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ORIGINAL ARTICLES

Vitamin D levels in pregnant women in southern Brazil during Summer and Winter

Níveis séricos de vitamina D em gestantes do sul do Brasil durante o verão e inverno

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ABSTRACT

Objective: To evaluate serum vitamin D levels and respective seasonality in pregnant women in southern Brazil.

Methods: This was an analytical prospective cross-sectional study set at prenatal outpatient clinics of two public teaching hospitals. A total of 520 pregnant women were included, 256 in the Summer and 264 in the Winter of 2016. Women were divided into a high-risk group of patients with preeclampsia, gestational diabetes mellitus, or human immunodeficiency virus seropositivity, and a low-risk group of disease-free patients. Sociodemographic, epidemiologic, and clinical data were collected, and blood was sampled for assessment of vitamin D levels. Serum 25 hydroxyvitamin D levels were measured by chemiluminescence.

Results: The mean serum vitamin D levels were 22.5 ± 8.70 ng/mL in the high-risk group and 56.2 ± 21.75 nmol/L in the low-risk group, with significantly higher levels in Summer (26.7 ± 7.80 ng/mL and 66.7 ± 19.50 nmol/L, respectively) compared with Winter (18.3 ± 7.50 ng/mL and 45.7 ± 18.75 nmol/L, respectively; p <0.001). The prevalence of hypovitaminosis D was 69.9% in Summer and 91.3% in Winter (p <0.001). Both groups had a significantly higher prevalence of hypovitaminosis D in Winter compared to Summer (high-risk group, 92.4% vs. 71.2%, p <0.001; low-risk group, 87.0% vs. 64.7 p <0.011%).

Conclusion: A high prevalence of hypovitaminosis D was found in this cohort of pregnant women, particularly during Winter, raising awareness of the need to recommend adequate nutrition through a healthy and balanced diet and adequate sun exposure in prenatal care.

Keywords: deficiency; hypovitaminosis; insufficiency; pregnancy; vitamin D deficiency

RESUMO

Objetivo: Avaliar os níveis séricos de vitamina D e sua sazonalidade em gestantes do sul do Brasil.

Métodos: Estudo observacional transversal analítico realizado em contexto ambulatório pré-natal de dois hospitais públicos universitários. Foram incluídas 520 gestantes, 256 no verão e 264 no inverno de 2016, que foram divididas em dois grupos: um grupo de alto risco, de mulheres com preeclampsia, diabetes gestational ou soropositividade para o vírus da imunodeficiência humana, e um grupo de baixo risco, de mulheres sem doenças gravídicas ou associadas à gravidez. Foram recolhidos dados sociodemográficos, epidemiológicos e clínicos, bem como uma amostra de sangue para dosagem dos níveis séricos de vitamina D. Os níveis de 25-hydroxyvitamina D foram quantificados por quimioluminescência.

Resultados: A concentração média de vitamina D na população estudada foi de 22.5 ± 8.70 ng/mL no grupo de alto risco e 56.2 ± 21.75

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nmol/L no grupo de baixo risco, sendo significativamente mais elevada no verão (26.7 ± 7.80 ng/mL e 66.7 ± 19.50 nmol/L, respetivamente) do que no inverno (18.3 ± 7.50 ng/mL e 45.7 ± 18.75 nmol/L, respetivamente; p <0.001). A prevalência de hipovitaminose D foi de 69.9% no verão e 91.3% no inverno (p <0.001). Ambos os grupos registaram uma prevalência significativamente mais elevada de hipovitaminose D no inverno do que no verão (grupo de alto risco, 92.4% vs. 71.2%, p <0.001; grupo baixo risco, 87.0% vs. 64.7% p <0.011%).

Conclusão: Foi identificada uma elevada prevalência de hipovitaminose D na população estudada, particularmente durante o inverno, alertando para a necessidade de recomendar uma nutrição adequada, através de uma dieta saudável e equilibrada, e adequada exposição solar nos cuidados pré-natais.

