

CLINICAL SCIENCE

The impact of asymptomatic vertebral fractures on quality of life in older community-dwelling women: the São Paulo Ageing & Health Study

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OBJECTIVES: The aim of this study was to investigate the impact of asymptomatic vertebral fractures on the quality of life in older women as part of the Sao Paulo Ageing & Health Study.

METHODS: This study was a cross-sectional study with a random sample of 180 women 65 years of age or older with or without vertebral fractures. The Quality of Life Questionnaire of the European Foundation for Osteoporosis was administered to all subjects. Anthropometric data were obtained by physical examination, and the body mass index was calculated. Lateral thoracic and lumbar spine X-ray scans were obtained to identify asymptomatic vertebral fractures using a semi-quantitative method.

RESULTS: Women with asymptomatic vertebral fractures had lower total scores [61.4(15.3) vs. 67.1(14.2), $p=0.03$] and worse physical function domain scores [69.5(20.1) vs. 77.3(17.1), $p=0.02$] for the Quality of Life Questionnaire of the European Foundation for Osteoporosis compared with women without fractures. The total score of this questionnaire was also worse in women classified as obese than in women classified as overweight or normal. High physical activity was related to a better total score for this questionnaire ($p=0.01$). Likewise, lower physical function scores were observed in women with higher body mass index values ($p<0.05$) and lower physical activity levels ($p<0.05$). Generalized linear models with gamma distributions and logarithmic link functions, adjusted for age, showed that lower total scores and physical function domain scores for the Quality of Life Questionnaire of the European Foundation for Osteoporosis were related to a high body mass index, lower physical activity, and the presence of vertebral fractures ($p<0.05$).

CONCLUSION: Vertebral fractures are associated with decreased quality of life mainly physical functioning in older community-dwelling women regardless of age, body mass index, and physical activity. Therefore, the results highlight the importance of preventing and controlling asymptomatic vertebral fractures to reduce their impact on quality of life among older women.

KEYWORDS: Quality Of Life (QOL); Vertebral Fractures; Physical Activity; Body Mass Index (BMI); Old Women.

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INTRODUCTION

Health-related quality of life (HRQoL) is a multidimensional concept that defines a person's health based on specific aspects, such as physical, emotional, and social functioning and general welfare (1). The assessment of HRQoL consists of an evaluation of the degree to which these aspects are decreased by symptoms, incapacities, and limitations caused by disease (2). The assessment of HRQoL

has been used as a measure complementary to bone mineral density to evaluate and monitor the burden of osteoporosis on a patient's daily life (3).

There are several instruments that can be used to assess the quality of life of individuals with osteoporosis, including the Osteoporosis Assessment Questionnaire, the Quality of Life Questionnaire for Osteoporosis (OPTQoL), the Osteoporosis Quality of Life Questionnaire, and the Questionnaire of the European Foundation for Osteoporosis (QUALEFFO) (4-7).

The QUALEFFO, a specific tool used to evaluate subjects with vertebral fractures and that includes questions on pain, physical functioning, social functioning, general health perception and mental functioning, has been shown to be repeatable and consistent (7-8).

Vertebral fractures are the most frequent osteoporotic fractures, occurring in at least 30% of the elderly population

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(9), and have important clinical implications (10-14). These fractures are associated with increased risks of new osteoporotic fractures and mortality, especially in older women (10-11). Only one-third of vertebral fractures are symptomatic; therefore, patients may be unaware of their presence. Indeed, in studies based on the radiographic screening of populations, the incidence of all vertebral fractures has been estimated to be three times higher than the incidence of hip fractures, and only 30% of people with vertebral fractures were found to have received medical attention (12).

Women with vertebral fractures can also experience decreased HRQoL due to physical limitations and psychosocial disabilities (13-14).

Some studies have assessed the impact of vertebral fractures on HRQoL in older women in many countries (15,16), but few such epidemiological studies have been conducted in Brazil. Moreover, most of the studies conducted in this country have been performed in ambulatory or institutionalized individuals (17,18).

Therefore, this study evaluated the impact of vertebral fractures on the quality of life of healthy, community-dwelling women aged 65 years or older using the QUALEFFO.

SUBJECTS AND METHODS

Population

This study was performed using the framework of the São Paulo Ageing & Health Study (SPAH), which was a population-based, cross-sectional study (9). Based on the size sample calculation, 180 women were randomized and included.

