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Investigation of genetic polymorphisms in the SPP1 gene and pulp stones

Investigação de polimorfismos genéticos no gene SPP1 e nódulos pulpares

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ABSTRACT

Objective: This study aimed to assess the association between polymorphisms in the *SPP1* gene and the presence of pulp stones (PS). **Material and Methods:** This cross-sectional study involved individuals aged 18 years or older, divided into groups with and without PS, identified through panoramic and periapical radiographs. Saliva samples were collected as a source of genomic DNA. Polymorphisms in *SPP1* gene (rs9138, rs4754 and rs11730582) were genotyped by Real-Time PCR using the Taqman method. Data was analyzed using Pearson's Chi-square or Fisher's exact tests, followed by multivariate Poisson regression. P-values of < 0.05 were considered statistically significant. **Results:** A total of 116 individuals participated. No significant association was found between the groups concerning the presence of PS ($p > 0.05$). Allelic, genotype, and haplotype analysis also revealed no significant association between the presence of PS and the SNPs studied ($p > 0.05$). **Conclusion:** The genetic polymorphisms investigated were not associated with the presence of PS.

KEYWORDS

Dental pulp calcification; Genes; Genetic predisposition to disease; Human SPP1 protein; Osteopontin.

RESUMO

Objetivo: Este estudo teve como objetivo avaliar a associação entre polimorfismos no gene *SPP1* e a presença de nódulos pulpares (NP). **Material e Métodos:** Este estudo transversal envolveu indivíduos com 18 anos ou mais, divididos em grupos com e sem NP, identificados por meio de radiografias panorâmicas e periapicais. Amostras de saliva foram coletadas como fonte de DNA genômico. Os polimorfismos no gene *SPP1* (rs9138, rs4754 e rs11730582) foram genotipados por PCR em tempo real utilizando o método TaqMan. Os dados foram analisados utilizando os testes Qui-quadrado de Pearson ou exato de Fisher, seguidos por regressão de Poisson multivariada. Valores de $p < 0.05$ foram considerados estatisticamente significativos. **Resultados:** Participaram 116 indivíduos no total. Não foi encontrada associação significativa entre os grupos quanto à presença de NP ($p > 0.05$). As análises alélica, de genótipos e de haplótipos também não revelaram associação significativa entre a presença de NP e os SNPs estudados ($p > 0.05$). **Conclusão:** Os polimorfismos genéticos investigados não foram associados à presença de NP.

PALAVRAS-CHAVE

Calcificação da polpa dentária; Genes; Predisposição genética para doença; Proteína SPP1 humana; Osteopontina.

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INTRODUCTION

Pulp stones (PS) are calcified nodular aggregates found both in the crown and root of teeth, and can be found free in the pulp tissue, adhered and/or embedded in dentin [1]. They are usually asymptomatic and detected in radiographic examinations, being represented by radiopaque images inside the pulp chamber, which can make endodontic treatment difficult [2].

The prevalence of PS may vary according to the population and the diagnostic method adopted. Authors indicate a frequency between 3.5% and 83.3% [3-6]. There is also a predilection for females and for first molars, which are the teeth most frequently affected, possibly due to prolonged exposure to aggressive stimuli in the oral environment [1]. Several etiological factors are associated with the development of PS, including advancing age [7], circulatory disturbances in the pulp [7,8], orthodontic movement [8], chronic irritation of the pulp tissue due to dental caries [9], exposure to restorative materials [9], genetic factors [10] and the presence of nanobacteria [11], which can produce nucleated hydroxyapatite. Nanobacteria also increase the potential for pathological calcifications in the kidneys [12] and gallbladder [13].

Regarding the organic matrix of PS, the main components are type I collagen and osteopontin (OPN), a phosphorylated glycoprotein found in the peripheral areas of these calcifications [14]. OPN is also present in various body fluids, including saliva, blood, bile, urine, cerebrospinal fluid, and milk, and is the primary non-collagenous protein constituent of the organic matrix of bones [15]. OPN is encoded by the secreted phosphoprotein 1 (*SPP1*) gene, located on chromosome 4q21-25, which consists of seven exons [16]. Certain single nucleotide polymorphisms (SNPs) in this gene are situated in transcription factor binding sites that regulate the transcription of the *OPN* gene, making these SNPs potential candidates for studying abnormal calcifications [17].

