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Podoplanin immunoexpression in odontogenic lesions: a systematic review, meta-analysis, and integrated bioinformatic analysis

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Abstract

Background Podoplanin (PDPN) is a transmembrane glycoprotein implicated in the pathogenesis of odontogenic lesions (OL). It is localized at the membrane and cytoplasmic level, and its interaction with other proteins could trigger cell proliferation, invasion and migration. The main objective of this systematic review is to explore the immunoexpression pattern of podoplanin in OL. In addition, as secondary objectives, we aimed to compare the immunostaining intensity of PDPN in OL, to analyze its interaction networks by bioinformatic analysis and to highlight its importance as a potential diagnostic marker useful in the pathogenesis of OL.

Methods The protocol was developed following PRISMA and Cochrane guidelines. The digital search was performed in the databases: PubMed/MEDLINE, ScienceDirect, Scopus, Web of Science and Google Schoolar from August 15, 2010 to June 15, 2023. We included cross-sectional and cohort studies that will analyze the pattern of PDPN immunoexpression in OL. Two investigators independently searched for eligible articles, selected titles and abstracts, analyzed full text, conducted data collection, and performed assessment of study quality and risk of bias. In addition, part of the results were summarized through a random-effects meta-analysis. STRING database was used for protein-protein interaction analysis.

Results Twenty-nine relevant studies were included. The ages of the subjects ranged from 2 to 89 years, with a mean age of 33.41 years. Twenty-two point two percent were female, 21.4% were male, and in 56.4% the gender of the participants was not specified. A total of 1,337 OL samples were analyzed for PDPN immunoexpression pattern. Ninety-four (7.03%) were dental follicles and germs, 715 (53.47%) were odontogenic cysts, and 528 (39.49%) were odontogenic tumors. Meta-analysis indicated that the immunostaining intensity was significantly stronger in odontogenic keratocysts compared to dentigerous cysts (SMD=3.3(CI=1.85-4.82, p=0.000*). Furthermore, bioinformatic analysis revealed that PECAM-1, TNFRF10B, MSN, EZR and RDX interact directly with PDPN and their expression in OL was demonstrated.

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Conclusions The results of the present systematic review support the unique immunoexpression of PDPN as a potential useful diagnostic marker in the pathogenesis of OL.

Keywords PDPN Protein, Human, Odontogenic Tumors, Odontogenic Cysts, Systematic Review

Background

Odontogenic cysts and tumors are a heterogeneous group of lesions that affect the oral and maxillofacial region, and originate from complex molecular alterations that frequently occur after odontogenesis has been completed [1, 2]. Under normal conditions, the cells involved in this process remain dormant within the dental tissues, however, in some cases, these cells can reactivate and produce a wide range of lesions with diverse clinical, radiographic, histopathologic manifestations and behaviors within the maxillary bones [3]. These types of manifestations range from an innocuous lesion with minimal involvement of adjacent anatomical structures, to disastrous lesions such as severe facial disfigurements, accompanied by functional alterations that can compromise the patient's life [4, 5]. In 1971, the World Health Organization (WHO) published the first classification of odontogenic cysts and tumors, later revised and updated in 1992, then in 2005, 2017 and finally in 2022 considered to date the most current classification, which takes into account the tissue of origin and the biological behavior of each of the odontogenic lesions (OL) [6]. Thus, odontogenic cysts (OC) are a type of lesion comprising growths of inflammatory or developmental origin [7]. The radicular cyst (RC) remains the most frequent OC, followed by the dentigerous cyst (DC) and the odontogenic keratocyst (OKC), the latter usually presenting a remarkable growth potential producing massive bone destruction and most of them usually recur if not adequately removed [8, 9]. Regarding odontogenic tumors (OT), these types of lesions comprise solid tissue masses that are not necessarily usually neoplastic/malignant [10]. Odontoma is the most frequent OT, followed by solid/conventional ameloblastoma (AM), the latter, characterized by being slow-growing but locally invasive and destructive [11], and like OKC presents a high tendency to recurrence, if not completely excised [12].

The diagnosis of this type of lesions is clinical-radiographic and is confirmed by histopathological study [13], however, these tools alone are not able to predict the onset and potential for aggressive and neoplastic behavior such as, expansion and/or localized infiltration, as well as, some type of malignant transformation that may arise from a benign lesion [14]. In this sense, biomarkers are molecular features (proteins, lipids, carbohydrates and nucleic acids/genes) that have the ability to discriminate between a state of health and/or disease [15]. In fact, they currently play an important role in the diagnosis and management of patients with aggressive and tumorigenic cystic lesions [16]. Therefore, immunohistochemistry (IHC) is useful for oral and maxillofacial pathologists [17]. The use of this technique has helped to determine the presence of different specific markers, which increases the possibility of providing a correct diagnosis of some special types of OL and increase our knowledge about the pathogenesis and molecular genetic characteristics of this type of lesions [18]. However, the problem seems to be that there are few immunohistochemical markers with the ability to evaluate the proliferative and invasive activity of different odontogenic cysts and tumors. Despite this, scientific evidence has shown that the expression of p53 and Ki-67 proteins is altered in some OL such as AM and OKC, which is closely associated with uncontrolled cell growth giving rise to pathology [19, 20].

Thus, an arduous and long search for such an efficient marker drew the attention of some researchers to podoplanin (PDPN), an emerging IHC biomarker [21]. PDPN or D2-40 is a type I transmembrane glycoprotein, similar to mucin [22]. It is encoded by the *PDPN* gene located on chromosome 1p36.21, has a molecular weight of 16.69 kDa and consists of 162 amino acids [23]. As for its structure, it consists of an O-glycosylated ectodomain, a hydrophobic transmembrane domain and a short cytoplasmic domain of nine amino acids. The extracellular domain contains four platelet aggregation domain repeats (PLAG) that interact with C-type lectin 2 receptors (CLEC-2) on the platelet surface, the intracellular region contains basic amino acids and serine residues. When this region binds to ezrin-radixin-moesin (ERM) it directs RhoA GTPases to reorganize the cytoskeleton, hence its importance in mechanisms of cell infiltration and invasion [24]. PDPN is expressed in different tissues and cells such as glomerular podocytes, type I alveolar cells, osteocytes, mesothelial cells, choroid plexus, glia cells, as well as neurons and fibroblasts [25]. Among its main functions are its participation in embryonic development, in lymphangiogenesis (as a lymphatic endothelial biomarker), in the production of platelets by the bone marrow, in the immune response, as well as stimulating invasion and metastasis of cancer cells [26]. In relation to immune function, strong PDPN immunoreactivity has been demonstrated in all layers of the sulcus and

junctional epithelium associated with severe inflammatory reaction in the connective tissue, suggesting that PDPN expression in the gingival epithelium is associated with the progression of chronic periodontitis [27]. On the other hand, PDPN has been shown to be involved in oral oncogenesis and may be a predictor of invasion and progression of lymph node metastases in asymptomatic oral cancer patients [28].

In relation to OL, numerous studies have demonstrated differences in the immunoexpression pattern as well as in the intensity of PDPN immunostaining between different odontogenic cysts and tumors, suggesting that this protein is involved in the development of this type of lesions [29–57]. Taking into account the above, the aim and objectives of the present study were:

1. To know and evaluate the immunoexpression pattern of podoplanin in odontogenic cysts and tumors.
2. To compare the intensity of podoplanin immunostaining in odontogenic cysts and tumors.
3. To analyze podoplanin interaction networks in normal biological processes and those associated with OL through bioinformatic analysis using the STRING database.
4. To highlight the importance of podoplanin and target proteins as possible immunohistochemical biomarkers to evaluate the proliferative potential and aggressiveness among different odontogenic cysts and tumors.

Materials and methods

Protocol, registration and PECO strategy

The protocol for this systematic review was developed following the Preferred Reporting Items for Systematic Reviews and Mea-Analyses (PRISMA) [58] and Cochrane Handbook for Systematic Reviews guidelines [59]. In addition, it was registered in the Open Science Framework (OSF) platform, accessed on February 02, 2024; https://doi.org/10.17605/OSF.IO/E2ZJW.

PECO items were taken into account as part of the PRISMA requirements and the central question was as follows: 1)Are there differences in the immunoexpression pattern and immunostaining intensity of PDPN in odontogenic cysts and tumors? In addition, the following two sub-questions were formulated: 2)Does PDPN interact with other proteins that contribute to the processes of tumor proliferation and invasion associated with the development of odontogenic cysts and tumors? And 3) What would be the importance of PDPN and its target proteins as possible immunohistochemical biomarkers in the assessment of proliferative potential and aggressiveness among different odontogenic cysts and tumors?

1. Population: Biopsies of odontogenic cysts and tumors.
2. Exposure: Podoplanin immunoexpression.
3. Comparison: Differences in the pattern of podoplanin immunoexpression in odontogenic cysts and odontogenic tumors.
4. Results: Type of antibody most commonly used, pattern of immunoexpression at the tissue and cellular level, number of positive/negative cases, intensity and immunostaining score of each of the OL.

Eligibility criteria

Inclusion criteria

- Cross-sectional or cohort clinical studies.
- Studies published after 2010.
- Studies written in the English language.
- Clinical studies approved by the institucional ethics committee.
- Subjects with OL (odontogenic cysts and tumors) basing the diagnosis according to the clinical-radiographic appearance and histopathological study.
- Formalin-fixed, paraffin-embedded specimens for immunohistochemistry analysis of PDPN.