Palavras-chave: deficiência; deficiência de vitamina D; gestação; hipovitaminose; insuficiência

INTRODUCTION

Hypovitaminosis D, defined as vitamin D insufficiency, deficiency, or both, is a global health problem affecting all age groups and several at-risk groups in particular. Pregnant women are one of these high-risk groups, with a reported prevalence of hypovitaminosis D between 20% and 40%.(1) Serum 25(OH)D concentration is the best indicator of vitamin D levels in the body, as it has a half-life of 15 days.(2)

A growing number of studies in the literature have assessed vitamin D deficiency during pregnancy and its association with maternal-fetal health. Several authors have performed systematic reviews, meta-analyses, and other studies demonstrating that low serum 25(OH)D levels in pregnant women are associated with obstetric and neonatal complications, such as increased risk of preeclampsia (PE), gestational diabetes mellitus (GDM), bacterial vaginosis, prematurity, and low birth weight.(3-8) Children of women with hypovitaminosis D during pregnancy have been reported to be at increased risk for autoimmune diseases in childhood and adulthood, including diabetes mellitus type I, multiple sclerosis, allergies, and atopic diseases.(9)

Brazil is a tropical country with high sun exposure, but the climate varies widely throughout the country due to its geographic extension. In addition, the Brazilian population is characterized by ethnic diversity. Studies of the prevalence of hypovitaminosis D in the general Brazilian population are scarce, but some studies focusing on specific regions and groups have been published. These have shown a higher frequency of hypovitaminosis D in institutionalized elderly and postmenopausal women living in the city of São Paulo (latitude 23ºS).(10,11) No Brazilian studies have evaluated serum vitamin D levels in pregnant women to date.

The present study aimed to investigate serum 25(OH)D levels in pregnant women in South Brazil and explore the impact of seasonality on those levels. Vitamin D levels are currently not routinely assessed in prenatal care in Brazil, and this study can help to inform whether it should be part of routine clinical care.

MATERIALS AND METHODS

This was a prospective cross-sectional study. Sample calculation was based on the number of births in the Curitiba metropolitan region in 2015, according to data from the Secretary of Health of the State of Paraná. The estimated sample size to obtain a 95% confidence level was 382 individuals. Serum vitamin D levels were tested at least four weeks after the beginning of the Summer or Winter season. All women were enrolled in prenatal outpatient clinics of public hospitals, specifically in Hospital de Clínicas (Clinics Hospital) of the Universidade do Paraná (Federal University of Paraná) and Hospital Universitário Evangélico (Evangelical University Hospital) in Curitiba, which are tertiary care hospitals.

Inclusion criteria comprised age between 18 and 40 years, Brazilian nationality with residence in the Curitiba metropolitan region, singleton pregnancy, and willingness to sign informed consent. Exclusion criteria included current use of vitamin D supplementation or chronic medications, such as anticonvulsants or glucocorticoids, and diseases other than those defining the high-risk group: preeclampsia (PE), gestational diabetes mellitus (GDM), or human immunodeficiency virus seropositivity (HIV+). Women in the low-risk group had no known conditions.

A questionnaire was developed by researchers and applied to participating women during an interview to collect sociodemographic, epidemiologic, and clinical data, including age, educational level, monthly household income, self-declared ethnicity, skin type according to Fitzpatrick´s 1988 classification, menarche, parity, date of the last menstrual period, gestational age according to ultrasonography, lifestyle habits (smoking, alcohol consumption, sedentary habits), weight at the first prenatal visit, height, body mass index (BMI), and current weight.(12)

Assessment of serum vitamin D levels

Venous blood samples were collected from all subjects in the morning. Samples were maintained at 2–8 °C and protected from light after collection and during transportation. Blood was centrifuged for 15 minutes at 3000 rpm for serum separation. All blood samples collected were analyzed on the same day of collection at the same laboratory using the microparticle chemiluminescence method with Architect i2000SR (Abbott, Illinois, USA).

Endocrine Society criteria were used to classify vitamin D status, with sufficiency being defined as vitamin D levels ≥30 ng/mL or 75 nmol/L, insufficiency as vitamin D levels between 20 and 30 ng/mL or 50 and 75 nmol/L, and deficiency as vitamin D levels below 20 ng/mL or 50 nmol/L.[13]

Statistical analysis

Data were collected in frequency and contingency tables using Microsoft Excel 2013®. Quantitative data were described as mean and standard deviation or median and minimum and maximum values. Qualitative data were described as frequencies and percentages. The Kolmogorov-Smirnov test was used to assess the normality of quantitative variables. T-test for independent samples was used for comparison of quantitative data between the two groups assessed. One-way analysis of variance model and the least significant difference test were used for multiple comparisons. Qualitative variables were compared through Chi-square or Fisher's exact test. A p-value ≤0.05 indicated statistical significance. Data were analyzed using Stata v.13.1 software (Texas, USA).