Previous studies have investigated the role of *OPN* as a serum biomarker for arterial calcifications [18], inflammatory processes [19], kidney stones formation [20], and neoplastic proliferations [21]. Also, a study assessing the level and diagnostic accuracy of osteopontin and oral health status in periodontitis patients with/without type-2 diabetes mellitus, revealed that osteopontin could be used as a diagnostic biomarker of periodontal disease [22].

Since the formation of PS is multifactorial [1] and its chemical composition closely resembles that of kidney stones [20], there is biological plausibility to suggest that SNPs in the *SPP1* gene, which have been associated with other systemic conditions, may also be linked to the etiology of PS. Therefore, the aim of this study was to determine whether an association exists between the presence of PS and the polymorphisms rs9138, rs4754, and rs11730582 in the *SPP1* gene. The null hypothesis is that no such association exists.

MATERIAL AND METHODS

Ethical aspects

This cross-sectional study was approved by the Research Ethics Committee of Universidade Positivo (CAAE registration 94846918.5.0000.0093; approval no. 2.805.133) and was conducted in accordance with the Strengthening the Reporting of Genetic Association Studies (STREGA) guidelines [23]. All participants provided written informed consent.

Sample

The sample was composed of the same one used by Romano et al. [24], that is, from the random stratification of digital panoramic radiographs of individuals aged between 18 and 65 years, treated at the dental radiology service of a private university in Curitiba, PR, Brazil, between 2018 and 2019.

A power analysis was performed (https://clincalc.com/stats/samplesize.aspx), assuming a 0.15 difference in the distribution between the groups and an error probability of 0.05. The results indicated that a sample size of 54 patients per group would provide a power of 0.80 for analysing allele distribution.

A total of 1,047 digital panoramic radiographs were analysed by three trained and calibrated researchers (Cohen's Kappa = 0.88). These examinations were divided into two groups: "with PS", where image suggestive of PS (radiopaque, circular or oval, single or multiple) was observed in one or more posterior teeth (premolars and/or molars) free of caries, and if the teeth had restorations, the depth should

be shallow (compromise of up to 1 mm of the dentin-enamel junction); and "without PS", any permanent teeth (except third molars) presenting no images suggestive of PS. Figure 1 presents the study flowchart.

From the initial analysis of the images, individuals who met the inclusion criteria (age ≥ 18 years, no cognitive problems, not pregnant, and with PS in the complementary exams) were contacted by telephone and invited to participate.

Questionnaire application and saliva collection

A questionnaire adapted from Bhattarai et al. [25] was administered, which included demographic variables: sex (male or female), observed skin color (white, yellow, black or brown - dichotomized into white or non-white), age (in years) and systemic conditions: heart attack (yes or no), kidney stone (yes or no), gallstone (yes or no). For female participants, two additional questions were included: diagnosis of endometriosis (yes or no) and presence of ovarian cysts (yes or no). The questionnaires were administered by two trained researchers. Individuals previously allocated into "with PS" group underwent a complementary periapical radiographic examination to confirm the presence of PS.

Afterwards, saliva samples were collected from all participants as a source of genetic material by three trained researchers. The samples were stored at -20 °C until the DNA extraction and genotyping.

DNA extraction and genotyping

Genomic DNA was extracted from buccal cells isolated from the saliva samples according to the protocol previously described by Küchler et al. [26]. The quantity and purity of the DNA were assessed using a spectrophotometer (Nanodrop 1000; ThermoFisher Scientific, Wilmington, NC, USA). Candidate polymorphisms were selected by consulting the UCSC Genome Browser to identify those already characterized for the candidate gene, considering a minimum allele frequency of 0.30. In this manner, three SNPs in the *SPP1* gene (rs9138, rs4754 and rs11730582) were chosen for investigation, and their characteristics are presented in Table I.