Exclusion criteria

- Letters to the editor.
- Short communications.
- Conference papers.
- Review papers.
- Case reports.
- Case series.

Information sources and search strategy

A comprehensive literature search was performed in PubMed/MEDLINE, ScienceDirect, Scopus, Web of Science and Google Schoolar databases from August 15, 2010 to June 15, 2023. For the first database (PubMed) the following search strategy was used: ((((("PDPN protein, human" [Mesh] AND "Odontogenic Cysts"[Mesh]) OR "Odontogenic Tumors"[Mesh]) AND "Immunohistochemistry"[Mesh]. While for the rest, the following keywords "podoplanin", "PDPN", "immunohistochemistry", "odontogenic cysts" and "odontogenic tumors" were used. Thus, the search strategy employed allowed the identification of relevant research and the development of an extensive study library (Fig. 1).

Finally, to complement and further enrich the search strategy, a manual search was carried out in the following journals: *Oral Surgery Oral Medicine Oral*

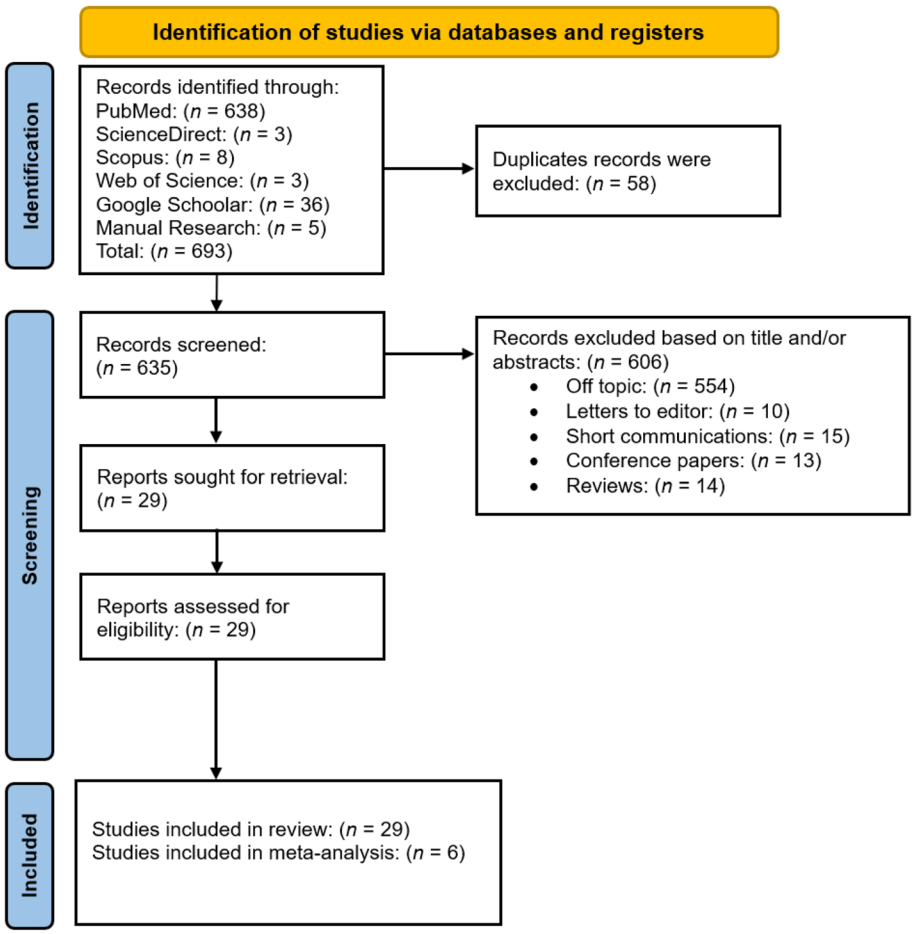


Fig. 1 PRISMA flow diagram. PRISMA: Preferred Reporting Items for Systematic and Meta-Analyses

Pathology Oral Radiology, *Journal of Oral Pathology & Medicine*, *Oral Medicine*, *Oral Pathology and Oral Surgery* and *Journal of Stomatology Oral and Maxillofacial Surgery*.

Study selection

The titles and abstracts of the records retrieved from the different search engines were examined by two reviewers (M.A.A.S and G.L.B) independently, in order to find relevant documents for inclusion. The same researchers then accessed the full text of the articles, taking into account the previously established inclusion and exclusion criteria. Papers with insufficient information in their title/abstract were discarded. In addition, if there were any differences or disagreements among the reviewers, these were clarified by discussion in consultation with a third expert researcher (A.H). Thus, all articles that met the inclusion criteria were included in the present review, after which all information of interest was extracted and the risk of bias was assessed.

Data collection process and quality assessment

Two investigators (M.A.A.S and S.R.S) carried out the data extraction process on predefined tables independently. Disagreements were resolved by discussion in consultation with a third investigator (A.H).

The following information was extracted:

- First author and year of publication.
- Country.
- Study design.
- Age.
- Gender.
- Ethics committee approval.
- Immunoassay technique used.
- Type of odontogenic lesion (odontogenic cysts and tumors).
- Number and size of sample.
- Anti-PDPN antibody used.
- Localization of the podoplanin marker in tissues and at the cellular level.

- Immunostaining intensity (weak, moderate and strong) and mean score.
- The number of positive and negative cases.
- The main results of the investigation.

The adapted Newcastle-Ottawa scale (NOS) was used to assess the quality and risk of bias of the included cross-sectional and cohort studies [60]. This tool is based on scoring using a star system on three domains mainly selection (4 stars), comparability (2 stars) and exposure/outcome (3 stars) of included studies. For practical purposes, quality was rated as "Very good" when the score was >5, "Good" with a score of 4, "Satisfactory" with a score of 3, or "Unsatisfactory" with a score of 0-2. Finally, in the absence of data, the authors of the articles were contacted for additional information [61].

Data synthesis

A meta-analysis was performed, which calculated and compared the mean PDPN score in relation to immunostaining intensity, this by constructing a forest plot using a random effects model about standardized mean difference (SMD) with a 95% confidence interval (CI). Heterogeneity was calculated using Cochran's Q test and Higgins' I^2. A value of $p=\leq 0.05^*$ was considered statistically significant. Percentages for I^2 of 0-40% were considered as low heterogeneity, 41-75% as moderate heterogeneity and ≥76% as high heterogeneity. In addition, funnel plot and Egger linear regression were used to investigate the existence of publication bias. All statistical analysis was performed using STATA V17 software (Stata Corp, College Station, TX, USA).

Protein interaction network prediction

Interaction network analysis was performed in STRING to gain insight into the relationship of PDPN with other proteins and their association with biological processes. STRING (http://string-db.org/) is a database of known and predicted protein-protein interactions [62]. Interactions can be direct (physical) and indirect (functional) associations; they come from computational prediction, inter-organism knowledge transfer and aggregated primary base interactions.

Results

Initially 693 articles were found in the five databases consulted, including PubMed/MEDLINE (638 articles), ScienceDirect (3 articles), Scopus (8 articles), Web of Science (3 articles), Google Schoolar (36 articles) and in the manual search 5 articles were found. Duplicates were removed and, based on title and abstract, the remaining 635 articles were reviewed. After analyzing the full text of the remaining articles, 606 records were excluded as irrelevant (off topic n=554; letters to editor n=10; short communications n=15; conference papers n=13 and reviews n=14). A total of 29 articles were assessed for eligibility. Therefore, a total of 29 articles were included for the qualitative analysis and 6 articles for the quantitative analysis of the present review. Details of the study selection are sampled in Fig. 1.

Quality assessment

According to the criteria established by NOS, 7 (24.1%) [31, 34, 37, 40, 41, 51, 56] articles achieved a score of very good quality, while the rest (75.9%) [29, 30, 32, 33, 35, 36, 38, 39, 42–50, 52–55, 57] achieved a score of good quality (Table 1).

Description of the included studies

Twenty-nine articles were reviewed in this study, of which 26 studies were cross-sectional [29–34, 36–48, 50–54, 57] and 3 studies were cohort studies [35, 49, 56]. The total number of individuals studied in the included investigations was 1,337. The ages of the subjects ranged from 2 to 89 years, with a mean age ± standard deviation (SD) of 33.41 ± 5.38 years, of which 22.2% were female, 21.4% were male, and in 56.4% the gender of the participants was not specified. All studies were approved by the ethics committee of their respective institutions. Most of the articles were published after 2012 (25:86.2%) [29–53]. The oldest study was from 2010 [57], and the most recent from 2023 [29]. The investigations were conducted in eight different countries. Nine (31.03%) studies were conducted in India [29–32, 34, 37, 37, 39–41], six (20.68%) in Japan [38, 50, 52, 54, 55, 57], five (17.24%) in Brazil [33, 46–48, 51], three (10.34%) in Germany [43, 53, 56], two (6.89%) in Malaysia [35, 45] and Iran [36, 44] and other studies (3.44%) in Mexico [42] and China [49] (Table 2).