Ethics

This study was approved by the Research Ethics Committee of the Clinics Hospital of the Federal University of Paraná and of the Evangelical University Hospital of Curitiba. All subjects agreed to participate in the study and signed informed consent**.**

RESULTS

A total of 520 pregnant women were included in the study in 2016. Of these, 256 (49.2%) were assessed in the Summer (January through March) and 264 (50.8%) in the Winter (July through August; **Table 1**). Twenty percent of women (n=105) were included in the low-risk group, and the remaining 80% (n=415) in the high-risk group, with 228 women in this group presenting with PE, 154 with GDM, and 33 with HIV+.

The overall study population had a mean age of 29.5 ± 6.1 years, a mean age of menarche of 12.6 ± 1.6 years, a mean chronological gestational age of 32.0 ± 6.4 weeks, and a mean ultrasound gestational age of 32.1 ± 6.4 weeks. Nearly half of women had Fitzpatrick skin type II, and most were overweight at the beginning of gestation, with a mean BMI of 28.6 ± 6.7 kg/m^2, a mean weight gain until the date of data collection of 8.2 ± 6.8 kg, and a mean BMI at that time of consultation of 30.3 ± 5.9 kg/m^2. Sedentary lifestyle was common in this cohort (85.5%). Most women were nonsmokers and had no tobacco or alcohol consumption habits.

The mean serum 25(OH)D level of the study cohort was 22.5 ± 8.7 ng/mL, being significantly higher in Summer (mean 26.7 ± 7.8 ng/mL, range 8.8–56.6 ng/mL) compared to Winter (mean 18.3 ± 7.5 ng/mL, range 4.5–51.9 ng/mL; p <0.001) months. A total of 19.2% of women had vitamin D deficiency in the Summer compared to 67.4% in the Winter. Vitamin D insufficiency was observed in 50.8% of cases in the Summer and 23.9% in the Winter, and vitamin D sufficiency in 30.0% and 8.7%, respectively. All these differences were statistically significant (p <0.001; **Table 2**).

As shown in **Table 3**, age, education, ethnicity, skin type, and smoking were not significantly associated with serum vitamin D levels during Summer. Primiparous women had a significantly higher incidence of vitamin D deficiency than multiparous counterparts (p=0.026). Women with an initial BMI greater than 30 kg/m^2 were significantly less likely to be vitamin D-deficient (p=0.014).

No association was found between education, ethnicity, skin type, BMI, and smoking and vitamin D sufficiency in Winter (**Table 4**). On the other hand, subjects aged ≥30 years had a significantly higher incidence of vitamin D sufficiency (p=0.032). Primiparous women had a higher incidence of vitamin D deficiency than multiparous women, but the difference was not statistically significant (p=0.079).

HIV+ women had significantly higher vitamin D levels than women in other disease groups (p=0.001), while women with GDM had significantly lower vitamin D levels than women with low-risk pregnancy or PE (**Table 5**).

Table 1 - Clinical, epidemiological, and demographic data of pregnant Brazilian women

Characteristic	n (% or SD)
Total study population	520 (100%)
Women tested in Summer	256 (49.2%)
Women tested in Winter	264 (50.7%)

Mean age (years)	29.5 ± 6.1
Education	
Below/Under high school	170 (32.6%)
High school	301 (57.8%)
College	49 (9.4%)
Monthly household income	
Minimum wage	170 (32.6%)
Up to 3 times the minimum wage	300 (57.6%)
>3 times the minimum wage	50 (9.6%)
Ethnicity	
White	271 (52.1%)
Mixed	234 (45.0%)
Black	15 (2.8%)
Fitzpatrick skin types	
I	34 (6.5%)
II	237 (45.5%)
III	132 (25.3%)
IV	58 (11.1%)
V	44 (8.4%)
VI	15 (2.8%)
Menarche (years)	12.6 ± 1.6
Parity (gestations)	2.6 ± 1.4
Parity	
Primiparous	113 (21.7%)
Multiparous	407 (78.2%)
Gestational age by US (weeks)	32.1 ± 6.4
Gestational age by LMP (weeks)	32.0 ± 6.4
Weight at early pregnancy (kg)	70.0 ± 16.1
BMI in early pregnancy (kg/m²)	28.6 ± 6.7
Current weight	78.3 ± 16.0
Current BMI (kg/m²)	30.3 ± 5.9
Weight gain (kg)	8.2 ± 6.8
BMI gain (kg/m²)	3.2 ± 2.6
Lifestyle	
Smoking habits	37 (7.1%)
Alcohol consumption	4 (0.7%)
Sedentary lifestyle	445 (85.5%)