Genotyping was performed using TaqMan SNP Genotyping Assays (Life Technologies; ThermoFisher Scientific, Wilmington, NC, USA) in Stratagene Mx3005P (Agilent Technologies, Santa Clara, CA, USA). Real-time PCR was performed in a total volume of 3 μL (4 ng DNA/reaction, 1.5 μL Taqman PCR master mix, and 0.075 SNP assay) (Applied Biosystems, Foster

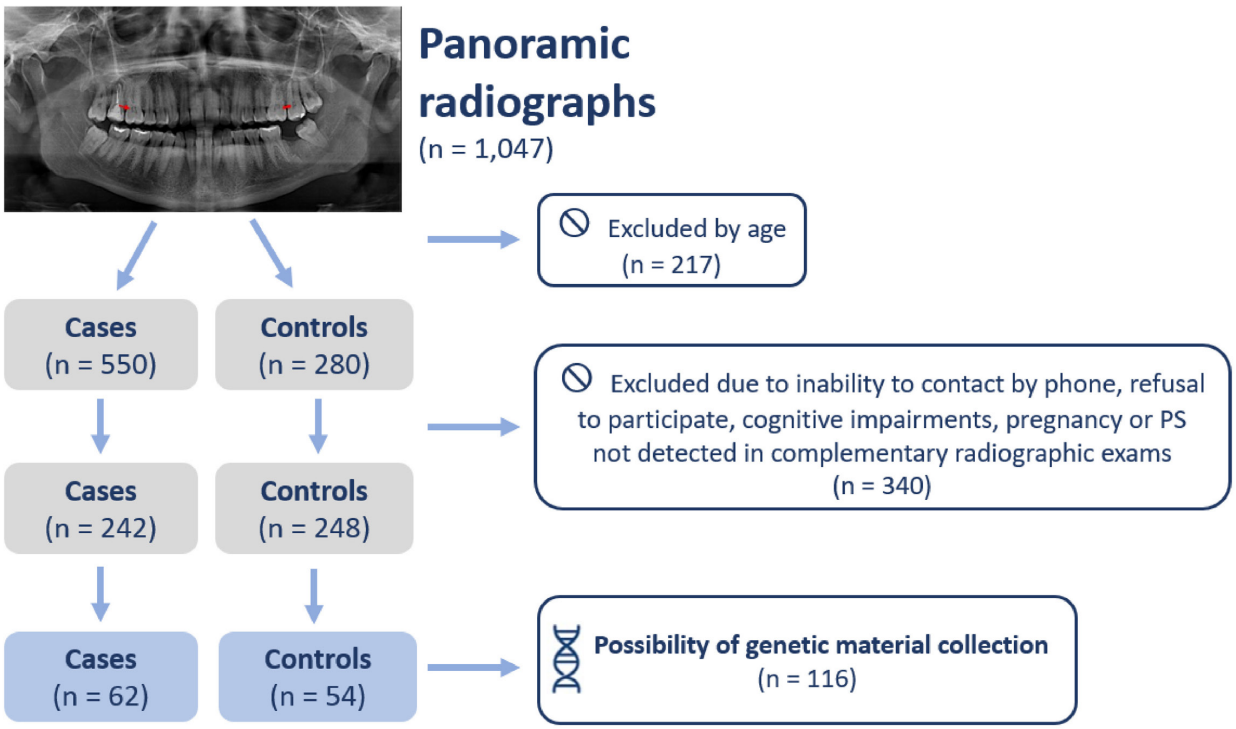


Figure 1 - Flowchart of the study.

Table I - Characteristics of selected polymorphisms

Polymorphism	Base substitution	Position	Global MAF
rs9138	A/C/T	4:87983190	0.36
rs4754	A/C/T	4:87981540	0.36
rs11730582	C/T	4:87975269	0.33

Note: MAF - Minor Allele Frequency.
Source: National Library of Medicine [27].

City, CA, USA). Thermal cycling was performed with one cycle of maintenance at 95 °C for 10 minutes, followed by 45 cycles of amplification at 92 °C for 15 seconds and 60 °C for one minute. The assays and reagents were obtained from Applied Biosystems (Foster City, CA, USA).

The processing of the genetic material was blindly performed by an experienced researcher specialized in this type of analysis, in March 2023, at the Clinical Research Unit of the Antonio Pedro University Hospital of the Universidade Federal Fluminense, Rio de Janeiro, Brazil.

Statistical analysis

Pearson's Chi-square test without correction or Fisher's exact test was applied to compare sex, skin color, kidney stones, gallstones, endometriosis, and ovarian cysts between the groups with and without PS. After analysing the data distribution (Shapiro-Wilk test; $p > 0.05$), age was compared between the groups using the Mann-Whitney test.