The inclusion and exclusion criteria were the same as those of the core study (9). Specifically, only well-functioning elderly women were recruited to participate. All of the individuals were apparently healthy and showed no evidence of malabsorption, chronic diarrhea, hepatic disease, severe chronic diseases, or cancer. Current or previous bisphosphonate use was also an exclusion criterion (9).

Quality of life assessment

Quality of life was assessed through individual interviews using the validated QUALEFFO with 41 questions covering five domains: pain (5 questions), physical functioning (17 questions), social functioning (seven questions), general health perception (three questions), and mental functioning (nine questions) (7). The total score for each domain was obtained by summing the scores of all questions for that domain and submitting this sum to a linear transformation to a scale ranging from 0 to 100, where 0 corresponds to the worst HRQoL and 100 to the best HRQoL. The interviewers were blinded to the presence of vertebral fractures.

Assessment of vertebral fractures

Radiographs of the lumbar and thoracic spine centered on L2 and T7, respectively, were obtained for all participants, with 40" between the tube and the film. All of the obtained images provided good visibility of the T4 to L4 segment. The identification of vertebral fractures was performed by two individuals with experience in the field of analyzing vertebral fractures. They were blinded to each other's assessments, and when the results conflicted, a consensus

between the two individuals was reached. The agreement between the assessments of the two individuals was 96%, and the Kappa coefficient was 0.82. Vertebral fractures were classified using a Genant SQ approach (19). Each identified fractured vertebra was assigned a grade based on the Genant SQ scale, where mild (grade 1) corresponds to a 20-25% reduction in the anterior, middle, and/or posterior height; moderate (grade 2) corresponds to a 26-40% reduction in any height; and severe (grade 3) corresponds to a reduction of over 40% in any height.

Anthropometry

The height (without shoes) of each participant was measured to the nearest 0.1 cm with a wall-mounted stadiometer. The weight of each participant (without shoes and wearing only light clothing) was measured to the nearest 0.25 kg using a double-beam balance scale. The body mass index (BMI) was calculated by dividing the participant's weight (kilograms) by her height squared (square meters), and the subjects were categorized using the following cutoff points proposed by the World Health Organization (WHO): normal weight = $BMI < 25$; overweight = $25 \leq BMI < 30$; and obese = $BMI \geq 30$ (20).

Other variables and definitions

Information regarding health, lifestyle and risk factors for osteoporosis/fractures was obtained through individual interviews. Women who had had two or more falls in the last 12 months were defined as chronic fallers (21).

Physical activity was classified as (a) low, does not even perform housework; (b) moderate, performs regular housework, walks irregularly, and gardens; and (c) high, performs regular physical activity aside from her daily routine at least twice a week for 30 min (22).

Regarding concomitant diseases, those mentioned at the time of the interview were noted, as well as those diagnosed during the physical examination. Systemic arterial hypertension (SAH) was defined as a history of hypertension with the use of antihypertensive drugs or a systolic blood pressure > 140 mmHg and/or diastolic blood pressure > 90 mmHg, which was measured with a standard sphygmomanometer with the subject seated for at least 5 minutes prior to the measurement (23). Participants taking oral hypoglycemic agents or insulin or those with fasting blood glucose levels ≥ 126 mg/dL were considered to be diabetic (24).

Bone mineral density (BMD) assessment

The BMD was measured by dual X-ray absorptiometry (DXA) using Hologic densitometry equipment (Hologic Inc. Bedford, MA, USA, Discovery model) in the following regions: lumbar spine, femoral neck, and total femur. All BMD measurements were performed by the same experienced technologist. Anatomically abnormal vertebrae were excluded from the analysis of the lumbar spine only if they were clearly abnormal and were not assessable within the resolution of the system or if there was a difference in the T-score of more than 1.0 between the vertebra in question and adjacent vertebrae, as recommended by the International Society for Clinical Densitometry (ISCD) (25). The precision of the BMD measurements was determined based on standard ISCD protocols (26). We calculated the least significant change with 95% confidence to be 0.033 g/cm² for the spine, 0.047 g/cm² for the femoral neck, and 0.039 g/cm² for the total femur.

