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Association between diabetes, hypertension, activities of daily living and physical activity among elderly users of primary healthcare facilities

Associação entre diabetes, hipertensão, atividades da vida diária e atividade física entre idosos usuários de serviços de atenção primária à saúde

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Abstract – The aim of this cross-sectional study was to estimate the prevalence of self-reported hypertension and diabetes among elderly users of primary healthcare facilities in Ribeirão Preto, Brazil, and to investigate the association between these chronic conditions and physical activity and the degree of independence on the performance of activities of daily living. The study included 357 subjects aged 60 years or older. The classification of physical activity was based on the International Physical Activity Questionnaire (IPAQ) and the Pfeffer Functional Activities Questionnaire (PFAQ) was applied to assess activities of daily living. Prevalence of diabetes was associated with the level of physical activity, the self-perception of health and the degree of independence on the performance of activities of daily living. No significant associations were found between the prevalence of hypertension and these variables. These findings reinforce the relevance of continuous strategies of management of diabetes in the primary healthcare facilities based on the promotion of physical and occupational activities.

Key words: Activities of daily living; Aged; Diabetes; Hypertension; Physical activity.

Resumo — O objetivo deste estudo transversal foi estimar a prevalência auto-reportada de hipertensão e diabetes entre idosos usuários das unidades de atenção primária à saúde de Ribeirão Preto, Brasil, e investigar a associação entre estas condições crônicas e a atividade física e o grau de independência na realização das atividades da vida diária. O estudo incluiu 357 participantes com idade de 60 anos ou mais. A classificação da atividade física baseou-se no International Physical Activity Questionnaire (IPAQ), e o Pfeffer Functional Activities Questionnaire (PFAQ) foi aplicado para avaliar a realização das atividades da vida diária. Prevalência de diabetes foi associada com o nível de atividade física, a autopercepção da saúde e o grau de independência na realização das atividades da vida diária. Não foram encontradas associações significantes entre a prevalência de hipertensão e estas variáveis. Estes achados reforçam a relevância de estratégias contínuas de manejo da diabetes nas unidades de atenção primária à saúde, baseadas na promoção de atividades físicas e ocupacionais.

Palavras-chave: Atividades cotidianas; Atividade física; Diabetes; Hipertensão; Idosos.

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INTRODUCTION

An article published in the early 1970s reported that one of the main characteristics of Brazilian population was its extreme youth¹, or rather, 42.7% of its population were composed of children under 14 years old, while only 4.9% were over 60. However, since the early 1960s the Brazilian population has been aging rapidly, when a decline in fertility rates began to change its age structure and progressively narrowed the base of the population pyramid². Official data³ show that in 2010, people with 60 years or older accounted for 11.75% in the female population and 9.8% in the male population in Brazil. In addition, while in the 1950s the life expectancy at birth in Brazil for both sexes was less than 50 years, in 2013 it was estimated at 74.8 years⁴. The aging of the Brazilian population and the subsequent changes in its epidemiological profile led to the need for establishing special strategies aimed at the elderly, including public policies for health promotion in the face of the increased occurrence of chronic illnesses and functional disabilities and the need of studies in order to know the demand for health care.

Along with complications such as mood disorders, dementia and falls, the development of chronic diseases as diabetes mellitus (DM) lower the chances of successful ageing, increase functional limitation, and impair quality of life⁵. In 2013, the International Diabetes Federation (IDF) estimated a prevalence of diabetes in Brazil of 9.19%. In elderly population, the prevalence of DM is higher than that in the general population. A cross-sectional study conducted in Viçosa, Minas Gerais, Brazil, with elderly people aged 60 years or older, estimated a self-reported prevalence of diabetes of 22.4%. DM prevalence among the elderly living in the city of São Paulo, Brazil, was estimated to be 17.9%. In a population-based study with a probabilistic sample of elderlies in Goiânia, Goiás, Brazil, 19.0% self-reported DM9. Arterial hypertension (AH) is also highly prevalent in Brazil. The National Health Survey, 2013, estimated the self-reported AH prevalence in the Brazilian population aged 60 to 64 years to be 44.4%¹⁰. Among the elderly aged 65 to 74 years, the AH prevalence was 52.7%¹⁰. In Brazil, the prevalence of chronic diseases has been also associated with black or indigenous ethnicity, lower schooling levels and lack of private health insurance^{11,12}.