For allelic distribution and the haplotype analyses, the Pearson's Chi-square test without correction was used. The genotypes distribution between the groups was analysed using either the Chi-square test or Fisher's Exact test. Finally, multivariate Poisson regression was performed to adjust for age and endometriosis regarding the genotypes. The prevalence ratio (PR) and 95% confidence interval (95% CI) were calculated for both univariate and multivariate tests.

Haplotype analyses were performed using PLINK software version 1.06 [28]. For the other analyses, the SPSS® version 25.0 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp) was used. Values of $p < 0.05$ were considered with statistically significant difference.

The Hardy–Weinberg equilibrium was evaluated using Pearson's chi-square test within each SNP.

RESULTS

After applying the inclusion criteria, a total of 116 individuals were investigated from June 2019 to February 2020. Of these, 62 (53.4%) presented PS and 54 (46.6%) had no PS (Figure 1).

Regarding the demographic data, 82 participants (70.7%) were women and white individuals comprised 96 (82.7%). The mean age of individuals in the "with PS" group was significantly higher ($p < 0.001$) than that of individuals in the "without PS" group (36.8 ± 11.3 and 27.1 ± 8.9 years, respectively). The variables presence or absence of kidney stones and gallstones had no significant difference between the groups ($p > 0.05$) when both sexes were analyzed. However, in the analysis adjusted by sex, women with endometriosis had a higher prevalence of PS ($p = 0.018$) (Table II).

All the polymorphisms evaluated were in Hardy-Weinberg equilibrium (data not shown).

The genotype distributions in the co-dominant, dominant, and recessive models among the groups presented no statistically significant association ($p > 0.05$) (Table III).

Table IV shows the allelic distributions and frequency according to the groups "with PS" and "without PS". No alleles were associated "with PS" ($p > 0.05$).

Haplotypes frequency comparisons between the three polymorphisms showed no statistically significant differences ($p > 0.05$) (Table V).

DISCUSSION

Root canal obliteration caused by PS is a recurrent topic in Endodontics, as it can directly interfere with the diagnosis and prognosis of the endodontic treatment [29]. The formation of PS is multifactorial, and little is known about the genes involved in this condition.

Table II - Distribution of sample characteristics comparing groups with and without NP (n = 116)

	With PS n (%)	Without PS n (%)	PR (95% CI)	p-value*
Variables				
Sex				
Male	22 (35.5)	12 (22.2)	0.69 (0.41 – 1.13)	> 0.05
Female	40 (64.5)	42 (77.8)		
Skin				
White	49 (79.0)	47 (87.0)	1.04 (0.77 – 1.40)	> 0.05
Non-white	13 (21.0)	7 (13.0)		
Kidney stone				
No	46 (74.2)	47 (87.0)	1.66 (0.86 – 3.18)	> 0.05
Yes	16 (25.8)	7 (13.0)		
Gallbladde				
No	57 (91.9)	51 (94.4)	1.25 (0.50 – 3.14)	> 0.05
Yes	5 (8.1)	3 (5.6)		
*Endometriosis***				
No	31 (77.5)	40 (95.2)	**3.09 (1.87 – 11.03)**	**< 0.05**
Yes	9 (22.5)	2 (4.8)		
*Ovarian cyst***				
No	27 (67.5)	32 (76.2)	1.24 (0.74 – 2.10)	> 0.05
Yes	13 (32.5)	10 (23.8)		

*Pearson´s Chi-square or Fisher's exact test was used. **Only women (n = 82) were included in the analysis. Bold values indicate statistical significance (p < 0.05).