BMI, body mass index; LMP, last menstrual period; SD, standard deviation; US, ultrasound

Table 2 - Serum vitamin D levels in pregnant women during Summer and Winter

Vitamin D	Summer (n = 256)	Winter (n = 264)	p-value*
<20 ng/mL (deficiency)	49 (19.2%)	178 (67.4%)	0.001
20–30 ng/mL (insufficiency)	130 (50.8%)	63 (23.9%)	0.001
≥30 ng/mL (sufficiency)	77 (30.0%)	23 (8.7%)	0.001

**t*-test for independent samples

Table 3 - Association between maternal characteristics and serum vitamin D levels during Summer in Brazil

Characteristic (n)		Vitamin D (ng/mL)			p-value*
		Deficiency (<20)	Insufficiency (20–29.9)	Sufficiency (≥30)	
Age					
<20 years	10	5 (50.0%)	1 (10.0%)	4 (40.0%)	
20–29 years	114	40 (35.1%)	36 (31.6%)	38 (33.3%)	
≥30 years	132	49 (37.1%)	41 (31.1%)	42 (31.8%)	0.697
Education					
Below high school	68	20 (29.4%)	19 (27.9%)	29 (42.7%)	
High school	159	59 (37.2%)	50 (31.4%)	50 (31.4%)	
College	29	15 (51.8%)	9 (31.0%)	5 (17.2%)	0.125
Ethnicity					
White	132	58 (43.9%)	34 (25.8%)	40 (30.3%)	
Mixed	118	33 (27.9%)	42 (35.6%)	43 (36.5%)	
Black	6	3 (50.0%)	2 (33.3%)	1 (16.7%)	0.098
Parity					
Primiparous	58	30 (51.7%)	13 (22.4%)	15 (25.9%)	
Multiparous	198	64 (32.3%)	65 (32.8%)	69 (34.9%)	0.026
Initial BMI (kg/m²)					
<18.5	4	4 (100.0%)	0 (0%)	0 (0%)	
18.5–24.9	88	36 (40.9%)	24 (27.3%)	28 (31.8%)	
25.0–29.9	77	32 (41.6%)	18 (23.4%)	27 (35.0%)	
>30.0	87	22 (25.3%)	36 (41.4%)	29 (33.3%)	0.014
Skin type					
I/II	132	58 (43.9%)	34 (25.8%)	40 (30.3%)	
III/IV	93	26 (27.9%)	32 (34.4%)	35 (37.7%)	
V/VI	31	10 (32.3%)	12 (38.7%)	9 (29.0%)	0.128
Smoker					
No	247	91 (36.8%)	74 (29.9%)	82 (33.3%)	
Yes	9	3 (33.3%)	4 (44.4%)	2 (22.3%)	0.623

*p <0.05 based on Pearson's X^2 test
BMI, body mass index

Table 4 - Association between maternal characteristics and serum vitamin D levels during Winter in Brazil