Clinical characteristics of the included studies

A total of 1,337 OL samples were analyzed for PDPN immunoexpression. Ninety-four (7.03%) [31, 34, 41, 42, 51, 56] samples corresponded to dental follicles and germs, 715 (53.47%) [29–39, 41, 44, 47–50, 52, 53, 55–57] to OC and 528 (39.49%) [29, 34, 36, 37, 40–46, 48, 50, 51, 54, 56, 57] to OT. Regarding jaw cysts, 53 (3.96%) [34, 35, 37, 44, 56] samples corresponded to RC, 163 (12.19%) [29–32, 34, 35, 37, 37, 39, 44, 55–57] to DC, 43 (3.21%) [29, 32, 33, 48, 55] to orthokeratinized odontogenic cysts (OOC), 404 (30.21%) [29–39, 47, 50, 53, 55, 56] to OKC, 5 (0.37%) [44] to glandular odontogenic cysts (GOC) and 47 (3.51%) [36, 41, 48, 50, 52] to calcifying odontogenic cysts (COC). Regarding OT, 266 (19.89%) [29, 34, 36, 37, 40–42, 45, 46, 48, 50, 51, 56, 57] samples corresponded to AM, 66 (4.93%) [37, 40–42, 44–46, 51, 57] to ameloblastomas of the

Table 1 Quality assessment of the included studies according to Newcastle-Ottawa Scale (NOS)

Author's and year	Selection	Comparability	Outcome	Total Stars
Anjum *et al.*, 2023[29]	★★	★	★	**4**
Zolfaghari *et al.*, 2023[30]	★★	★	★	**4**
Nayar *et al.*, 2022[31]	★★★★	★★	★★	**8**
Chahar *et al.*, 2021[32]	★★	★	★	**4**
Malaguez *et al.*, 2020[33]	★★	★	★	**4**
Singh *et al.*, 2020 [34]	★★★★	★★	★★	**8**
Kechik *et al.*, 2018[35]	★★	★	★	**4**
Etemad-Moghadam and Alaeddini, 2018[36]	★★	★	★	**4**
Singhal *et al.*, 2017[37]	★★★★	★★	★★	**8**
Naruse *et al.*, 2017[38]	★★	★	★	**4**
Gupta *et al.*, 2017[39]	★★	★	★	**4**
Habba *et al.*, 2017[40]	★★	★	★	**4**
Ganvir *et al.*, 2016[41]	★★★★	★★	★★	**8**
Sánchez-Romero *et al.*, 2016[42]	★★★★	★★	★★	**8**
Friedrich *et al.*, 2016[43]	★★	★	★	**4**
Alaeddini *et al.*, 2016[44]	★★	★	★	**4**
Siar *et al.*, 2015[45]	★★	★	★	**4**
Costa *et al.*, 2015[46]	★★	★	★	**4**
Oliveira *et al.*, 2014[47]	★★	★	★	**4**
Caetano *et al.*, 2013[48]	★★	★	★	**4**
Zhang et al., 2013[49]	★★	★	★	**4**
Tsuneki *et al.*, 2012[50]	★★	★	★	**4**
Tjioe *et al.*, 2012[51]	★★★★	★★	★★	**8**
Kikuchi *et al.*, 2012[52]	★★	★	★	**4**
Friedrich *et al.*, 2012[53]	★★	★	★	**4**
González-Alva *et al.*, 2011[54]	★★	★	★	**4**
Okamoto *et al.*, 2010[55]	★★	★	★	**4**
Zustin *et al.*, 2010[56]	★★★★	★★	★★	**8**
González-Alva *et al.*, 2010[57]	★★	★	★	**4**

unicystic type (AMU), and 4 (0.29%) [40] of the peripheral/extraosseous type (APe). Thirty-seven (2.76%) [36, 41, 48, 50, 56] samples corresponded to adenomatoid odontogenic tumors (AOT), 20 (1.49%) [36, 41, 48] to calcifying epithelial odontogenic tumors (CEOT), 86 (6.43%) [54] to odontomas (ODS), 9 (0.67%) [36.48] to ameloblastic fibromas (AF), 4 (0.29%) [48] to ameloblastic fibro-odontomas (AFO), 10 (0.74%) [36.43] to odontogenic myxomas (OM) and 26 (1.94%) [40–42] to ameloblastic carcinomas (AMC). On average the section size was 4µm and all samples were analyzed by immunohistochemistry (100%). In addition, the most commonly used antibody was Mouse monoclonal PDPN D2-40; DAKO brand (68.96%) [29, 33, 36–38, 40–44, 46–48, 50–55, 57] (Table 2).

Characteristics of PDPN immunoexpression in odontogenic cysts and tumors

The characteristics of PDPN immunoexpression, such as the localization of the protein marker in tissues and at the cellular level, the intensity and mean immunostaining score, the number of positive and negative cases, the mean score and the main results of the investigation were evaluated (Table 3). In relation to dental follicles (DF), PDPN was strongly expressed in the dental lamina and reduced enamel epithelium [31, 34, 41, 42, 51, 56]. Whereas, in relation to OC, PDPN expression was weak-moderate in the basal layer of the RC and DC [29–32, 34, 35, 37, 39, 44, 55–57], it was weak in the basal and suprabasal layer of the OOC [29, 32, 33, 48, 55], strong in the basal and suprabasal layer of OKC [29–39, 47, 50, 53, 55, 56], negative in

Table 2 Clinical characteristics and related data from included studies in this systematic review

Author's and Year	Country	Design study	Age M/R	Sex F/M	Ethical	Technique	Odontogenic lesion	No. of samples	Samples size	Abs model
Anjum *et al.,* 2023[29]	India	CS	NI	NI	Yes	IHC	AMs, OKC, OOC, DC	10, 37, 13, 10	4µm	Mouse Mo PDPN D2-40 (DAKO)
Zolfaghari *et al.,* 2023[30]	India	CS	31.47 8-59	24/36	Yes	IHC	OKC, DC	30, 30	4µm	Mouse Mo PDPN D2-40 (Medaysis)
Nayar *et al.,* 2022[31]	India	CS	NI	NI	Yes	IHC	OKC, DC, DF	20, 20, 20	4µm	Mouse Mo PDPN D2-40
Chahar *et al.,* 2021[32]	India	CS	NI	NI	Yes	IHC	OKC, OOC, DC	10, 10, 10	3µm	Mouse Mo PDPN D2-40
Malaguez *et al.,* 2020[33]	Brazil	CS	32.2 4-79	25/25	Yes	IHC	OKC, OOC	28, 4	4µm	Mouse Mo PDPN D2-40 (DAKO)
Singh *et al.,* 2020 [34]	India	CS	NI	NI	Yes	IHC	AMs, OKC, DC, RC, DF	10, 12, 8, 8, 8	NI	Mouse Mo PDPN D2-40
Kechik *et al.,* 2018[35]	Malaysia	CH	36.46 11-67	20/20	Yes	IHC	OKC, DC, RC	20, 10, 10	5µm	Mouse Mo PDPN (Abcam)
Etemad-Moghadam and Alaeddini, 2018[36]	Iran	CS	NI	NI	Yes	IHC	AMs, AOT, CEOT, AF, OM, OKC, COC	16, 9, 4, 7, 5, 28, 7	3µm	Mouse Mo PDPN D2-40 (DAKO)
Singhal *et al.,* 2017[37]	India	CS	NI	NI	Yes	IHC	AMs, AMU, OKC, DC, RC	9, 6, 10, 5, 5	NI	Mouse Mo PDPN D2-40 (DAKO)
Naruse *et al.,* 2017[38]	Japan	CS	41 10-87	26/37	Yes	IHC	OKC	65	4µm	Mouse Mo PDPN D2-40 (DAKO)
Gupta *et al.,* 2017[39]	India	CS	NI	NI	Yes	IHC	OKC, DC	20, 20	3µm	Mo anti- PDP
Habba *et al.,* 2017[40]	India	CS	NI	NI	Yes	IHC	AMs, AMPe, AMU AMC	26, 4, 7, 8	4µm	Mouse Mo PDPN D2-40 (DAKO)
Ganvir *et al.,* 2016[41]	India	CS	44 9-65	40/50	Yes	IHC	AMs, AMU, AOT, CEOT, COC, DF, AMC	12, 3, 15, 15, 15, 15, 15	4µm	Mouse Mo PDPN D2-40 (DAKO)
Sánchez-Romero *et al.,* 2016[42]	Mexico	CS	NI	NI	Yes	IHC	AMs, AMU, DF, AMC	38, 15, 10, 3	3µm	Mouse Mo PDPN D2-40 (DAKO)
Friedrich *et al.,* 2016[43]	Germany	CS	NI	NI	Yes	IHC	OM	5	NI	Mouse Mo PDPN D2-40 (DAKO)
Alaeddini *et al.,* 2016[44]	Iran	CS	NI	NI	Yes	IHC	AMU, GOC, DC, RC	8, 5, 10, 20	NI	Mouse Mo PDPN D2-40 (DAKO)
Siar *et al.,* 2015[45]	Malaysia	CS	32.8	22/28	Yes	IHC	AMs, AMU	20, 5	5µm	Mouse Mo PDPN (Abcam)
Costa *et al.,* 2015[46]	Brazil	CS	32.2 9-68	32/15	Yes	IHC	AMs, AMU	35, 11	4µm	Mouse Mo PDPN D2-40 (DAKO)
Oliveira *et al.,* 2014[47]	Brazil	CS	28 9-68	9/7	Yes	IHC	OKC	18	3µm	Mouse Mo PDPN D2-40 (DAKO)
Caetano *et al.,* 2013[48]	Brazil	CS	NI	NI	Yes	IHC	AMs, AOT, OKC, OOC, CEOT, AF, AFO, COC	8, 9, 20, 5, 1, 2, 4, 5	4µm	Mouse Mo PDPN D2-40 (DAKO)
Zhang et al., 2013[49]	China	CS	30.1 13-55	9/7	Yes	IHC	OKC	16	4µm	Rabbit Mo PDPN (Protein-tech group)
Tsuneki *et al.,* 2012[50]	Japan	CS	NI	NI	Yes	IHC	AMs, AOT, COC, OKC	18, 2, 5, 15	4µm	Mouse Mo PDPN D2-40 (DAKO)