Table III - Genotypic distribution under co-dominant, dominant, and recessive models was compared between groups with and without PS (n = 116)

SNP SPP1 gene	Model	Genotype	With PS n (%)	Without PS n (%)	p-value*	PR (95% CI)	p-value**	PR (95% CI)
rs 4754	Co-dominant	CC	29 (46.8)	32 (59.3)		Ref.		
		CT	31 (50.0)	19 (35.2)	> 0.05	0.76 (0.54 – 1.07)	> 0.05	1.03 (0.70 – 1.53)
		TT	2 (3.2)	3 (5.6)	> 0.05	1.18 (0.39 – 3.59)	> 0.05	0.89 (0.40 – 1.97)
	Dominant	CC	29 (46.8)	32 (59.3)	> 0.05	1.31 (0.87 – 1.96)	> 0.05	1.01 (0.69 – 1.48)
		CT + TT	33 (53.2)	22 (40.7)				
	Recessive	CC + CT	60 (96.8)	51 (94.4)	> 0.05	0.76 (0.36 – 1.61)	> 0.05	0.87 (0.40 – 1.88)
		TT	2 (3.2)	3 (5.6)				
rs 9138	Co-dominant	AA	30 (48.4)	32 (59.3)		Ref.		
		AC	30 (48.4)	19 (35.2)	> 0.05	0.79 (0.56 – 1.11)	> 0.05	1.02 (0.69 – 1.49)
		CC	2 (3.2)	3 (5.6)	> 0.05	1.20 (0.40 – 3.64)	> 0.05	0.88 (0.40 – 1.94)
	Dominant	AA	30 (48.4)	32 (59.3)	> 0.05	1.26 (0.84 – 1.89)	> 0.05	1.00 (0.69 – 1.45)
		AC + CC	32 (51.6)	22 (40.7)				
	Recessive	AA + AC	60 (96.8)	51 (94.4)	> 0.05	0.76 (0.36 – 1.61)	> 0.05	0.87 (0.40 – 1.88)
		CC	2 (3.2)	3 (5.6)				
rs 11730582	Co-dominant	TT	23 (37.1)	19 (35.2)		Ref.		
		CT	24 (38.7)	22 (40.7)	> 0.05	1.04 (0.71 – 1.55)	> 0.05	0.93 (0.62 – 1.39)
		CC	15 (24.2)	13 (24.1)	> 0.05	1.02 (0.65 – 1.58)	> 0.05	0.99 (0.62 – 1.59)
	Dominant	TT	23 (37.1)	19 (35.2)	> 0.05	0.95 (0.63 – 1.44)	> 0.05	0.95 (0.66 – 1.36)
		CT + CC	39 (62.9)	35 (64.8)				
	Recessive	TT + CT	47 (75.8)	41 (75.9)	> 0.05	1.00 (0.63 – 1.58)	> 0.05	1.03 (0.66 – 1.58)
		CC	15 (24.2)	13 (24.1)				

Note: Ref.- reference. *Pearson´s Chi-square or Fisher's exact test was used. **Multivariate Poisson regression adjusted for endometriosis and age (p < 0.05).

Table IV - Analysis of the studied polymorphisms in the allelic model

SNP	Allele	With PS (n%)	Without PS (n%)	p-value*	PR (95% CI)
rs 4754	C	89 (71.8)	83 (76.8)		
	T	35 (28.2)	25 (23.2)	> 0.05	0.88 (0.62 – 1.14)
rs 9138	A	90 (72.6)	83 (76.8)		
	C	34 (27.4)	25 (23.2)	> 0.05	0.90 (0.69 – 1.17)
rs 11730582	T	70 (56.4)	60 (55.5)		
	C	54 (43.6)	48 (44.5)	> 0.05	1.01 (0.79 – 1.29)

*Pearson´s Chi-square without correction - univarite ($p < 0.05$).

Table V - Haplotype frequency comparisons between groups

Haplotypes		Frequency (%)		p-value*
		With PS	Without PS	
rs4754|rs9138|rs11730582	CCC	0.2084	0.1736	0.504
rs4754|rs9138|rs11730582	TAC	0.2225	0.2709	0.394
rs4754|rs9138|rs11730582	CCT	0.06801	0.05789	0.753
rs4754|rs9138|rs11730582	TAT	0.5011	0.4977	0.958
rs4754|rs9138	CC	0.2764	0.2315	0.434
rs4754|rs9138	TA	0.7236	0.7685	0.4345
rs4754|rs11730582	CC	0.2154	0.1739	0.428
rs4754|rs11730582	TC	0.2201	0.2705	0.372
rs4754|rs11730582	CT	0.0669	0.05755	0.769
rs4754|rs11730582	TT	0.4976	0.498	0.995
rs4754|rs11730582	CC	0.2063	0.1733	0.524
rs4754|rs11730582	AC	0.2292	0.2711	0.461
rs4754|rs11730582	CT	0.06792	0.05817	0.761
rs4754|rs11730582	AT	0.4966	0.4974	0.990

*Pearson's Chi-square test ($p < 0.05$).