Evidence from epidemiological studies suggests that diabetes is also a risk factor for functional limitations and disability in elderly subjects^{13,14}. One hypothesis that can explain this relation is that diabetes has been associated with a faster decline in cognitive function, as well as increased incidence of falls^{15,16}. In addition, chronic hyperglycemia is an indicator of diabetes and it is associated with muscle weakness, which may cause difficulties in performing daily activities¹⁶.

Therefore, the aim of the present observational cross-sectional study was to estimate the prevalence of self-reported hypertension and diabetes among elderly users of primary healthcare facilities in Ribeirão Preto, Brazil, and to identify variables that may be associated with these chronic conditions. These variables include the physical activity classified by the

International Physical Activity Questionnaire (IPAQ) and the degree of independence on the performance of activities of daily living, measured by the Pfeffer Functional Activities Questionnaire (PFAQ). The present study differs from most previous studies in three ways. First, it was focused on a specific population of interest rather than general population, which allows us to study in detail the elderly people who attended the public health system. Second, the information was collected through face-to-face interviews carried out at health care facilities. Third, there are few articles describing the concurrent prevalence of self-reported DM and AH.

METHOD

Study design and place

This cross-sectional study included 357 subjects aged 60 years or older living in the municipality of Ribeirão Preto, Southeast region of Brazil, users of primary health care services. The overall population of Ribeirão Preto is about 670 thousand inhabitants, being among the thirty largest Brazilian municipalities. Nearly 11% of its population is composed of people aged 60 and over. Healthcare facilities in Ribeirão Preto are organized into five geographical areas (North, South, East, West and Central Health Districts) with distinct demographic characteristics. It is estimated that 52% of its urban population has exclusive use of the public healthcare resources and 80% use these facilities at some time. In each Health Districts there is a Basic and District Health Unit (UBDS or, in Portuguese, Unidade Básica e Distrital de Saúde) that is the reference point for some medical specialties in the respective region. Assuming that the elderly attending the UBDS to seek primary care services represent the population of elderly users of the units located in the coverage area of the corresponding Health District, the data collection was performed in each of the five UBDS of Ribeirão Preto. Using a formula for sample size for a proportion estimate with relative precision¹⁷, the sample size of 357 subjects is sufficient to estimate a prevalence rate close to 0.5 (corresponding to the maximum variance) with precision of approximately 10% and a confidence coefficient of 95%.

Data collection instruments and measures

People with a given self-reported condition were defined as those who responded "yes" to the question: "Have you ever heard from a doctor, at some moment in your life, that you have/had X?", where X denotes the respective condition (DM or AH). Socioeconomic status was assessed based on the acquisition of material goods and the schooling of the head of the household, as proposed by the Brazilian Association of Research Institutions. In an order from the most privileged socioeconomic class to the least privileged class, the possible classifications are as follows: A, B1, B2, C1, C2, D and E. The educational level was classified into four groups: no schooling (including illiterates and people who have never attended school), elementary/middle (incomplete or complete), high school

(incomplete or complete), and higher education (incomplete or complete). Smoking was coded as never smoked, ex-smoker, or current smoker.

The classification of physical activity was based on the International Physical Activity Questionnaire (IPAQ) modified for the elderly and adapted to the Brazilian-Portuguese language by Mazo and Benedetti¹⁸. IPAQ classified the participants into three categories: low, moderate and high (or vigorous) physical activity.

The Pfeffer Functional Activities Questionnaire (PFAQ)^{19,20} was applied to assess activities of daily living. PFAQ evaluates of the degree of independence on the performance of ten instrumental activities of daily living: (a) writing checks, paying bills, or keeping financial records; (b) assembling tax records, business affairs, or papers; (c) shopping alone for clothes, household necessities or groceries; (d) playing a game of skill or working on a hobby; (e) heating water, making a cup of coffee, or turning off the stove; (f) preparing meals; (g) keeping track of current events; (h) watching news reports and discussing them; (i) remembering appointments, family occasions, holidays, or medications; and (j) traveling out of the neighborhood, driving, or arranging to take airplane, train or buses. The performance in each of these activities is assessed with a scoring ranging from 0-3, according to increasing severity. The maximum possible score is 30, and subjects who score higher than five are considered to have functional impairment²¹.