Characteristic (N)		Vitamin D (ng/mL)			p-value*
		Deficiency (<20)	Insufficiency (20–29.9)	Sufficiency (≥30)	
Age					
<20 years	13	3 (23.1%)	7 (53.8%)	3 (23.1%)	
20–29 years	120	54 (45.0%)	33 (27.5%)	33 (27.5%)	
≥30 year	131	47 (35.9%)	30 (22.9%)	54 (41.2%)	0.032
Education					
Below high school	102	38 (37.2%)	27 (26.5%)	37 (36.3%)	
High school	142	54 (38.0%)	38 (26.8%)	50 (35.2%)	
College	20	12 (60.0%)	5 (25.0%)	3 (15.0%)	0.320
Ethnicity					
White	139	55 (39.5%)	37 (26.6%)	47 (33.9%)	
Mixed	116	45 (38.8%)	30 (25.9%)	41 (35.3%)	
Blcak	9	4 (44.4%)	3 (33.3%)	2 (22.2%)	0.954
Parity					
Primiparous	55	24 (43.6%)	19 (34.5%)	12 (21.9%)	
Multiparous	209	80 (38.3%)	51 (24.4%)	78 (37.3%)	0.079
Initial BMI (kg/m²)					
<18.5	9	3 (33.3%)	3 (33.3%)	3 (33.3%)	
18.5–24.9	82	33 (40.2%)	26 (31.7%)	23 (28.1%)	
25.0– 9.9	68	31 (45.6%)	18 (26.5%)	19 (27.9%)	
>30.0	105	37 (35.2%)	23 (21.9%)	45 (42.9%)	0.294
Skin type					
I/II	139	55 (39.6%)	37 (26.6%)	47 (33.8%)	
III/IV	97	36 (37.1%)	24 (24.7%)	37 (38.2%)	
V/VI	28	13 (46.4%)	9 (32.1%)	6 (21.5%)	0.606
Smoker					
No	236	93 (90.3%)	62 (88.6%)	81 (88.9%)	
Yes	28	10 (35.7%)	8 (28.6%)	10 (35.7%)	0.923

*p <0.05 based on Pearson's X^2 test
BMI, body mass index

Table 5 - Serum vitamin D levels in healthy and at-risk pregnancies in Brazilian women

Group	n	Mean	Medium	Minimum	Maximum	Standard deviation	p value
Healthy	105	22.9	21.8	4.5	47.3	9.5	<0.001
PE	228	21.9 [a]	21.2	6.5	43.7	7.5	
GDM	154	21.6 [b]	21.2	4.6	51.9	8.7	
HIV+	33	28.5 [c]	27.7	10.1	56.6	11.8	

ANOVA, analysis of variance; GDM, gestational diabetes mellitus; HIV, human immunodeficiency virus; PE, preeclampsia
[a]Healthy vs. PE: p=0.326
[b]Healthy vs GDM: p=0.247
[c]Healthy vs HIV+: p=0.001

DISCUSSION

As far as the authors are aware, this is the first study of serum vitamin D levels in pregnant women in south Brazil and shows a high prevalence of hypovitaminosis D in the Summer months, which becomes worse in Winter.

Hypovitaminosis D during pregnancy has attracted much interest in recent years due to its association with gestational and neonatal complications. Around 1 billion people globally are estimated to have the condition.[(14)]

In a review including several countries, a high prevalence of hypovitaminosis D was reported globally in infants, children, adolescents, adults, and the elderly throughout the year, including in sunny countries.[(15)] The highest prevalence was found in the Middle East. In most South American and African countries, there is not enough data.

Another review included seventeen studies on pregnant and lactating women, two from America, six from Europe, one from Africa, seven from Asia, and one from Oceania.[(15)] The authors reported vitamin D insufficiency in 33% of pregnant women in the USA, 24% in Canada, 45% in Belgium, 35% in the United Kingdom, 44% in the Netherlands, 20% in Spain, and 77% in Germany. In addition, vitamin D deficiency was reported in 12% of women in Belgium, 4% in England, and 23% in the Netherlands. A single study in Africa reported that 1% of pregnant women presented with hypovitaminosis D. In Asia, the prevalence of hypovitaminosis D in pregnant women was found to be 90% in Turkey, 67% in Iran, 72% in Pakistan, 70–83% in Kuwait, 96% in India, and 69% in China. In Australia, vitamin D insufficiency was found in 48% and vitamin D deficiency in 15% of pregnant women.[15] In the present study in tropical Brazil, the prevalence of vitamin D insufficiency and deficiency was high, with only 8.7% of subjects having sufficient vitamin D levels in Winter. Regardless of the season, more than half of subjects did not have adequate levels of this vitamin.