Table 2 (continued)

Author's and Year	Country	Design study	Age M/R	Sex F/M	Ethical	Technique	Odontogenic lesion	No. of samples	Samples size	Abs model
Tjioe *et al.*, 2012[51]	Brazil	CS	34 9-68	49/16	Yes	IHC	AMs, AMU, DF	24, 8, 32	4µm	Mouse Mo PDPN D2-40 (DAKO)
Kikuchi *et al.*, 2012[52]	Japan	CS	NI	NI	Yes	IHC	COC	15	NI	Mouse Mo PDPN D2-40 (DAKO)
Friedrich *et al.*, 2012[53]	Germany	CS	NI	NI	Yes	IHC	OKC	6	NI	Mouse Mo PDPN D2-40 (DAKO)
González-Alva *et al.*, 2011[54]	Japan	CS	23.9 2-89	41/45	Yes	IHC	ODS	86	NI	Mouse Mo PDPN D2-40 (DAKO)
Okamoto *et al.*, 2010[55]	Japan	CS	NI	NI	Yes	IHC	OKC, OOC, DC	46, 11, 15	NI	Mouse Mo PDPN D2-40 (DAKO)
Zustin *et al.*, 2010[56]	Germany	CH	U	U	Yes	IHC	AMs, OKC, AOT, RC, DC, DF	5, 3, 2, 10, 10, 9	NI	Mouse Mo PDPN D2-40 (SIGNET)
González-Alva *et al.*, 2010[57]	Japan	CS	34.9 6-76	NI	Yes	IHC	AMs, AMU, DC	35, 3, 15	4µm	Mouse Mo PDPN D2-40 (DAKO)
The data presented below were the most prevalent →	India 31,03%	CS 100%	33,41 2-89 years	NI 56,4%	Yes 100%	IHC 100%	Odontogenic cyst 53,47%	1,337	4µm	Mouse Mo PDPN D2-40 (DAKO) 68,96%

NI No Information; *M* Mean; *R* Range; F^e Female; M^a Male; *CS* Cross-sectional study; *CH* Cohort; *U* Unclear; *IHC* Immunohistochemistry; *Mo* Monoclonal; *PDPN* Podoplanin; *AMs* Ameloblastoma solid; *AMU* Ameloblastoma, Unicystic; *AMP* Ameloblastoma, Peripheral; *AMC* Ameloblastic carcinoma; *OKC* Odontogenic keratocyst; *OOC* Orthokeratinized odontogenic cyst; *AOT* Adenomatoid odontogenic tumor; *CEOT* Calcifying epithelial odontogenic tumor; *AF* Ameloblastic fibroma; *AFO* Ameloblastic fibro-odontoma; *COC* Calcifying odontogenic cyst; *ODS* Odontoma; *OM* Odontogenic mixoma; *GOC* Glandular odontogenic cyst; *RC* Radicular cyst; *DC* Dentigerous cyst; *DF* Dental follicle

Table 3 Features of podoplanin immunoexpression in different odontogenic lesions

Reference	Odontogenic lesion	Tissue	Cellular	Intensity	(*n*) Negative	(*n*) Positive	Score (mean)	Outcome
[29]	AMs	Peripheral cells-Islands	M-C	Moderate	NI	NI	7.10	↑ PDPN in AMs and OKC compared to OOC and DC
	OKC	Basal and suprabasal layer	M-C	Weak			4.97	
	OOC	Basal and suprabasal layer	M-C	Weak			2.54	
	DC	Basal and suprabasal layer	M-C	Weak			0.30	
[30]	OKC	Basal and suprabasal layer	M-C	Strong	0-27	27	2.87	↑ PDPN in OKC compared to DC
	DC	Basal layer	M-C	Weak	4-13	9-13	1.97	
[31]	OKC	Basal and suprabasal layer	M-C	Strong	2-20	18-20	2.63	↑ PDPN in OKC compared to DC and DF
	DC	Basal layer	M-C	Weak	3-20	17-20	2.18	
	DF	Basal layer	M-C	Weak	0-20	20	2.18	
[32]	OKC	Basal layer	M-C	Strong	1-10	9-10	2.4	↑ PDPN in OKC compared to OOC and DC
	OOC	Basal layer	M-C	Strong	3-10	7-10	2.1	
	DC	Basal layer	M-C	Moderate	3-10	7-10	1.9	
[33]	OKC	Basal and suprabasal layer	M-C	Strong	1-28	27-28	NI	↑ PDPN in OKC compared to OOC
	OOC	Basal and suprabasal layer	M-C	Weak	0-4	4		
[34]	AMs	Peripheral cells-Islands	M-C	Strong	NI	NI	NI	↑ PDPN in AMs and OKC compared to DC, RC and DF
	OKC	Basal and suprabasal layer	M-C	Strong				
	DC	Basal layer	M-C	Weak				
	RC	Basal layer	M-C	Weak				
	DF	Basal layer	M-C	Weak				
[35]	OKC	Basal and suprabasal layer	M-C	Strong	0-20	20	2	↑ PDPN in OKC compared to DC and RC
	DC	Basal layer	M-C	Weak	0-10	10	1	
	RC	Basal layer	M-C	Weak	0-10	10	1	
[36]	AMs	Peripheral cells-Islands	M-C	Strong	0-16	16	NI	A greater number of OKC cases presented strong immunoexpression of PDPN, followed by AMs, compared to the rest of the odontogenic lesions
	AOT	Epithelial cells, rosettes, duct-like structures	M-C	Strong	0-9	9		
	CEOT	Peripheral cells	M-C	Moderate	0-4	4		
	AF	Epithelial strands and cords	M-C	Strong	0-7	7		
	OM	Negative/lymphatic vessels	M-C	Weak	5	0-5		
	OKC	Basal and suprabasal layer	M-C	Strong	0-28	28		
	COC	Epithelial lining, stellate-reticulum-like cells and ghost cells	M-C	Moderate	0-7	7		
[37]	AMs	Peripheral cells-Islands	M-C	Strong	NI	NI	NI	↑ PDPN in AMs and OKC compared to DC and DF
	AMU	Basal and suprabasal layer	M-C	Strong				
	OKC	Basal and suprabasal layer	M-C	Strong				
	DC	Basal layer	M-C	Weak				
	RC	Basal layer	M-C	Moderate				
	DF	Reduced enamel epithelium	M-C	Weak				
[38]	OKC	Basal and suprabasal layer	M-C	Strong	6-65	59-65	NI	↑ PDPN in OKC
[39]	OKC	Basal and suprabasal layer	M-C	Strong	2-20	18-20	3.20	↑ PDPN in OKC compared to DC
	DC	Basal layer	M-C	Strong	3-20	17-20	1.20	
[40]	AMs	Peripheral cells-Islands	M-C	Strong	0-6	26	29.6	PDPN is associated with invasive behavior of AM as well as progression from benign to malignant AM
	AMPe	Basal and spinous layer	M-C	Strong	0-4	4	28.45	
	AMU	Basal and suprabasal layer	M-C	Strong	0-7	7	24.83	
	AMC	Central/peripheral cells	M-C	Strong	0-8	8	63.10	

Table 3 (continued)

