I/D polymorphic variant of the \textit{ACE} gene. Studies assessing the association of this polymorphic variant with hypertension in elderly individuals are scarce and show no relationship; in this sense, this study corroborated the results of this literature suggesting, perhaps, a slight genetic influence of this variant in increased BP in this age range.\textsuperscript{14}

Our findings corroborate those of another Brazilian epidemiological study that showed a prevalence of hypertension of approximately 65% in the elderly, with a higher prevalence among women older than 75 years, potentially reaching 80%\textsuperscript{15}. In the present research, this clinical condition was similar to that observed in other population studies.\textsuperscript{9,16}

The greatest chance of presenting hypertension was observed in women (p = 0.009). The literature highlights a greater prevalence of hypertension among elder women when compared with men at the same age.\textsuperscript{9} A previous study had already demonstrated this greater probability of association with hypertension.\textsuperscript{17} Up until menopause, women are hemodynamically younger than men at the same age and, thus, less vulnerable to BP increases. However, after menopause and due to weight gain and hormonal changes, women become more vulnerable to increased BP.\textsuperscript{18}

Despite the direct and linear relationship of BP with increased age, this association did not remain, possibly due to the fact that the age range in this population was more homogeneous. The literature highlights a greater chance of hypertension in Blacks.\textsuperscript{19} In the present study, the skin color was not associated with hypertension, which was comparable with another study that included this variable in its analyses.\textsuperscript{17} This shows that the extension of the impact of the Brazilian miscegenation on hypertension is still unclear. A recent study on the genomic ancestry in our country observed that the Brazilian genome is much more uniform than otherwise expected with its large racial mixture over the past two centuries.\textsuperscript{20}
Table 1 – Distribution of non-hypertensive and hypertensive elderly individuals according to individual and socioeconomic factors (Ibiaí, MG)

|                | Non-hypertensive | Hypertensive | Total | OR \( \text{crude} \) | 95%CI | p value |
|----------------|------------------|--------------|-------|-------------------------|-------|---------|
| **Individual factors** |                  |              |       |                         |       |         |
| Age            |                  |              |       |                         |       |         |
| 60-69 years    | 44               | 21.3         | 163   | 78.7                    | 207   | 1.00    |
| ≥ 70 years     | 49               | 27.2         | 131   | 71.8                    | 180   | 1.39 (0.87-2.21) |
| Sex            |                  |              |       |                         |       |         |
| Male           | 49               | 30.8         | 110   | 69.2                    | 159   | 1.00    |
| Female         | 44               | 19.3         | 184   | 80.7                    | 228   | 1.86 (1.16-2.98) |
| Skin color     |                  |              |       |                         |       |         |
| White/Yellow   | 19               | 31.1         | 42    | 68.9                    | 61    | 1.00    |
| Brown/Black    | 74               | 22.7         | 252   | 77.3                    | 326   | 1.54 (0.84-2.80) |
| ACE*           |                  |              |       |                         |       |         |
| II             | 14               | 17.3         | 67    | 82.7                    | 81    | 1.00    |
| DI             | 31               | 24.8         | 94    | 75.2                    | 125   | 0.63 (0.31-1.28) |
| DD             | 35               | 25.9         | 100   | 74.1                    | 135   | 0.60 (0.30-1.19) |
| **Socioeconomic factors** |              |              |       |                         |       |         |
| Marital status |                  |              |       |                         |       |         |
| With a partner | 59               | 29.8         | 139   | 70.2                    | 198   | 1.00    |
| Without a partner | 34             | 18.0         | 155   | 82.0                    | 189   | 1.93 (1.20-3.13) |
| Education*     |                  |              |       |                         |       |         |
| ≥ 4 years      | 19               | 26.4         | 53    | 73.6                    | 72    | 1.00    |
| 0-3 years      | 72               | 23.8         | 231   | 76.2                    | 303   | 1.15 (0.64-2.07) |
| Home crowding* |                  |              |       |                         |       |         |
| < 1 person/room | 80              | 24.2         | 251   | 75.8                    | 331   | 1.00    |
| ≥ 1 person/room | 12              | 25.5         | 35    | 74.5                    | 47    | 0.93 (0.46-1.88) |
| Material goods* |                 |              |       |                         |       |         |
| Has            | 86               | 24.2         | 269   | 75.8                    | 355   | 1.00    |
| Does not have  | 6                | 26.1         | 17    | 73.9                    | 23    | 0.90 (0.35-2.37) |
| Work*          |                  |              |       |                         |       |         |
| Works          | 30               | 29.1         | 73    | 70.9                    | 103   | 1.00    |
| Does not work  | 62               | 22.6         | 212   | 77.4                    | 274   | 1.40 (0.84-2.34) |
| Income (mean ± SD) | 511.73±506.42 | 531.97±219.86 | 1,000 | 1.00 (0.99-1.00) | |