Therefore, the present study found no association between the polymorphisms rs9138, rs4754 and rs11730582 in the *SPP1* gene and the presence of PS, confirming the null hypothesis. However, these findings provide new insights into the etiology of PS, which remains largely unknown.

Considering the factors that contribute to the formation of PS, one recurrent factor is a higher prevalence associated with advancing age [7]. The demographic analysis of the sample in this study also supports this observation, as a direct relationship was found between increasing age and the presence of PS. Regarding the relationship between sex and PS, the frequency was higher in women in the studied sample. This finding has been previously noted in the systematic review by Jannati et al. [1] in which more than half of the primary studies analyzed reported a high prevalence of PS among women. Some authors who have indicated that PS are more frequently observed in females have hypothesized that the heightened incidence of bruxism among women may result in chronic irritation of the dental structures, thereby facilitating the PS formation [4].

For the skin color variable, no significant difference was found, and there is no report in the literature indicating that this variable is associated with the incidence of PS. However, when considering ethnic and geographic aspects, studies [4,9] have reported a greater relationship with environmental factors rather than genetic ones.

Researchers have been working to elucidate the involvement of genes in the etiology of PS. In the study by Ramirez et al. [30], SNPs of the *WNT6* and *WNT10A* genes were investigated concerning the risk of dentin calcification in orthodontic

patients. The authors observed that the genetic polymorphisms studied were associated with the presence of PS in molars post-treatment and could be considered biomarkers of dentin calcification. Thuller et al. [10] analyzed the association of SNPs of the *BMP2*, *BMP4*, *SMAD6*, and *RUNX2* genes with the presence of PS, and found that the polymorphisms rs2119261 and rs3934908 of the *SMAD6* gene showed significant associations in both genotypic distribution and haplotype analysis. These findings suggest that these polymorphisms could also serve as biomarkers for PS. Contrastingly, Meyfarth et al. [31], found no association between polymorphisms in genes encoding growth factors and dental pulp calcification in orthodontic patients.

In this study, genetic polymorphisms in the *SPP1* gene were studied. This gene encodes OPN, a glycoprotein that is expressed in endothelial cells, muscle cells, and bone tissue, especially by osteoblasts and osteoclasts, playing an essential role in the regulation of bone metabolism [31], adhesion, migration, and activation [19]. OPN has been associated with a number of pathological events, including endometriosis [18,21] and tumor metastasis [21]. Regarding to some pathologies involving dental pulp, OPN appears to be associated with calcification of pulp tissues [14], apical periodontitis [32] and, may act as a biomarker for pulpitis [33]. In this sense, OPN seemed to be a promising candidate gene to be studied in conditions involving inflammatory responses and abnormal calcifications.

In the present study, no associations were observed between the studied polymorphisms rs9138, rs4754, and rs11730582 in the *SPP1* gene and the presence of PS. However, among the investigated systemic conditions, a statistically significant association was found between endometriosis and the presence of PS. This finding may be related to the pathogenesis of immune-mediated diseases that increase the expression of OPN [19,22]. Endometriosis is a systemic chronic inflammatory condition characterized by an altered immune microenvironment and elevated levels of pro-inflammatory cytokines and OPN in both peritoneal fluid and serum [34,35].

OPN is a multifunctional glycoprotein involved in cell adhesion, tissue remodeling, and the regulation of biomineralization [36]. In the highly vascularized pulpal microenvironment, chronic exposure to systemic inflammatory signaling can alter local cellular behavior. Elevated OPN levels along with systemic pro-inflammatory mediators may stimulate pulpal mesenchymal stem cells toward osteo/odontogenic differentiation [37]. This sequence of events facilitates matrix deposition and crystal nucleation, sharing mechanistic features with pathological calcification dynamics elsewhere in the body [19].