Reference	Odontogenic lesion	Tissue	Cellular	Intensity	(*n*) Negative	(*n*) Positive	Score (mean)	Outcome
[41]	AMs AMU AOT CEOT COC DF AMC	Peripheral cells-Islands Basal and suprabasal layer Epithelial cells, rosettes and duct-like structures Peripheral cells Epithelial lining Reduced enamel epithelium Central/peripheral cells	M-C M-C M-C M-C M-C M-C M-C	Moderate Moderate Moderate Moderate Moderate Moderate Strong	0-12 0-3 0-15 0-15 0-15 0-15 0-15	12 3 15 15 15 15 15	NI	A greater number of AMC cases presented strong immunoexpression of PDPN, followed by AM, compared to the rest of the odontogenic lesions
[42]	AMs AMU DF AMC	Peripheral cells-Islands Peripheral cells-Islands Reduced enamel epithelium Central/peripheral cells	M-C M-C M-C M-C	Strong Strong Strong Strong	0-24 0-15 0-10 0-3	38 15 10 3	NI	↑ PDPN in AM and DF compared to AMC, which suggesting that PDPN may not be related with the aggressiveness of this tumor.
[43]	OM	Negative	Negative	Negative	5	0-5	NI	Negative for expression of PDPN
[44]	AMU GOC DC RC	Basal and suprabasal layer Negative Basal layer Basal layer	M Negative M-C M	Weak Negative Weak Weak	0-8 5 2-10 5-20	8 0-5 8-10 15-20	NI	↑ PDPN in AM (U) compared to DC, RC and GOC.
[45]	AMs AMU	Peripheral cells-Islands Basal layer	M-C M-C	Strong Strong	6-20 0-5	14-20 5	NI	↑ PDPN in AM
[46]	AMs AMU	Peripheral cells-Islands Basal and suprabasal layer	M-C M-C	Strong Strong	0-35 0-11	35 11	NI	↑ PDPN in AM
[47]	OKC	Basal layer	M-C	Strong	0-16	16	NI	↑ PDPN in OKC
[48]	AMs AOT OKC OOC CEOT AF AFO COC	Peripheral cells-Islands Epithelial cells, rosettes and duct-like structures Basal and suprabasal layer Few cells from basal layer Peripheral cells Central/Peripheral cells Epithelial strands and cords, reticulum stellate-like cells Epithelial lining	M-C M-C M-C M-C M-C M-C M-C M-C	Strong Strong Strong Weak Strong Strong Strong Strong	0-8 0-9 0-20 0-5 0-1 0-2 0-4 0-5	8 9 20 5 1 2 4 5	NI	↑ PDPN in AM, AOT, OKC, CEOT, AF, AFO, COC ↓ PDPN in OOC
[49]	OKC	Basal and suprabasal layer	M-C	Strong	1-16	15-16	NI	↑ PDPN in OKC
[50]	AMs AOT COC OKC	Peripheral cells-Islands Epithelial cells, rosettes and duct-like structures Epithelial lining Basal and suprabasal layer	M-C M-C M-C M-C	Strong Strong Strong Strong	0-18 0-2 0-5 0-15	18 2 5 15	NI	↑ PDPN in AMs, AOT, OKC and COC
[51]	AMs AMU DF	Peripheral cells of the islands Basal and suprabasal cells Epithelial remnants of the dental lamina, the reduced enamel epithelium	M-C M-C MC	Strong Strong Strong	0-24 0-8 0-32	24 8 32	NI	↑ PDPN in AM and DF
[52]	COC	Epithelial lining	MC	Strong	0-15	15	NI	↑ PDPN in COC

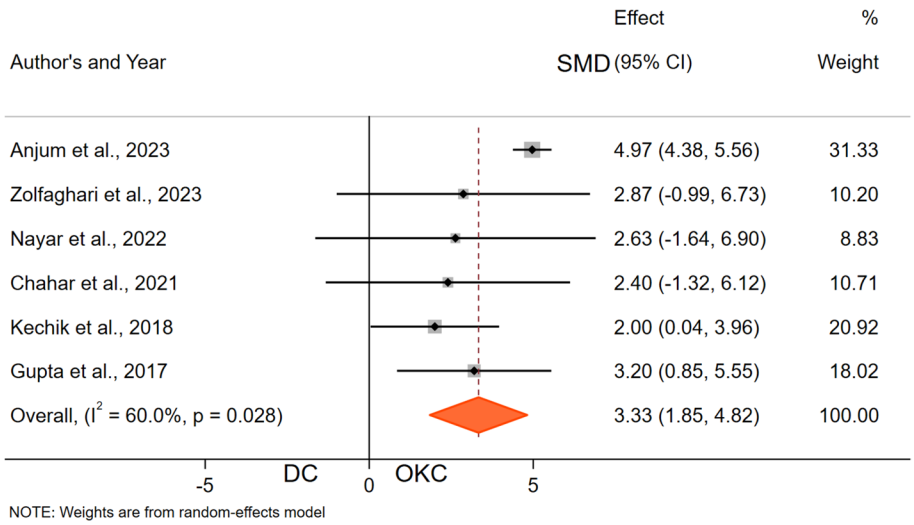
Table 3 (continued)

Reference	Odontogenic lesion	Tissue	Cellular	Intensity	(*n*) Negative	(*n*) Positive	Score (mean)	Outcome
[53]	OKC	Basal and suprabasal cells	M-C	Strong	0-6	6	NI	↑ PDPN in OKC
[54]	ODS Compound Complex	Columnar odontoblasts adjacent to the dentin matrix, Tomes' fibers and pulp cells Sparse flat cells adhering to dentin-like, irregular dentin tubules or Liesegang's ring calcifications	M-C M-C	Strong Strong	0-57 0-29	57 29	NI	↑ PDPN in ODS
[55]	OKC OOC DC	Basal and suprabasal cells Basal layer Basal layer	M-C M-C M-C	Strong Weak Strong	4-46 8-11 4-15	42-46 3-11 11-15	NI	↑ PDPN in OKC compared to OOC and DC
[56]	AMs OKC AOT RC DC DF	Basal and suprabasal cells Basal and suprabasal cells Basal and suprabasal cells Basal layer Basal layer Epithelial remnants of the dental lamina, the reduced enamel epithelium	M-C M-C M-C M-C M-C M-C	Strong Strong Strong Strong Strong Strong	0-5 0-3 0-2 0-10 0-10 0-9	5 3 2 10 10 9	NI	↑ PDPN in AM, OKC, AOT, DC, RC and DF
[57]	AMs AMU DC	Peripheral cells of the islands Basal layer Basal layer	M-C M-C M-C	Strong Strong Weak	3-35 0-3 9-15	32-35 3 6-15	NI	↑ PDPN in AM compared to DC

NI No Information; ↑ Overexpression; ↓ Decreased expression; *M* Mean; *R* Range; *F*[e] Female; *M*[a] Male; *CS* Cross-sectional study; *U* Unclear; *IHC* Immunohistochemistry; *Mo* Monoclonal; *PDPN* Podoplanin; *AMs* Ameloblastoma solid; *AMU* Ameloblastoma, Unicystic; *AMP* Ameloblastoma, Peripheral; *AMC* Ameloblastic carcinoma; *OKC* Odontogenic keratocyst; *OOC* Orthokeratinized odontogenic cyst; *AOT* Adenomatoid odontogenic tumor; *CEOT* Calcifying epithelial odontogenic tumor; *AF* Ameloblastic fibroma; *AFO* Ameloblastic fibro-odontoma; *COC* Calcifying odontogenic cyst; *ODS* Odontoma; *OM* Odontogenic mixoma; *GOC* Glandular odontogenic cyst; *RC* Radicular cyst; *DC* Dentigerous cyst; *DF* Dental follicle

GOC [44] and moderate-strong in the epithelial lining, of COC [36, 41, 48, 50, 52]. In relation to OT, PDPN was strongly expressed in the peripheral columnar cells of the epithelial islands of the AM [29, 34, 36, 37, 37, 40–42, 45, 46, 48, 50, 51, 56, 57], whereas, in its malignant counterpart the AMC, this protein was also strongly expressed in the peripheral and central cells of the epithelial islands [40–42]. On the other hand, in AMU and APe, PDPN was strongly expressed in the basal and suprabasal layer [37, 40–42, 44–46, 51, 57]. In AOT, PDPN was strongly expressed in epithelial cells, rosettes and duct-like structures [36, 41, 48, 50, 56], whereas in CEOT, PDPN was moderately to strongly expressed in peripheral cells of the tumor epithelium [36, 41, 48]. In ODS, PDPN is expressed in developing and mature odontoblasts, Tomes fibers, and in secretory ameloblasts [36, 48]. In AF, PDPN was expressed in epithelial filaments and cords [48], whereas, in AFO, PDPN was expressed in epithelial filaments and cords, as well as in stellate reticular cells [36, 43]. PDPN expression in the OM was negative [36, 43]. PDPN expression at the cellular level in most DF and OL, was in the cytoplasm and cell membrane [29–57]. Also, the number of PDPN positive and negative cases was found to be 1,079 and 85 respectively.

Finally, because PDPN was more frequently evaluated in OKC and also, in the face of reduced data availability. Meta-analysis was only possible to compare the mean score between DC vs OKC. Thus, six studies [29–32, 35, 39] compared the immunostaining intensity of PDPN and the meta-analysis indicated a SMD=3.3(CI=1.85-4.82 p=0.000*), demonstrating that the immunostaining intensity was significantly stronger in OKC compared to DC (Fig. 2, panel A).



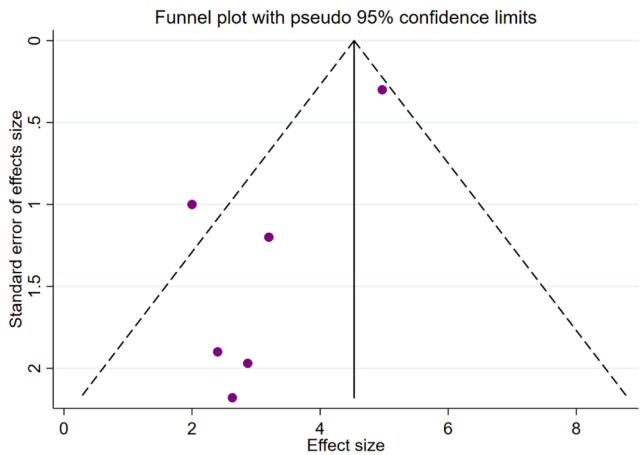


Fig. 2 Forest plot comparing the PDPN immunoexpression of **A** OKC vs DC. **B** Funnel plot to check the publication bias. OKC=Odontogenic keratocyst, DC= Dentigerous cyst

Publication bias

Based on the chi-square test, moderate heterogeneity was found among the analyzed studies (I^2=60.0%, p=0.028*). Therefore, Egger's test was used to assess publication bias (CI=3.36-0.31, p=0.029*), which showed evidence of bias. Figure 2, panel B shows the funnel plot highlighting asymmetry and publication bias.