According to the classification criteria of the ISCD (International Society of Clinical Densitometry), the lowest T-score among the three sites (lumbar spine, femoral neck, and total femur) was used to classify each participant as having osteoporosis, osteopenia, or normal bone density. Thus, the individuals with T-scores that were 2.5 standard deviations or more below the scores for healthy controls for the peak bone mass were diagnosed with osteoporosis, individuals with T-scores between 2.5 and 1.0 standard deviations below the scores for healthy controls were diagnosed with osteopenia, and individuals with T-scores greater than 1.0 standard deviation below the scores for healthy controls were classified as normal (26).

Statistical analysis

The sample size of 180 was based on a standard deviation of 15% (27) for the total QUALEFFO score and a two-sided 5% significance level. The study had 95% power to detect a difference of 10 points in the total QUALEFFO score.

The results for the quantitative variables are expressed as the mean (standard deviation), and results for the qualitative variables are described by the absolute and relative (%) frequencies. Demographic characteristics and the QUALEFFO results were compared between women with and without fractures using the Mann-Whitney-Wilcoxon test for quantitative variables and the chi-square test for qualitative variables. The associations between the QUALEFFO scores and potential determinants of quality of life were assessed using the Wilcoxon rank-sum test or the Kruskal-Wallis test. Correlations between continuous variables and the QUALEFFO questionnaire data were tested using the Spearman correlation coefficient (r_s).

Generalized linear models with gamma distributions and logarithmic link functions were performed to determine the influence of the vertebral fractures on the QUALEFFO score. Variables with a statistical significance better than 0.1 ($p < 0.1$) in the bivariate tests were included in these models,

and statistically significant variables ($p < 0.05$) were retained in the final model.

All tests were performed using STATA 10.0.

Ethics

This study was conducted in compliance with the ethical principles of the Helsinki Declaration (2008) and local applicable laws and regulations. This study was approved by the Local Ethics and Research Committee (Research Protocol 1110/07).

RESULTS

Forty-one women (22.8%) had vertebral fractures. The demographic, anthropometric and clinical data for all participants in the study, grouped based on the presence (Vertebral Fracture) or absence of vertebral fractures (No Vertebral Fracture), are shown in Table 1.

There were no significant differences with respect to the mean BMI or the percentage of Caucasian individuals between the groups ($p > 0.05$) (Table 1). Regarding the BMI classification, 22.8% and approximately 38% of the subjects were classified as normal and obese, respectively, with no significant difference between the two groups ($p > 0.05$). A tendency of older age in the vertebral fracture group was observed ($p = 0.057$).

The frequencies of hypertension ($p = 0.224$), diabetes ($p = 0.672$), hypothyroidism ($p = 0.723$), and two or more concomitant diseases ($p = 0.216$) and the average number of medications used ($p = 0.497$) were comparable between the groups.

Interestingly, the Vertebral Fracture group contained a higher frequency of women defined as chronic fallers than the No Vertebral Fracture group (64.7 vs. 32.1%, $p = 0.017$). As expected, the Vertebral Fracture group had a higher frequency of osteoporosis (73.2 vs. 51.1%, $p = 0.012$) and a

Table 1 - Demographic, anthropometric, and clinical data of the Vertebral Fracture (VF) and No Vertebral Fracture (NVF) groups.