*OR = odds ratio; CI = confidence interval; ACE = angiotensin-converting enzyme; SD = standard deviation.*
Table 2 – Distribution of non-hypertensive and hypertensive elderly individuals according to behavioral, metabolic, and health-related factors (Ibiaí, MG)

| Behavioral habits                | Non-hypertensive | Hypertensive | Total | OR_{crude} | 95% CI | p value |
|----------------------------------|------------------|--------------|-------|------------|--------|---------|
| Fruit*                           |                  |              |       |            |        |         |
| More than 3x/day                 | 11               | 39           | 50    | 1.00       |        | 0.756   |
| Less than 3x/day                 | 73               | 231          | 304   | 0.89       | 0.43-1.83 | 0.800   |
| Vegetables*                      |                  |              |       |            |        |         |
| More than 3x/day                 | 15               | 45           | 60    | 1.00       |        |         |
| Less than 3x/day                 | 69               | 225          | 294   | 1.09       | 0.57-2.07 | 0.004   |
| Salt intake*                     |                  |              |       |            |        |         |
| One portion/day                  | 51               | 206          | 257   | 1.00       |        | 0.099   |
| More than one portion/day        | 33               | 63           | 96    | 0.47       | 0.28-0.80 | 0.126   |
| Alcoholism*                      |                  |              |       |            |        |         |
| Does not drink alcohol           | 50               | 173          | 223   | 1.00       |        |         |
| Drinks alcohol                   | 15               | 29           | 44    | 0.56       | 0.28-1.12 | 0.000   |
| Smoking*                         |                  |              |       |            |        |         |
| Non-smokers                      | 48               | 178          | 226   | 1.00       |        |         |
| Smokers                          | 44               | 113          | 157   | 0.69       | 0.43-1.11 | 0.126   |
| Physical activity*               |                  |              |       |            |        |         |
| Active                           | 22               | 69           | 91    | 1.00       |        | 0.000   |
| Inactive                         | 69               | 216          | 285   | 1.00       | 0.57-1.73 | 0.995   |

| Metabolic and health-related conditions | Non-hypertensive | Hypertensive | Total | OR_{crude} | 95% CI | p value |
|-----------------------------------------|------------------|--------------|-------|------------|--------|---------|
| WHR*                                    |                  |              |       |            |        |         |
| Normal                                  | 41               | 68           | 109   | 1.00       |        | 0.000   |
| Altered                                 | 52               | 209          | 261   | 2.42       | 1.48-3.96 | 0.000   |
| BMI*                                    |                  |              |       |            |        |         |
| Normal                                  | 55               | 131          | 186   | 1.00       |        | 0.019   |
| Altered                                 | 31               | 134          | 165   | 1.81       | 1.10-3.00 | 0.000   |
| Cholesterol*                            |                  |              |       |            |        |         |
| Optimal                                 | 42               | 123          | 165   | 1.00       |        | 0.807   |
| Risk                                    | 40               | 110          | 150   | 0.94       | 0.57-1.55 | 0.156   |
| HDL*                                    |                  |              |       |            |        |         |
| Optimal                                 | 35               | 78           | 113   | 1.00       |        | 0.156   |
| Risk                                    | 44               | 143          | 187   | 1.46       | 0.86-2.46 | 0.939   |
| LDL*                                    |                  |              |       |            |        |         |
| Optimal                                 | 40               | 113          | 153   | 1.00       |        | 0.807   |
| Risk                                    | 39               | 108          | 147   | 0.98       | 0.59-1.64 | 0.151   |
| Triglycerides*                          |                  |              |       |            |        |         |
| Optimal                                 | 38               | 87           | 125   | 1.00       |        | 0.151   |
| Risk                                    | 43               | 143          | 186   | 1.45       | 0.87-2.42 | 0.151   |
| GFR*                                    |                  |              |       |            |        |         |
| Normal                                  | 38               | 87           | 125   | 1.00       |        | 0.151   |
| Altered                                 | 43               | 143          | 186   | 1.45       | 0.87-2.42 | 0.151   |

*n < 387. OR: odds ratio; CI: confidence interval; WHR: waist-to-hip ratio; BMI: body mass index; HDL: high-density lipoprotein; LDL: low-density lipoprotein; GFR: glomerular filtration rate.
### Table 3 – Results of the hierarchical logistic multiple regression analysis (Ibiaí, MG)

| Block 1 - Individuals\(^1\) | Model 1 |
|----------------------------|---------|
|                            | Block 1 |
|                            | OR\(_{\text{crude}}\) | OR\(_{\text{adjusted}}\) | 95%CI | p value | \(\chi^2\) |
| Sex                       |         |         |      |         |         |
| Male                      | 1.00    | 1.00    |      | 0.402   | 0.009   |
| Female                    | 1.39    | 1.88 (1.17-3.02) |      |         |         |

| Block 2 - Socioeconomic\(^2\) | Model 2 |
|-------------------------------|---------|
|                               | Blocks 1 and 2 |
| Sex                           |         |         |      |         |         |
| Male                          | 1.00    | 0.567   |      |         |         |
| Female                        | 1.59 (0.97-2.63) |      |         |         |
| Marital status                |         |         |      |         |         |
| With a partner                | 1.00    | 1.00    |      | 0.032   |         |
| Without a partner             | 1.93    | 1.73 (1.05-2.86) |      |         |         |