Bioinformatic analysis results

Protein-protein interactions were identified in the STRING database, which showed 20 prominent interactions (Fig. 3). The lines indicate that directly bound proteins are part of the same physical complex; however, in large complexes this may not mean that they bind directly to each other. The thickness of the line indicates the confidence level of that interaction and, in agreement with what has been reported in the literature we observe that for PDPN and CLEC1B there is experimental evidence of their direct interaction with a score of 0.999, followed by PROX1 (0.811), TNFSRF10B (0.774), MSN (0.747) and PCAM1(0.702) proteins. While a mean confidence score for GP6 (0.673), LGALS8 (0.642), TNFSF4 (0.567), CCL21(0.564), SYK (0.499) and PODXL (0.436). With respect to the rest of the proteins (LCP2, PLCG2, LYVE1, FLT4, PTPRC, VEGF-C, SELP, EZR and RDX) the score was low. Importantly, immunoexpression of PECAM1, TNFRF10B, MSN, EZR and RDX associated with PDPN has been studied in some odontogenic cysts and tumors [46, 47, 63–65] (Table 4).

Discussion

A systematic review was carried out to learn about the role and significance of podoplanin as a potential immunohistochemical biomarker with the main purpose of evaluating the proliferation and invasion capacity with respect to the aggressiveness presented by different odontogenic cysts and tumors. Based on the results obtained, a quantitative analysis (meta-analysis) could also be performed, which compared the mean score in relation to the intensity of immunostaining between the odontogenic keratocyst and the dentigerous cyst mainly. Both odontogenic cysts along with ameloblastoma were the most frequently evaluated odontogenic lesions, this partly due to their high prevalence in the general population [8–10] and also due to their potential for neoplastic behavior such as aggressive and localized expansion or infiltration of such lesions [11, 12], however, due to the lack of data (mean score), it was not possible to make comparisons with ameloblastoma, therefore, the meta-analysis was limited only to comparing these two types of odontogenic cysts. A protein-protein interaction analysis was also performed, with the main objective of identifying those target proteins with which PDPN interacts that participate in both physiological and pathological

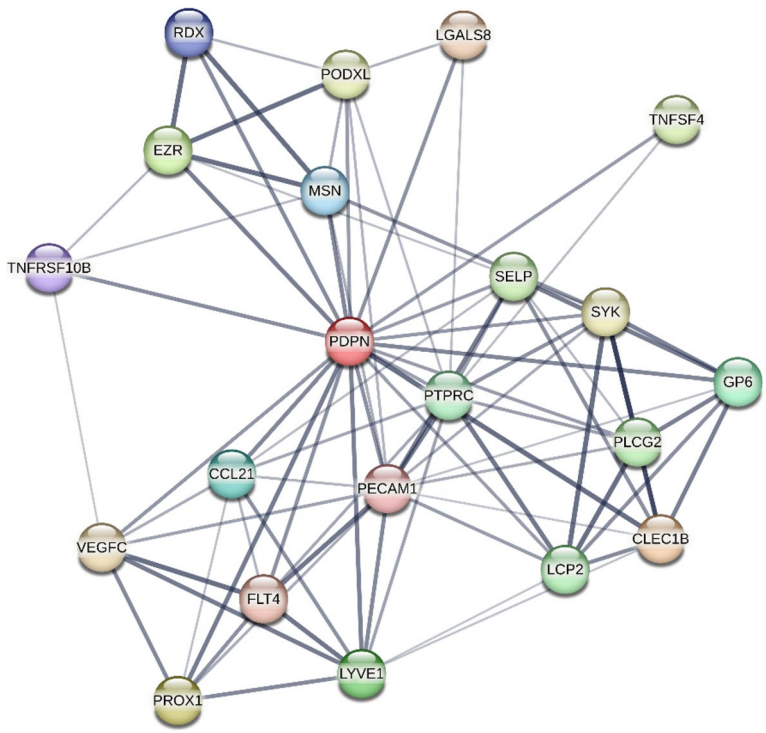


Fig. 3 Podoplanin interactome

Table 4 Features of the proteins that interact most with PDPN and their relationship with odontogenic lesions

Protein	Biological function	Immunoexpression in odontogenic cysts and tumors, cancer and other conditions
CLEC1B	Platelet activation, angiogenesis, and immune and inflammatory responses	Potential prognostic biomarker in hepatocellular carcinoma [66]
LCP2/SLP-76	Regulates immunoreceptor signaling (such as T-cell receptors) and is also required for integrin signaling in neutrophils and platelets	Potential prognostic biomarker in metastatic melanoma [67]
PLCG2	Transmitting signals from growth factor receptors and immune system receptors across the cell membrane	Potential diagnostic and prognostic biomarker in non-small cell lung cancer [68]
GP6	Collagen-induced platelet adhesion and activation	Potential biomarker of bleeding [69]
LYVE1	Lymphatic trafficking of immune cells to the development of lymphatic vasculature	Potential prognostic biomarker in head and neck squamous cell carcinoma, oral squamous cell carcinoma, oral tongue squamous cell carcinoma, and others [70]
PROX1	Embryogenesis and lymphangiogenesis	Potential biomarker related to wound healing in periodontal pockets and oral cancer [71, 72]
FLT4	Lymphangiogenesis and maintenance of the lymphatic endothelium	Potential biomarker for early detection of oral squamous cell carcinoma [73]
PECAM1 /CD31	Leukocyte transendothelial migration, trombosis and angiogenesis	Potential diagnostic and treatment biomarker for the bone metastases of tumors [74] as well as, OKC and RC [63]
PTPRC/CD45	Hematopoietic stem cells proliferation and differentiation	Negative expression of CD45 has been reported in mesenchymal stem cells derived from periapical cysts and dentigerous cysts, as well as in clear cell odontogenic carcinoma [75–77]
SYK	Lymphocyte development and activation of immune cells	Potential treatment biomarker for the human papilomavirus-positive head and neck squamous cell carcinoma [78]
VEGFC	Lymphangiogenesis	Potential diagnosis biomarker for the head and neck squamous cell carcinoma [79]
CCL21	Inhibits hemopoiesis and stimulates chemotaxis	Potential treatment biomarker for the tongue squamous cell carcinoma [80]
SELP	Cell adhesion molecule	Increased serum P-selectin levels in subjects with periodontitis associated with platelet activation could be a contributing factor to the development of cardiovascular disease [81]
TNFSF4/OXO40	T cell survival and development of memory T cells	Potential diagnosis and prognostic biomarker for the head and neck squamous cell carcinoma [82, 83]
TNFRF10B /DR5/ TRAIL-R2	Cancer cell apoptosis	Could be involved in the neoplastic transformation of the odontogenic epithelium and could suggest some intrinsic regulation of neoplastic cell proliferation and death in AM, which would explain their slow growth and inability to metastásico [64]
MSN	Connects the actin cytoskeleton to the plasma membrane and thereby regulates the structure and function of specific domains of the cell cortex	Involvement in the development of benign odontogenic lesions such as AM, AOT, OKC, OOC, CEOT, AF, ODS, COC. However, their role in the expansive growth and local invasiveness of these lesions remains unclear [65]
EZR	Connections of major Cytoskeletal structures to the plasma membrane	May contribute to the expansive growth and local invasiveness of OKC and AM [46, 47]

Table 4 (continued)

Protein	Biological function	Immunoexpression in odontogenic cysts and tumors, cancer and other conditions
PODXL	Cell migration, invasion and extravasation, as well as chemoresistance	Potential diagnosis biomarker for the oral squamous cell carcinoma [84]
LGALS8	Modulate cell adhesion, cell proliferation, apoptosis, and Immun responses	Potential diagnosis biomarker for the Warthin's tumor of the parotid gland [85]
RDX	Binding of the barbed end of actin filaments to the plasma membrane	Role in the control of invasion and migration of papillary thyroid carcinoma cells [86]

NI: No Information; PDPN: Podoplanin; GP6: Glycoprotein VI; LYVE1: Lymphatic vessel endothelial hyaluronic acid receptor 1; PROX1: Prospero homeobox protein 1; FLT4: Fms-related tyrosine kinase 4; PECAM1: Platelet endothelial cell adhesion molecule;
PTPRC: Protein phosphatase receptor type C; SYK: Tyrosine-protein kinase; VEGFC: Vascular endothelial growth factor C; CCL21: C-C motif chemokine 21; SELP: P-selectine; TNFSF4: Tumor necrosis factor (receptor) superfamily, member 4; TNFRF10B: TNF receptor superfamily member 10B; MSN: Moesin; EZR: Ezrin; PODXL: Podocalyxin-like protein 1; LGALS8: Galectin 8 RDX: Radixin; AMs: Ameloblastoma solid; OKC: Odontogenic keratocyst; OOC: Orthokeratinized odontogenic cyst; AOT: Adenomatoid odontogenic tumor; CEOT: Calcifying epithelial odontogenic tumor; AF: Ameloblastic fibroma; COC: Calcifying odontogenic cyst; ODS: Odontoma; RC: Radicular cyst

processes, and to associate them with the development of the different odontogenic cysts and tumors.

Based on the background and results previously obtained, the discussion was divided into subtopics with the main objective of answering the research questions that had been proposed at the beginning.