| | All (n = 180) | Vertebral Fracture (n = 41) | No Vertebral Fracture (n = 139) | p-value VF vs. NVF |
|------------------------------------|---------------|-----------------------------|---------------------------------|--------------------|
| Age, years | 75.2 (4.5) | 76.3 (5.4) | 74.8 (4.2) | 0.057 |
| Caucasian individuals, n (%) | 121 (67.2) | 27 (65.9) | 94 (67.6) | 0.832 |
| Body Mass Index, kg/m ² | 28.8 (4.9) | 29.1 (5.1) | 28.7 (4.8) | 0.572 |
| Classification according to BMI | | | | |
| Normal, n (%) | 44 (22.8) | 9 (22.0) | 35 (25.6) | |
| Overweight, n (%) | 67 (37.6) | 16 (39.0) | 51 (37.2) | 0.896 |
| Obese, n (%) | 67 (37.6) | 16 (39.0) | 51 (37.2) | |
| Currently smoking, n (%) | 18 (10) | 3 (7.3) | 15 (10.8) | 0.515 |
| Physical activity level, n (%) | | | | |
| Low | 8 (4.4) | 3 (7.3) | 5 (3.6) | |
| Moderate | 120 (66.7) | 27 (65.9) | 93 (66.9) | 0.586 |
| High | 52 (28.9) | 11 (26.8) | 41 (29.5) | |
| Chronic faller, n (%) | 28 (40) | 11 (64.7) | 17 (32.1) | 0.017 |
| SAH, n (%) | 126 (74.6) | 25 (83.3) | 101 (72.7) | 0.224 |
| Diabetes, n (%) | 44 (24.4) | 9 (22.0) | 35 (25.2) | 0.672 |
| Hyperthyroidism, n (%) | 14 (8.3) | 2 (6.7) | 12 (8.6) | 0.723 |
| Two or more concomitant diseases | 128 (71.1) | 26 (63.4) | 102 (73.4) | 0.216 |
| Number of medications | 1.5 (1.4) | 1.7(1.4) | 1.5(1.4) | 0.497 |
| Densitometric criteria (ISCD) | | | | |
| Osteopenia, n (%) | 61 (33.9) | 8 (19.5) | 53 (38.1) | 0.027 |
| Osteoporosis, n (%) | 101 (56.1) | 30 (73.2) | 71 (51.1) | 0.012 |

Data are presented as the mean (standard deviation) or n (percentage).
SAH = Systemic Arterial Hypertension.

lower frequency of osteopenia (19.5 vs. 38.1%, $p=0.027$) than the No Vertebral Fracture group.

The results for each domain of the QUALEFFO and the total QUALEFFO score in both groups are shown in Table 2. The total QUALEFFO score was lower in the Vertebral Fracture group than in the No Vertebral Fracture group [61.4 (15.4) vs. 67.1 (14.2), $p=0.031$]. Likewise, the physical function domain score of the QUALEFFO was worse in the Vertebral Fracture group compared with the No Vertebral Fracture group [69.5 (20.1) vs. 77.3 (17.1), $p=0.018$]. No difference was observed regarding the other QUALEFFO domains (pain, social functioning, health perception, and mental functioning) (Table 2).

The total QUALEFFO score was inversely related to BMI ($r_s=-0.21$, $p=0.005$) and weight ($r_s=-0.22$, $p=0.009$). The total QUALEFFO score was worse in women classified as obese than in those classified as overweight or normal [61.7 (15.4) vs. 66.4 (13.8) vs. 70.8 (13.5), respectively, $p=0.008$]. A lower total QUALEFFO score was also observed in women with low physical activity than in those with moderate or high activity [51.8 (19.1) vs. 64.8 (14.3) vs. 70.3 (13.2), respectively, $p=0.010$] (Table 3).

Similarly, the physical function domain of the QUALEFFO was inversely related to the BMI ($r_s=-0.24$, $p=0.001$). In this domain, women classified as obese were found to have lower scores than women classified as overweight or normal [70.7 (19.3) vs. 76.1 (16.1) vs. 81.4 (17.4), respectively, $p=0.002$]. Finally, lower scores for the physical function domain were found among women with low physical activity compared with those with moderate or high activity [49.6(24.9) vs. 75.1(18.1) vs. 80.7(12.6), respectively, $p=0.002$] (Table 3).

A generalized linear model with gamma distributions and logarithmic link functions was developed to identify patient characteristics that were related to the total and physical function domain scores of the QUALEFFO. Variables with a $p<0.10$ in the univariate analysis (age, BMI classification, physical activity, diabetes, presence of at least one fracture) were included as independent variables. The presence of obesity was negatively associated with the total QUALEFFO score ($p=0.001$). A high physical activity level was positively associated with the total QUALEFFO score ($p=0.001$). The presence of at least one fracture was associated with a worse total QUALEFFO score, independent of age, BMI classification and physical activity level ($p=0.030$). Likewise, the presence of at least one fracture was negatively associated with the physical function domain, independent of these same variables ($p=0.041$) (Table 4).

DISCUSSION

This study was the first study conducted in Brazil that specifically assessed the impact of vertebral fractures on the quality of life in older, community-dwelling women using a specific questionnaire. In this study, we demonstrated that the presence of vertebral fractures in this population is related to worse HRQoL, particularly with respect to physical functioning.

The major advantage of the present study is the homogenous selection of community-dwelling women, unlike previous studies in which individuals were recruited from clinics or from populations included in clinical trials. These latter populations represent sick individuals with a high risk of fractures.