Therefore, while the specific SPP1 genetic variants evaluated here did not confer direct genetic susceptibility to PS, the biological crosstalk between systemic chronic inflammation in endometriosis and OPN regulation at the protein level provides a plausible mechanism for pulpal calcification. To our knowledge, this is the first study to report a clinical link between these conditions, underscoring the importance of future functional and proteomic studies to further clarify this interplay.

In the case of kidney stones, the literature highlights similarities in both chemical composition and developmental processes with other calcifications, with calcium and phosphorus ions as major components. Amorphous aggregates of phosphorylated, including OPN, act as initial sites for calcium crystal precipitation, which also leads to the formation of PS. In kidney stones, there is a supersaturation of urine with calcium oxalate or calcium phosphate, followed by nucleation, growth, and aggregation of crystals [19]. This process may explain the co-occurrence of kidney stones and PS in the same individuals, as reported by Jia et al. [20].

The haplotype comparisons in this study showed no statistical association, although some evidence points to a potential role of polymorphisms in genes related to the extracellular matrix in the formation of PS [16,17,21,30]. However, it is important to emphasize that the lack of statistical significance does not completely exclude the possibility that haplotypes may influence the formation of PS. Studies with larger sample sizes and analysis of genetic interactions could provide more information. In addition, nongenetic factors, such as age, type of pulp stimulation, and time of exposure to dental treatments, may play a significant role and should be considered in future investigations [1,7,9].

This research had limitations related to the diagnostic methods used, which consisted

of panoramic and periapical radiographs analysis. It is known that the most accurate examinations to confirm the presence of PS are computed tomography and/or histological evaluations [6]. However, these resources are not routinely indicated due to their high costs and their status as complementary exams to radiographic analysis [6]. Consequently, the number of cases of PS may be underestimated. Additionally, two-dimensional images can present overlapping structures and distortion, which make PS visualization difficult or sometimes impossible [3]. Another potential limitation arises from the fact that the history of previous orthodontic treatment was not investigated in this study, despite this variable has been associated with the occurrence of PS [8]. Furthermore, the failure to request clinical examinations to confirm systemic alterations, relying solely on self-reports, presents limitations, as other morbidities, such as autoimmune diseases and/or neoplasms, may be present and influence the presence or absence of PS.

This is the first study to investigate polymorphisms in the *SPP1* gene and PS bringing a novel data. A strength of this study is that the sample analyzed in this study had similar socioeconomic, ethnic, and cultural backgrounds. Additionally, the accuracy and rigor in both the selection of images and the genetic tests, along with an adequate sample size, enable precise observations that may stimulate further research with a genetic focus, given the evidence of an association between systemic alterations and the presence of PS. Finally, the genetic tests used enable visualization of the individual contributions of each SNP to the PS outcome; however, this analysis is limited. Understanding the hereditary contribution to the risk of PS formation and identifying the genes that contribute to the pathogenesis of this condition may be useful for risk assessment. Future studies could employ other genic analysis to elucidate the role of the *SPP1* gene in the etiology of PS.

CONCLUSION

The polymorphisms rs9138, rs4754 and rs11730582 in the *SPP1* gene were not associated with PS. However, a high prevalence of the condition was observed in women and older individuals, and a significant association occurred between the presence of PS and endometriosis.

Data availability

The datasets generated and/or analyzed during the current study are not publicly available due to ethical restrictions involving patient confidentiality but are available from the corresponding author on reasonable request and with approval from the Institutional Review Board.

Author's Contributions

GMB: Data Curation, Formal Analysis, Investigation, Writing – Original Draft Preparation, Writing – Review & Editing. PMOK: Investigation. SRSM: Data Curation, Formal Analysis, Writing – Original Draft Preparation. VGM: Formal Analysis, Investigation, Writing – Original Draft Preparation. JAB: Conceptualization, Methodology, Resources, Supervision, Validation, Visualization, Writing – Original Draft Preparation. LSA: Methodology, Validation, Writing – Original Draft Preparation. MCLG: Conceptualization, Funding Acquisition, Methodology, Project Administration, Resources, Supervision, Validation, Visualization, Writing – Review & Editing.

Conflict of Interest

No conflicts of interest declared concerning the publication of this article.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local Human Research Ethics Committee of Universidade Positivo (CAAE registration 94846918.5.0000.0093; approval No. 2.805.133). All participants provided written informed consent.

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