PDPN immunoexpression pattern in dental follicles/germs, cysts and odontogenic tumors

A total of 29 investigations were analyzed, which were carried out in eight different countries [29–57]. In relation to dental follicles and germs, it has been shown that PDPN is mainly expressed in dental lamina, reduced enamel epithelium, preameloblasts, active secretory ameloblasts, developing and mature odontoblasts, Tomes' process, pulp cells and in the terminal portion of Hertwig's root sheath, because these cells have high mitotic activity (increased proliferative activity) [31, 34, 41, 42, 51, 56]. In relation to OC, PDPN is expressed in the basal layer of the RC and DC, as well as in the basal and suprabasal layer of the OKC. These cysts show a similar type of response in presence of inflammation. In this regard, it has been shown that, the expression of PDPN in the epithelium varies according to the amount of inflammatory changes present in the connective tissue wall, therefore, it is understood that in areas of mild to moderate inflammation, the expression of PDPN is weak-moderate and is mainly limited to the basal cell layer, whereas, in areas of severe inflammation the expression of PDPN is stronger. This could suggest that the inflammatory reaction plays an important role in the expansion and growth of OC. However, other authors have found that PDPN expression in these OL is restricted to the basal layer even in areas of severe inflammation, suggesting that probably morphological changes such as regeneration and reparative process have an impact on the proliferative activity of the lining epithelium [29–32, 34, 35, 37, 39, 44, 55–57]. In OKC, PDPN is strongly expressed in the basal and suprabasal cell layer, this could be due to the possible presence of a subpopulation of cells presenting sites of constant remodeling of the actin cytoskeleton, promoting cell migration activities, therefore, the proliferative activity of these cells, would increase their growth potential, influencing their tumorigenic behavior, i.e., making them locally more invasive and aggressive compared to RC, DC and OOC [29–39, 47, 50, 53, 55, 56]. In fact, our quantitative analysis showed that the immunostaining intensity was higher in OKC compared to DC (p=0.000*). This would partly explain the difference between the aggressive behavior of OKC with respect to DC. PDPN expression was negative in GOC [44], which could be explained by the fact that, despite its aggressive potential, GOC has low proliferative activity in its epithelium. In fact, a study showed that the apoptosis inhibitor protein bcl-2 is expressed in the basal and suprabasal layer of GOC, with low expression of Ki-67 and p53 in its epithelial lining [87]. With respect to the GOC, PDPN is expressed in the epithelial lining of this cyst, constituted by a layer of columnar basal cells. Whereas, central polyhedral stellate reticulum-like cells, ghost cells, eosinophilic material and areas of calcification were negative for PDPN. Their expression in that area, as well as OKC and AM could be related to cell migration and local invasion of these tumors [36, 41, 48, 50, 52]. In relation to OT, PDPN is expressed in the epithelial islands constituted by columnar cells of follicular AM, whereas, stellate reticulum cells do not express this marker. In the plexiform variant, PDPN is expressed in both peripheral cuboidal cells and central cells. Interestingly, in the acanthomatous variant, peripheral cells express PDPN, whereas keratinized acanthomatous cells do not express PDPN. In the granular cell variant, PDPN is expressed in the central cells, whereas, in the basal cell variant and in desmoplastic AM, PDPN is expressed in both peripheral cells and central cells [29, 34, 36, 37, 40–42, 45, 46, 48, 50, 51, 56, 57]. In AOT, PDPN is expressed in epithelial cells, rosettes and duct-like structures [36, 41, 48, 48, 50, 56], whereas in CEOT, PDPN is expressed in peripheral cells of the tumor epithelium. This demonstrates that, when there is intense proliferative activity by odontogenic cells, PDPN expression is increased, whereas, when these cells mature, stabilize or enter a quiescent state, mitotic activity decreases and therefore, so does PDPN expression [36, 41, 41, 48]. In relation to CAM, PDPN is expressed in both peripheral and central cells, indicating that the entire invasive front is composed of more aggressive cells and is a region where a variety of active molecular interactions take place that could potentially affect tumor progression [40–42]. In ODS, it appears that the expression pattern of PDPN corresponds to the development of the tooth germ and may be influenced by the differentiation stage of the lesion, suggesting that this protein may participate in the differentiation process [36, 48]. Interestingly, PDPN is not expressed in the MO, which has a locally aggressive behavior. Therefore, it is likely that other molecules and different signaling pathways are involved in this neoplasm [36, 43].

PDPN interaction networks associated with the development of odontogenic cysts and tumors, *cancer*, and other oral conditions

In our bioinformatics analysis, we found that PDPN interacts with 20 different target proteins directly (CLE1B, CCL21, LGALS8, MSN, EZR, RDX, LCP2, PLCG2, GP6, LYVE1, PROX1, FLT4, PECAM-1, PTPRC, SYK, VEGF-C, SELP, TNFSF4, TNFRF10B and PODXL). The proteins

that interact with the ectodomain present in the extracellular region of PDPN are CLEC-2, CCL21 and LGALS8 [88–90]. On the one hand, the C-type lectin superfamily member (CLEC-2) is a type II transmembrane protein encoded by the *CLE1B* gene. When CLEC-2 interacts with PDPN different biological processes are carried out, such as platelet biogenesis and activation, vascular blood integrity and development of the lymphatic vasculature, however, this interaction also results in activation of the immune response, thrombosis and invasion and metastasis of cancer cells [91]. The interaction between CCL21 and PDPN is also involved in the immune response, mainly in the development of regulatory T cells, as well as a potent chemoattractant in the tumor microenvironment, and in immune escape [89]. While the interaction between LGALS8 and PDPN is involved in lymphangiogenesis [90]. CD9 and CD44 interact with the transmembrane domain of PDPN, and have been shown to inhibit CLEC-2/PDPN interaction and also direct cell migration [22, 92]. Finally, matrix metalloprotease-14 (MMP14) has been shown to interact with the cytosolic domain and leads to mechanisms related to cancer invasion and metastasis. Meanwhile, the interaction of this region with the ezrin-radixin-myosin (ERM) family of proteins gives rise to processes related to heart development, lymphangiogenesis, in the activation of the immune response, in the epithelium-mesenchyme transition and in cell metastasis and invasion, this through mechanisms such as the regulation of the cytoskeleton and cell motility, favoring the local dissemination of odontogenic cysts and tumors with a more aggressive behavior [46, 47, 91, 93].

Importantly, CLEC1B, LCP2, PLCG2, GP6, LYVE1, PROX1, FLT4, PTPRC, SYK, VEGF-C, CCL21, SELP, TNFSF4, PODXL, LGALS8 and RDX proteins are expressed in different types of cancer such as breast cancer, hepatocellular carcinoma, melanoma, lung cancer, non-small cell lung cancer, papillary cell thyroid carcinoma, endometrial carcinoma, ovarian carcinoma, head and neck squamous cell carcinoma, oral carcinoma and in particular also squamous cell carcinoma of the head and neck in particular also squamous cell carcinoma of the tongue, as well as in some bone tumors [66–86], suggesting an important role in tumor proliferation, invasion and metastasis [22, 24–28, 91]. These proteins are also expressed in noncancerous and immunoinflammatory diseases such as periodontitis [71]. Current literature suggests that only four proteins (PECAM-1, TNFRF10B, MSN, EZR) with which PDPN interacts are expressed in odontogenic cysts and tumors [46, 47, 63–65]. PECAM-1 or CD31 is a type I transmembrane glycoprotein encoded by the *PECAM1* gene located on chromosome 17q23. It has a molecular weight of 140 kDa and consists of 738 amino acids [94]. It is strongly expressed in endothelial cells and weakly in megakaryocytes, platelets, plasma cells, marginal zone B cells, peripheral T cells and neutrophils. Its main functions include an important role in thrombosis and angiogenesis [95]. In this sense, a study investigated the spatial heterogeneity of blood vessels comparing the tumor center and the invasion front, as well as its prognostic value in samples of oral squamous cell carcinoma, by means of CD31 immunoexpression. Thus, the authors found a significantly higher presence of blood vessels in the invasion front of oral squamous cell carcinoma compared to the tumor center, considering this molecule as a possible prognostic marker for this type of lesions [96]. On the other hand, in relation to the different OL, another study [63] evaluated the angiogenic processes related to RC and OKC expansion. The results showed differences in immunostaining intensity. In RC, intense CD31 expression was observed in both the inflamed zone and adjacent stroma. Whereas, OKC showed an increase in the immunoexpression of this protein associated with a newly formed vascularization process on the surface of the epithelium, close to the keratin zone, as well as in areas with inflammatory infiltrate. Finally, further follow-up is advisable to evaluate the potential of this molecule as a diagnostic biomarker for this type of lesions. On the other hand, TNFRF10B also called DR5 or TRAIL-R2 is a type I transmembrane glycoprotein, encoded by the *TNFRF10B* gene, located on chromosome 8p21.3. It has a molecular weight of 47.87 kDa and consists of 440 amino acids [97]. This receptor is highly expressed in the heart, peripheral blood lymphocytes, liver, pancreas, thymus, spleen, prostate, ovary, uterus, placenta, testis, gastrointestinal tract and tumor cells [98]. The interaction and binding between TNF-related apoptosis-inducing ligand (TRAIL) with its receptor (DR5) transduces the apoptosis signal, thus playing an important role in host immunosurveillance against tumor progression [99]. Thus, it has been shown that, DR5 expression is up-regulated in premalignant (oral leukoplakia) and malignant (COCE) oral epithelia compared to oral epithelium. Also, DR5 expression was significantly associated with increased tumor size, however, it does not sample significant correlations with nodal status and tumor cell apoptosis rates, so it does not seem to play a crucial role in these mechanisms, however, dysregulation of apoptosis is an early event contributing to oral carcinogenesis [100]. In relation to OL, DR4 and DR5 have been shown to be diffusely expressed in AM, confirming that TRAIL and its receptors could be involved in neoplastic transformation of the odontogenic epithelium. However, no clear correlation was established between the expression of these molecules with the apoptotic behavior of AM cells. Thus, both apoptosis and cell proliferation mechanisms are characteristic in central