Studies showing worse HRQoL in patients with vertebral fractures have been published in several countries (15,16). There are only two studies evaluating the HRQoL in patients with vertebral fractures in Brazil, but neither was specific to older community-dwelling individuals (17,18). The first of these two other studies was performed in 55 outpatient women divided into three groups: 1- women without osteoporosis, 2- women without osteoporosis and no vertebral fractures, 3- women with osteoporosis and vertebral fractures. In that study, the quality of life was assessed with the SF-36, and no difference was found among the three groups. One of the reasons for this finding was the inclusion of only women who were able to perform the spirometric tests, resulting in the exclusion of women in worse conditions who would most likely belong to the fracture group (17). Later, de Oliveira et al. evaluated the quality of life in ambulatory women with osteoporosis and found similar results for those who had vertebral fractures and those who did not. However, that study was not designed to assess the impact of vertebral fractures on quality of life, and the number of women with fractures was too small to enable an accurate assessment (18).

The assessment of quality of life in relation to the QUALEFFO pain domain was similar in women with and without vertebral fractures in our study. Some studies have found that the pain domain is worse in women with fractures; however, the patients included in those studies were recruited based on clinical symptoms related to fractures and were compared with those without back pain (28). Vertebral fractures do not always manifest with symptoms and are often diagnosed based on radiographs taken for other reasons. Gehlbach et al. evaluated the chest radiographs of older women who had been hospitalized for several causes, and they found that only a few of the vertebral fractures present had been previously identified

Table 2 - QUALEFFO questionnaire data: total score and score for each domain for the Vertebral Fracture (VF) and No Vertebral Fracture (NVF) groups.

| QUALEFFO | All (n = 180) | Vertebral Fracture (n = 41) | No Vertebral Fracture (n = 139) | p-value VF vs. NVF |
|-------------------|---------------|-----------------------------|---------------------------------|--------------------|
| Total | 65.8 (14.7) | 61.4 (15.4) | 67.1 (14.2) | 0.031 |
| Domains | | | | |
| Pain | 68.8 (27.5) | 63.1 (26.7) | 70.50 (27.7) | 0.115 |
| Physical function | 75.5 (18.1) | 69.5 (20.1) | 77.3 (17.1) | 0.018 |
| Social function | 32.9 (14.8) | 31.7 (15.6) | 33.2 (14.6) | 0.438 |
| Health Perception | 49.0 (21.9) | 44.5 (22.3) | 50.2 (21.7) | 0.118 |
| Mental Function | 69.6 (18.4) | 67.3 (19.3) | 70.3 (18.1) | 0.379 |

Data are presented as the mean (standard deviation).

Table 3 - Association between categorical variables and the quality of life questionnaire scores (QUALEFFO: Total score and physical function domain score).

| Variables | QUALEFFO Total score | p-value | QUALEFFO Physical Function | p-value |
|---------------------------------|----------------------|--------------|----------------------------|--------------|
| Classification according to BMI | | | | |
| Normal | 70.8 (13.5) | | 81.4 (17.4) | |
| Overweight | 66.4 (13.8) | 0.008 | 76.1 (16.1) | 0.002 |
| Obese | 61.7 (15.4) | | 70.7 (19.3) | |
| Currently smoking | | | | |
| Yes | 63.7 (14.2) | 0.419 | 72.1 (14.1) | 0.159 |
| No | 66.0 (14.7) | | 75.9 (18.4) | |
| Physical activity level | | | | |
| Low | 51.8 (19.1) | 0.010 | 49.6 (24.9) | 0.002 |
| Moderate | 64.8 (14.3) | | 75.1 (18.1) | |
| High | 70.3 (13.2) | | 80.7 (12.6) | |
| Hypertension | | | | |
| Yes | 68.6 (15.0) | 0.534 | 75.2 (17.1) | 0.317 |
| No | 69.9 (16.6) | | 76.4 (20.8) | |
| Diabetes | | | | |
| Yes | 62.1 (14.2) | 0.052 | 72.1 (17.4) | 0.073 |
| No | 70.0 (14.7) | | 76.6 (18.2) | |
| Osteoporosis | | | | |
| Yes | 67.7 (14.7) | 0.346 | 73.2 (17.2) | 0.027 |
| No | 69.8 (15.9) | | 77.3 (18.6) | |
| Severity of vertebral fracture | | | | |
| No Fracture | 67.1 (14.2) | 0.073 | 77.3 (17.1) | 0.053 |
| Grade 1 | 58.6 (16.1) | | 68.4 (17.8) | |
| Grade 2 or 3 | 62.5 (15.3) | | 69.9 (21.0) | |
| Chronic faller | | | | |
| Yes | 63.3 (13.9) | 0.230 | 72.3 (17.8) | 0.223 |
| No | 66.3 (14.8) | | 76.1 (18.1) | |