Data collection

Data collection was conducted between January and February 2017. First, authors contacted the Ribeirão Preto Municipal Health Secretariat in order to obtain authorization for the data collection. After approval by the Ethics Committee, data were collected by face-to-face interview by three trained interviewers. Participants were invited to participate in the study while they were waiting for medical care in the health facilities. Only elderly individuals (60 years old or more) who agreed and signed informed consent forms were interviewed.

Data analysis

DM and AH prevalence was estimated with their respective 95% confidence intervals (95%CI) based on the exact method¹⁷. Logistic regression models were used to assess the association between the prevalence of DM and AH and participants' characteristics, with age and sex adjustments. Considering the PFAQ scores as the dependent variable and age groups, sex and prevalence of DM (or AH) as the independent variables, beta-binomial models were evaluated using SAS PROC FMM. The use of these models is justified by the assumption that the PFAQ scores can be considered as a discrete variable. For statistical analysis, we used the SAS System statistical software version 9.4 for Windows (SAS Institute, Cary, North Carolina).

Ethical statement

The Ethics Committee from the Health School Center of the Ribeirão Preto Medical School (University of São Paulo, Brazil) reviewed and approved this research. Written informed consent was obtained from all the participants.

RESULTS

The sample was composed of 224 (63%) females with mean age of 71.4 years (standard deviation [SD] 7.5) and 133 (37%) males with mean age of 69.9 years (SD 6.8). Self-reported DM prevalence among females was 24.6% (95% CI: 19.0% – 30.7%) and among males was 28.6% (95% CI: 21.0% – 37.0%), and self-reported AH prevalence among females was 62.1% (95% CI: 55.3% – 68.4%) and among males was 53.4% (95% CI: 44.5% – 62.1%). According to the IPAQ instrument, the prevalence of high, moderate and low level of physical activity were, respectively, 28.9%, 54.9% and 16.2%.

Table 1 shows the prevalence of DM and AH according to the characteristics of the elderly participants, and the age and sex-adjusted prevalence ratios (PR) with their respective 95%CI. The results indicated that there was no evidence of association between DM prevalence and characteristics such as sex, age, educational level, marital status, and socioeconomic status. However, we can observe a higher prevalence of DM among the elderly individuals who reported regular or poor perception of health, among those with low level of physical activity, and among those considered to have functional impairment (PFAQ scores higher than five). Table 1 also shows that there was no evidence of association between AH prevalence and the variables considered, except for a significant effect of age (it is found a higher AH prevalence among elderly people aged 75 or more).

Table 2 presents the results of the descriptive measures (mean, standard deviation and minimum and maximum values) of the PFAQ scores according to the self-reports of DM and age groups. P values showed in Table 2 were obtained from beta-binomial regression models and compare PFAQ mean scores between participants who reported and did not report DM, in each age group. Considering the PFAQ scores as the dependent variable, this regression model detected a significant effect of sex, age groups, AH, and interaction terms between age groups and AH. Thus, it is possible to note that the degree of dependence on the activities of daily living tend to increase as the age of the participants increases. In addition, the PFAQ mean scores are statistically different between participants aged 75 years or older who reported and did not report DM (p<0.01).

Table 3, analogously to Table 2, shows the results of the descriptive of the PFAQ scores according to the self-reports of AH and age groups. A beta-binomial regression model detected a significant effect of sex and age groups on the PFAQ scores, but did not show a significant effect of AH.

Table 4 shows the DM prevalence according to the dependence in activities of daily living, given by each item of the PFAQ. We have observed significant associations between the DM prevalence and the items 3 (shopping alone for clothes, household necessities, and groceries), 4 (playing a game of skill, working on a hobby), and 9 (remembering out neighborhood, driving, arranging to take bus). We also studied the associations between AH prevalence and activities of daily living, but we did not find significant associations (these results were not shown, for parsimony).

Table 1. Age-and-sex adjusted prevalence ratios (PR) for self-reported DM and AH.