| Block 4 - Metabolic and health-related\(^3\) | Model 3 |
|---------------------------------------------|---------|
|                                             | Blocks 1, 2, and 4 |
| Sex                                         |         |         |      |         |         |
| Male                                        | 1.00    | 0.561   |      |         |         |
| Female                                      | 1.03 (0.52-2.02) |      |         |         |
| Marital status                              |         |         |      |         |         |
| With a partner                              | 1.00    | 0.126   |      |         |         |
| Without a partner                           | 1.59 (0.88-2.89) |      |         |         |
| WHR                                         |         |         |      |         |         |
| Normal                                      | 1.00    | 1.00    |      |         |         |
| Altered                                     | 2.42    | 2.01 (1.00-4.04) |      | 0.050   |         |

\(OR\): odds ratio; CI: confidence interval; \(\chi^2\): Hosmer-Lemeshow test; WHR: waist-to-hip ratio.

\(^1\)Adjusted by individual variables among each other; \(^2\)Set by individual factors; \(^3\)Adjusted by personal and socioeconomic factors.

After control for socioeconomic factors, the variable gender lost the strength of its association (\(p = 0.067\)), while marital status (\(p = 0.032\)) was shown to be statistically significant. There are few studies specifically correlating marital status with hypertension.\(^21,22\) In the present study, elderly individuals without a partner had a greater chance of hypertension and one of the possible explanations may be the fact that the elderly living alone has a greater probability of having emotional disturbances, influencing the BP increase.\(^23\) However, it is worth noting that in another study, the greatest risk of hypertension was observed among married individuals or among those living with a partner.\(^17\)

The WHR presented a positive correlation (\(p = 0.050\)) with hypertension. There are few studies specifically investigating the relationship between different fat indicators (body/corporal and/or abdominal) with hypertension.\(^24,25\) Excess weight among older
individuals is still a reality, especially among women.\textsuperscript{26} Munaretti et al.\textsuperscript{24} published the first population-based and domiciliar study verifying an association between hypertension and anthropometric indicators of fat (corporal and abdominal) in elderly Brazilians. In that study, fat anthropometric indicators (corporal and abdominal) were associated with hypertension, corroborating the results of other studies conducted with individuals from different populations and age groups, which found that excess fat, regardless of the anthropometric indicator used, is one of the main risk factors for hypertension.\textsuperscript{27-29} Although no consensus exists on an adequate criteria and values to define obesity among the elderly, BMI is the indicator most often used in epidemiological studies at this age range.\textsuperscript{28} However, some authors have suggested that BMI alone is unable to identify the association between body fat and hypertension.\textsuperscript{30}

Similarly, in a study conducted with 9,936 men and 12,154 women aged 45 to 79 years, the indicators WHR and BMI showed an association with hypertension.\textsuperscript{28} Such fact can probably be explained by the physiological changes that occur in obese individuals, such as the activation of the sympathetic nervous system, the SRA, endothelial dysfunction, and insulin resistance (increasing tubular sodium reabsorption).\textsuperscript{28} It should be emphasized that the predominance of obesity tends to be higher in lower socioeconomic classes, as was the case in the population investigated.\textsuperscript{30}

The fact that the other study variables showed no association strength with hypertension may be due to the sample size and the possibilities of selection biases and/or information involving confounding factors and occasional findings from the analysis, which constitute limitations of observational studies in general.

**Conclusion**

Although the polymorphic *ACE* variant did not influence the prevalence of hypertension in elderly individuals, this condition was associated with risk factors such as female gender and marital status (absence of a partner), and modifiable risk factors such as excessive salt consumption and alterations in WHR and body weight. The study proposes the adoption of a healthy lifestyle to prevent the disease, since modifiable factors correlated significantly with hypertension. In addition, studies addressing this issue in population groups at greatest risk are fundamental in improving the knowledge of genetic polymorphisms in the etiology of the disease.

**Author contributions**

Conception and design of the research: Ribeiro BB, Santos Neto PE, Nascimento JE, Santos SHS, Martins AMEBL; Acquisition of data: Ribeiro BB, Santos Neto PE, Nascimento JE, Andrade JMO, Paraíso AF, Silveira MF, Martins AMEBL; Analysis and interpretation of the data: Ribeiro BB, Santos Neto PE, Nascimento JE, Andrade JMO, Paraíso AF, Santos SHS, Silveira MF, Martins AMEBL; Statistical analysis: Ribeiro BB, Nascimento JE, Martins AMEBL; Writing of the manuscript: Ribeiro BB, Santos Neto PE, Andrade JMO, Paraíso AF, Martins AMEBL; Critical revision of the manuscript for intellectual contente: Ribeiro BB, Santos Neto PE, Andrade JMO, Paraíso AF, Santos SHS, Silveira MF, Martins AMEBL.

**Potential Conflict of Interest**

No potential conflict of interest relevant to this article was reported.

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