Data are presented as the mean (standard deviation).

by clinicians (29). Indeed, in our previous study performed in Brazil, 29.4% subjects had vertebral fractures, and none of these patients had prior knowledge of their vertebral fractures (9).

As observed in other studies, the mental function, social function, and health perception domains were not significantly different between women with and without fractures (30). The relatively small differences between the groups with and without fractures may be the result of the acceptance of poor health conditions due to the natural expectation of physical decline in older women (13).

In our study, we found that obesity and low physical activity were associated with lower quality of life. Other authors have reported that higher BMI and a sedentary lifestyle are factors that influence the quality of life in patients

with osteoporosis (18,31-32). It is important to highlight the fact that both obesity and a sedentary lifestyle are preventable factors and can be controlled by a change in lifestyle.

Some authors observed that physical exercise is associated with a better quality of life (33) and have demonstrated that a home exercise program for women with vertebral fractures (60 min/d, 3x/week for 12 months) significantly improves the HRQoL (34). A study performed at our institution in osteoporosis patients showed that an exercise program improved the quality of life in these women (35). Therefore, our data reinforce the need for all older women to be advised about the benefits of exercise. These women should be encouraged to exercise regularly to reduce their BMI and improve their general welfare.

Our study is the first in Brazil to conduct a thorough assessment of the relationship between quality of life and vertebral fractures. An important characteristic of this study was the use of standardized and reliable instruments for both the vertebral fracture assessment (Genant semi-quantitative method) and the HRQoL assessment (QUALEFFO). Although the QUALEFFO has not been validated in Brazil, which is a limitation of the present study, this tool is the most frequently used for assessing quality of life in osteoporosis and was recommended for the investigation of vertebral fracture subjects in multicentric studies that included centers in Brazil (36). This questionnaire is more sensitive in detecting differences between groups, and it provides a better discrimination between individuals with and without vertebral fractures compared with generic HRQoL instruments, such as the SF-36, particularly with respect to physical functioning, which is significantly affected in these patients (8).

In conclusion, this study demonstrated the negative impact of vertebral fractures on the quality of life in older women, independent of other factors such as BMI and

Table 4 - Generalized linear models.

| QUALEFFO | Independent Variables | Coefficient | p-value |
|--------------------------|----------------------------------|-------------|----------------|
| Total | Age | -0.01 | 0.055 |
| | Classification according to BMI* | -0.07 | 0.001 |
| | Physical activity level | 0.10 | 0.001 |
| | Diabetes | -0.05 | 0.208 |
| | Vertebral Fracture | -0.08 | 0.030 |
| Physical Function | Age | -0.01 | 0.060 |
| | Classification according to BMI* | -0.07 | 0.002 |
| | Physical activity level | 0.13 | < 0.001 |
| | Diabetes | -0.04 | 0.569 |
| | Vertebral Fracture | -0.10 | 0.041 |

*BMI = body mass index.

physical activity. Although the clinical relevance of vertebral fractures is well established, these results are important for assessing the burden of this disease and reinforce the need to reduce the underdiagnosis and undertreatment of these fractures. The results of this study also highlight the need for awareness of the importance of maintaining proper weight and promoting changes in lifestyle through physical activity and dietary control.

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AUTHOR CONTRIBUTIONS

Lopes JB and Pereira RM contributed to the study conception and design. Lopes JB, Fung LK, Cha CC, Gabriel GM, and Figueiredo CP assisted the recruitment of patients. Lopes JB, Fung LK, Cha CC, Gabriel GM, Fung LK, and Figueiredo CP acquired the data. Lopes JB, Fung LK, and Pereira RM contributed to the analysis and interpretation of the data. Lopes JB and Pereira RM prepared the manuscript.

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