	DM prevalence					AH prevalence			
Variables	Total	n	%	PR (95%CI)	n	%	PR (95%CI)		
Sex									
Women	224	55	24.6	Ref.	139	62.1	Ref.		
Men	133	38	28.6	1.2 (0.8 – 1.7)	71	53.4	0.8 (0.6 – 1.0)		
Age groups (years)									
60 – 65	93	23	24.7	Ref.	47	50.5	Ref.		
66 – 74	156	45	28.9	1.1 (0.7 – 1.8)	91	58.3	1.2 (0.9 – 1.5)		
75 or more	108	25	23.1	0.9(0.5-1.5)	72	66.7	1.3 (1.1 – 1.7)*		
Private health insurance									
No	303	77	25.4	Ref.	178	58.8	Ref.		
Yes	54	16	29.6	1.2 (0.8 – 1.7)	32	59.3	1.0 (0.7 – 1.3)		
Educational level				,			, ,		
No schooling	25	8	32.0	Ref.	18	72.0	Ref.		
Elementary school	238	60	25.2	0.7 (0.3 – 1.3)	146	61.3	0.9 (0.7 – 1.2)		
Intermediate school	68	21	30.9	0.9 (0.4 – 1.8)	36	52.9	0.8 (0.5 – 1.1)		
Higher education	26	4	15.4	0.4 (0.1 – 1.3)	10	38.5	0.5 (0.3 – 1.0)		
Marital status				3(3.1 1.0)		00.0	3.3 (3.3 1.0)		
Single	28	7	25.0	Ref.	12	42.9	Ref.		
Married	197	53	26.9	1.1 (0.5 – 2.1)	117	59.4	1.5 (0.9 – 2.3)		
Divorced	48	11	22.9	0.9 (0.3 – 2.1)	27	56.3	1.4 (0.8 – 2.3)		
Widowed	84	22	26.2	1.1 (0.5 – 2.4)	54	64.3	1.4 (0.8 – 2.2)		
Socioeconomic status	04	22	20.2	1.1 (0.3 – 2.4)	J 4	04.5	1.4 (0.0 – 2.2)		
	00	00	00.0	Dof	F.O.	E 4 .4	Dof		
A, B1 or B2	92	22	23.9	Ref.	50	54.4	Ref.		
C1	115	35	30.4	1.3 (0.8 – 2.0)	69	60.0	1.1 (0.8 – 1.3)		
C2	95	16	16.8	0.6 (0.3 – 1.2)	63	66.3	1.2 (0.9 – 1.5)		
D or E	55	20	36.4	1.6 (0.9 – 2.6)	28	50.9	0.9 (0.6 – 1.2)		
Self-perception of health	450	40	40.7	D (70	40.7	Б.		
Good	150	19	12.7	Ref.	70	46.7	Ref.		
Regular	153	51	33.3	3.0 (1.8 – 4.8)*	105	68.6	1.4 (1.1 – 1.8)		
Poor	54	23	42.6	3.6 (2.1 – 6.1)*	35	64.8	1.3 (1.0 – 1.8)		
Smoking status									
Current smoker	54	12	22.2	Ref.	26	48.2	Ref.		
Exsmoker	110	31	28.2	1.2 (0.6 – 2.2)	62	56.4	1.2 (0.8 – 1.6)		
Nonsmoker	193	50	25.9	1.8 (0.6 – 2.1)	122	63.2	1.3 (0.9 – 1.7)		
PAQ level									
High	103	20	19.4	Ref.	58	56.3	Ref.		
Moderate	196	50	25.5	1.3 (0.8 – 2.1)	116	59.2	1.0 (0.8 – 1.3)		
Low	58	23	39.7	2.1 (1.2 – 3.5)*	36	62.1	1.0 (0.7 – 1.3)		
Use of any device									
No	332	86	25.9	Ref.	193	58.1	Ref.		
Yes	25	7	28.0	1.1 (0.5 – 2.1)	17	68.0	1.1 (0.8 – 1.5)		
imitation due to pain									
Never	143	32	22.4	Ref.	75	52.5	Ref.		
Rarely	36	9	25.0	1.2 (0.6 – 2.3)	24	66.7	1.2 (0.8 – 1.6)		
Occasionally	73	22	30.1	1.4 (0.8 – 2.3)	44	60.3	1.1 (0.8 – 1.4)		
Oftentimes	47	9	19.2	0.8 (0.4 – 1.7)	27	57.5	1.1 (0.8 – 1.5)		
Always	58	21	36.2	1.8 (1.1 – 2.9)*	40	69.0	1.2 (0.9 – 1.6)		
Functional impairment (PFAQ)	- 30		JJ.L	2.0)		00.0	(0.0 1.0)		
No (score ≤ 5)	290	70	24.1	Ref.	166	57.2	Ref.		
Yes (score > 5)	67	23	34.2	1.5 (1.1 – 2.2)*	44	65.7	1.1 (0.8 – 1.3)		

Note. Confidence intervals that do not include 1 are marked with an asterisk (similar to p < 0.05); PR: prevalence ratio. CI: confidence interval. DM: diabetes mellitus. AH: arterial hypertension. PFAQ: Pfeffer Functional Activities Questionnaire Ref.: reference class.