According to some authors, seasonality increases the risk of hypovitaminosis D in pregnancy, with a higher prevalence of vitamin D deficiency during Winter compared to Summer months.[(16,17)] The results of this study support such findings. Serum vitamin D levels in pregnant women were significantly lower in Winter compared to Summer. The study evaluated pregnant women from the city of Curitiba and surrounding metropolitan region, located in the State of Paraná and in the southern region of Brazil, where Winter is most rigorous. Brazil is a country with continental dimensions, with latitudes between 3ºN and 33ºS. Curitiba is located at a latitude of 25ºS and at an altitude of 934 meters. Previous studies have shown that the latitude directly influences vitamin D levels, with the higher the latitude, the higher the prevalence of hypovitaminosis D.[(18)]

Some global health organizations recommend vitamin D supplementation during pregnancy.[(19,20)] The World Health Organization 2020 update of the recommendations for vitamin D supplementation suggests that supplementation during pregnancy improves maternal vitamin D status and may reduce the risk of PE, low birthweight, and preterm birth. However, the evidence currently available to directly assess the benefits and harms of the use of vitamin D supplementation alone in pregnancy for improving maternal and infant health outcomes is limited.[(21)] The American Academy of Pediatrics suggests monitoring serum 25(OH)D levels during gestation, but there is no consensus regarding the appropriate levels. The US Institute of Medicine considers 20 ng/mL to be adequate, although most researchers consider that ideal levels to be ≥30 ng/mL. The Endocrine Society recommends that pregnant women consume 1,500 to 2,000 (up to 4,000) IU/day, as this has been shown to be safe and effective.[(13)] Each 100 IU of vitamin D administered raises serum 25(OH)D levels by about 1.0 ng/mL (range 0.7–1.1 ng/mL).[(22)] The dose of vitamin D required to prevent or treat vitamin D deficiency has not been established. Some researchers suggest 1,000 IU/day, others 5,000 IU/week, and others a single dose of 200,000 IU or higher.[(22–25)] The Brazilian Endocrinology and Metabolism Society recommends daily oral vitamin D supplementation in the dose of 600 IU/d for pregnant women in general, 600 to 1,000 IU/d for pregnant women aged 14 to 18 years, and 1,500 to 2,000 IU/d for pregnant women at risk aged over 18 years.[(26)]

Nourishment guidance should be provided to pregnant women, advising on the adoption of a diet rich in natural sources of vitamin

D. Detailed next is the vitamin D uptake provided by 100 g of each of the following foods: fresh wild salmon (≈600-1000 UI of vitamin D3); fresh farmed salmon (≈100-250 UI of vitamin D3); canned sardines (≈300 UI de vitamin D3); canned mackerel (≈250 UI of vitamin D3); canned tuna (≈230 UI of vitamin D3); fresh shiitake mushrooms (≈100 UI of vitamin D2); sun-dried shiitake mushrooms (≈1600 UI of vitamin D2). Also importantly, 5 mL of cod liver oil provide ≈400-1000 UI of vitamin D3, and one unit of egg yolk provides ≈20 UI of vitamin D3.[27]

In Brazil, the assessment of serum of 25(OH)D levels is not part of routine prenatal care. However, in regions with a high prevalence of hypovitaminosis D, this assessment should be performed during gestation. Ideally, a woman who intends to become pregnant should have sufficient antenatal serum 25(OH)D levels. During pregnancy, levels should be periodically monitored in accordance with the pregnancy stage. Guidelines for the prevention or treatment of hypovitaminosis D during pregnancy through sun exposure and adequate nutrition, together with supplementation, if necessary, are required to avoid maternal-fetal complications and problems during the neonatal period, early childhood, or even adulthood. As there is no consensus regarding vitamin D supplementation during pregnancy, recommendations should be individualized according to serum 25(OH)D levels. When laboratory tests cannot be performed, sun exposure and adequate nutrition should be reinforced.

This study's cross-sectional design and the impossibility of evaluating samples from the same pregnant woman in both seasons were study limitations that should be acknowledged.

In conclusion, this study documents the importance of assessing women's serum vitamin D status during pregnancy, in particular considering seasonal effects, providing nutritional advice and recommending regular physical activity and adequate sun exposure, with or without vitamin D supplementation. Future studies are required to assess the need for vitamin D supplements during pregnancy and, if necessary, determine their most appropriate dose.

AUTHORSHIP

Kadija Rahal Chrisostomo – Conceptualization; Data curation; Formal Analysis; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization ; Writing – original draft; Writing – review & editing

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