Table 2. Descriptive measures of the PFAQ scores according to the self-reports of DM and age groups.

	Partic	Participants who did not reported DM			Participants who reported DM			
Age groups (years)	n	Mean (SD)	Min-Max	n	Mean (SD)	Min-Max	P value	
60 – 65	70	1.86 (3.15)	0 – 19	23	2.70 (3.60)	0 – 13	0.10	
66 – 74	111	2.69 (3.58)	0 – 18	45	2.84 (4.40)	0 – 26	0.99	
75 or more	83	3.87 (4.45)	0 - 20	25	7.48 (6.94)	0 – 25	< 0.01	

Note. DM: diabetes mellitus. SD: standard deviation.

Table 3. Descriptive measures of the PFAQ scores according to the self-reports of AH and age groups.

	Partic	Participants who did not reported AH			Participants who reported AH			
Age groups (years)	n	Mean (SD)	Min-Max	n	Mean (SD)	Min-Max	P value	
60 – 65	46	2.09 (3.66)	0 – 19	47	2.04 (2.87)	0 – 11	0.76	
66 – 74	65	2.25 (3.09)	0 – 16	91	3.09 (4.25)	0 - 26	0.60	
75 or more	36	4.67 (5.87)	0 – 25	72	4.72 (5.07)	0 – 19	0.95	

Note. AH: arterial hypertension. SD: standard deviation.

Table 4. Association between self-reported DM prevalence and the activities of daily living.

		DM prevalence		
Activities of daily living	Total	n	%	PR (95%CI)
1. Writing checks, paying bills, or keeping financial records				
Have no dependency	252	63	25.0	Ref.
Have some degree of dependency	105	30	28.6	1.2 (0.8 – 1.7)
2. Assembling tax records, business affairs, or papers				
Have no dependency	194	46	23.7	Ref.
Have some degree of dependency	163	47	28.8	1.2 (0.8 – 1.7)
3. Shopping alone for clothes, household necessities, and groceries				
Have no dependency	310	74	23.9	Ref.
Have some degree of dependency	47	19	40.4	1.8 (1.1 – 2.7)*
4. Playing a game of skill, working on a hobby				
Have no dependency	289	69	23.9	Ref.
Have some degree of dependency	68	24	35.3	1.5 (1.1 – 2.3)*
5. Heat water, make a cup of coffee, turn off stove				
Have no dependency	334	85	25.4	Ref.
Have some degree of dependency	23	8	34.8	1.4 (0.7 – 2.5)
6. Preparing a balanced meal				
Have no dependency	325	85	26.2	Ref.
Have some degree of dependency	32	8	25.0	0.9 (0.4 – 1.8)
7. Keeping track of current events				
Have no dependency	329	82	24.9	Ref.
Have some degree of dependency	28	11	39.3	1.7 (1.0 – 2.8)
8. Paying attention to, understanding, discussing TV, book, magazine				
Have no dependency	319	80	25.1	Ref.
Have some degree of dependency	38	13	34.2	1.4 (0.8 – 2.3)
9. Remembering appointments, family occasions, holidays, medications				
Have no dependency	253	58	22.9	Ref.
Have some degree of dependency	104	35	33.7	1.5 (1.1 – 2.1)*
10. Traveling out of neighborhood, driving, arranging to take buses				
Have no dependency	301	75	24.9	Ref.
Have some degree of dependency	56	18	32.1	1.4 (0.9 – 2.2)

Note. Confidence intervals that do not include 1 are marked with an asterisk (similar to p < 0.05); PR: prevalence ratio. CI: confidence interval. DM: diabetes mellitus.

DISCUSSION

Studies of the prevalence of chronic diseases among users of primary healthcare facilities are important for several reasons. Primary healthcare services have an essential role in various aspects of prevention, detection and management of chronic conditions in the general population. Thus, these studies can contribute to a better understanding of the dynamics of chronic diseases, describing the more susceptible segments of the population in which prevention strategies should be aimed at and implemented, or in which there is more demand for treatment and patient education toward health promotion. This knowledge can improve the decision-making processes in the public health system and the general health in the community.

In the present study, self-reported DM prevalence among females was 24.6% and among males was 28.6%. These values are very close to that obtained in a previous study²² among users of primary health care facilities of Ribeirão Preto (24.3% for the overall population of individuals aged 60 years or older). A former study based on home interviews conducted in 1996 in Ribeirão Preto, showed a self-reported DM prevalence among women of 23.1% among females and 19.2% among males aged 60 years or older²³. Although the prevalence of DM among females found in the present study is similar to that found by these authors²³, we estimate a higher prevalence among males. A possible explanation is that it is well known that, in Brazil, men use healthcare services less than women²⁴ and the prevalence of chronic conditions among men users of primary health care facilities can be higher to that founded in the general population, given that men tend to seek for medical care supposedly at a later stage of illness.

The present study estimated a prevalence of self-reported DM of 19.4% among participants with high level of physical activity, and of 39.7% among participants with low level of physical activity. In fact, a systematic review of published studies with meta-analysis²⁵ provided strong evidence for an inverse association between physical activity and risk of type 2 diabetes, which may partly be mediated by reduced adiposity. Another systematic review with meta-analysis²⁶ studied the effectiveness of physical activity intervention programs on different outcome measures in individuals with prediabetes and concluded that the intervention has a favorable effect on improving oral glucose tolerance and fasting blood sugar, and it showed a favorable effect on glycated hemoglobin, maximum oxygen uptake, and body composition. Considering that prediabetes is a strong risk factor for the development of DM, this systematic review suggests that the physical activity promotion can help to slow down the progression of disease in individuals with prediabetes. On the other hand, the present study did not find any association between physical activity and the prevalence of selfreported AH. However, a recent systematic review with meta-analysis of cohort studies²⁷ showed a significant association between physical activity and hypertension risk across studies. This study also shows that estimate the quantitative dose-response association between physical activity and hypertension is not an easy task, and may require more complex study designs and more sophisticated analytical tools. Anyway, these results suggest that modification in lifestyle plays an important role to avoid the prognosis of DM and AH and its complications in future.

Results in Table 1 show a higher prevalence of DM among the elderly people considered to have functional impairment (PFAQ scores higher than five). In addition, Table 4 shows that the activities of daily living whose dependence is significantly associated with prevalence of DM are: (a) shopping alone for clothes, household necessities or groceries; (b) playing a game of skill or working on a hobby; and (c) remembering appointments, family occasions, holidays, or medications. These activities have in common the use of cognitive capacities to be satisfactorily performed. In fact, a systematic overview of published prospective observational studies showed that, compared to people without diabetes, people with diabetes have a greater rate of decline in cognitive function and a greater risk of cognitive decline. Other studies have been shown that elderly people with diabetes have considerable functional impairment associated with reduced health status^{28,29}.

The main limitation of this study is its cross-sectional design, in which causal inferences cannot be made. We do not take into account important aspects of the chronic conditions, as the diagnostic time, chronicity of the disease and treatments. Other limitation is that estimates of self-reported DM and AH prevalence are always subject to underreporting. A Brazilian study showed a low sensitivity (57.1%) for self-reported DM, suggesting that this measure may not be suitable as an indicator of disease prevalence for the Brazilian population³⁰.

CONCLUSION

Among elderly people who attended the public health system, DM prevalence has association with the level of physical activity, the self-perception of health and the degree of independence on the performance of activities of daily living. These findings reinforce the relevance of continuous strategies of management of DM in the primary healthcare facilities based on the promotion of physical and occupational activities.

COMPLIANCE WITH ETHICAL STANDARDS

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Ethical approval

This study was approved by the Research Ethics Committee of the Ribeirão

Preto Medical School (CAAE: 61567416.7.0000.5414). This research is in accordance with the standards set by the Declaration of Helsinki.

Conflict of interest statement

The authors have no conflict of interests to declare.

Author Contributions

Conceived and designed the experiments: EZM, MLZ, SFS, NAYT. Performed the experiments: MLZ, SFS, NAYT. Analyzed the data: EZM, ASS, LJF, MLZ. Contributed reagents/materials/analysis tools: EZM, ASS, LJF, SFS, NAYT, MLZ. Wrote the paper: EZM, ASS, LJF, SFS, NAYT, MLZ.

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