and peripheral areas of AM, this could suggest a certain intrinsic regulation between both processes, which could control the development and progression of these lesions, thus explaining their slow growth and inability to metastasize [64]. Finally, ERM family proteins play a structural and regulatory role in the assembly and stabilization of plasma membrane interactions through their ability to interact with transmembrane proteins (PDPN) and the cytoskeleton [101]. MSN expression (67.82 kDa-577 amino acids) has been demonstrated in COCE [102], as well as, by odontogenic epithelial cells in AM, AOT, OKC, OOC, CEOT, AF, ODS, COC, suggesting its involvement in the development of these lesions, however, its role in the expansive growth and local invasion of these lesions is still unclear [65]. On the other hand, it has also been shown that EZR (69.41 kDa-586 amino acids) may be involved in the progression of squamous cell tongue carcinoma in situ [103]. Furthermore, as for OL, it has been shown that EZR and PDPN might have a synergistic role in the expansive growth and local invasiveness of AMs and OKC [46, 47], so their role in different odontogenic cysts and tumors needs to be further explored.

Significance of PDPN as a potential immunohistochemical biomarker assessing the proliferative potential and aggressiveness among different odontogenic cysts and tumors

PDPN through its role in cytoskeleton reorganization and cell migration, constitutes a possible molecular marker of cell proliferation, cystic expansion and cell invasion, in odontogenic cysts and tumors, therefore, it has potential implications for the diagnosis and prognosis of this type of lesions and the oral and maxillofacial pathologist should consider its application for clinical and research purposes [29–57, 63–65].

In addition, PDPN has also been used as a potential marker to predict the risk of different types of cancer, particularly oral cancer. Its immunoexpression has even been determined in potentially malignant oral disorders, such as in erythroplasia and oral leukoplakia, where, increased immunoexpression of this protein in this type of lesions would mean that this immunomarker could play a role in tumor cell differentiation and neoplastic progression of oral squamous cell carcinoma [91, 104, 105].

Limitations and future

The main limitations of the present review were the analysis of a high proportion of articles with a cross-sectional design and the lack of longitudinal clinical studies

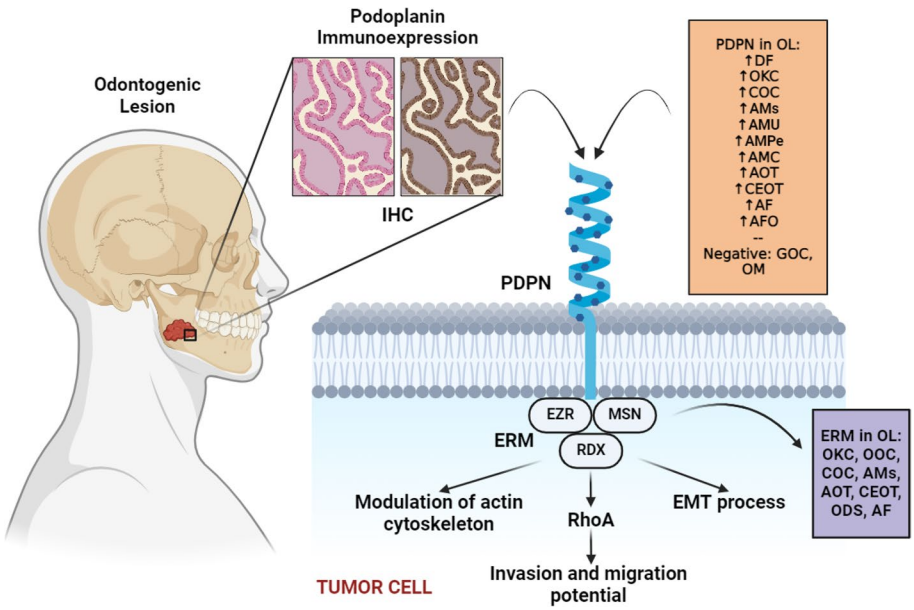


Fig. 4 Podoplanin immunoexpression in odontogenic lesions. PDPN is strongly expressed in DF, OKC, COC, AMs, AMU, AMPe, AMC, AOT, CEOT, AF and AFO. Its expression has been shown to be negative in GOC and OM. Furthermore, PDPN has been shown to participate in cell adhesion, migration and invasion through association with ERM proteins, which are expressed in different OL such as OKC, OOC, COC, AMs, AOT, CEOT, ODS and AF. IHC: Immunohistochemistry; OL: Odontogenic lesions; PDPN: Podoplanin; ERM: Ezrin, Radixin, Moesin; EZR: Ezrin; RDX: Radixin; MSN: Moesin; RhoA: Ras homolog gene family, member A; DF: Dental follicle; OKC: Odontogenic keratocyst; OOC: Orthokeratinized odontogenic cyst; COC: Calcifying odontogenic cyst; GOC: Glandular odontogenic cyst; AMs: Ameloblastoma solid; AMU: Ameloblastoma, Unicystic; AMPe: Ameloblastoma, Peripheral; AMC: Ameloblastic Carcinoma; AOT: Adenomatoid odontogenic tumor; CEOT: Calcifying epithelial odontogenic tumor; AF: Ameloblastic fibroma; AFO: Ameloblastic fibro-odontoma; ODS: Odontoma; OM: Odontogenic myxoma [29–57, 65, 91]

that will analyze the immunoexpression characteristics of PDPN in a larger number of odontogenic cysts and tumors that have undergone surgery through surgical procedures, this with the main objective of evaluating the possibilities of recurrence and thus being able to establish a prognosis; the high heterogeneity among study participants with respect to age and sex; the lack of numerical data (mean score) in relation to the intensity of immunostaining of the different OL to carry out quantitative analyzes (comparisons between subgroups of lesions); as well as the lack of articles that will investigate the PDPN immunostaining pattern in different types of OL, this based on the new classification of bone and maxillofacial tumors proposed by the WHO in 2022. In relation to OC, the expression of PDPN still needs to be investigated in the inflammatory colateral cyst, post-surgical ciliated cyst, nasopalatine duct cysts, gingival cysts, botryoid cyst and lateral periodontal cyst. While, in relation to OT, its expression in squamous odontogenic tumor, metastasizing ameloblastoma, primordial odontogenic tumor, dentinogenic phantom tumor, odontogenic fibroma, cementoblastoma and cement-ossifying fibroma still needs to be investigated. It would also be interesting to know its expression in malignant odontogenic tumors which with the exception of ameloblastic carcinoma, this protein still needs to be analyzed in sclerosing odontogenic carcinoma, odontogenic clear cell carcinoma, odontogenic ghost cell carcinoma, primary intraosseous carcinoma, odontogenic carcinosarcoma and sarcomas odontogenic. Therefore, we encourage researchers to carry out future work and provide information through immunohistochemical studies about possible differences between the PDPN immunoexpression pattern between different OL, with the aim of better understanding their possible pathogenesis and evaluating whether these differences can be taken into consideration as complementary criteria for diagnosis, prognosis, and treatment of OL that affect the maxillofacial region and thereby improve the quality of life of patients.

Conclusions

The results of the present systematic review support the unique immunoexpression of PDPN as a potentially useful diagnostic marker in the pathogenesis of OL. Additionally, the following conclusions are drawn:

1. PDPN is expressed in both follicles and tooth germs, in some OC such as RC, DC, OOC, OKC and COC. It seems that expression is negative in the GOC. On the other hand, it is also expressed in some OT such as AMs, AMU, AMPe, AMC, AOT, CEOT, ODS, FA and AFO. It also appears that its expression in the OM is negative, however more studies are needed to confirm these findings.
2. According to the results of the meta-analysis, the intensity of immunostaining was greater in the OKC compared to the DC.
3. PDPN interact with several molecules directly, leading to altered behavior of cystic tumor and cancer cells. This behavior is different for each of them. Therefore, determining the function and interaction of PDPN in various types of OL leading to a reduction in cell proliferation and motility may be of clinical importance (Fig. 4).

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Authors' contributions
Conceptualization, M.A.A.-S.; methodology, M.A.A.-S-.; software, M.A.A.-S.; validation, M.A.A.-S, and G.L.-B.; formal analysis, M.A.A.-S, A.H. and G.L.-B.; investigation, M.A.A.-S.; resources, M.A.A.-S, and G.L.-B.; data curation, M.A.A.-S.; writing—original draft preparation, M.A.A.-S, and G.L.-B.; writing—review and editing, M.A.A.-S, G.L.-B.; and A.H; visualization, M.A.A.-S, G.L.-B, S.R.S; and A.H; supervision, M.A.A.-S, G.L.-B.; and A.H; project administration, M.A.A.-S. All authors have read and agreed to the published version of the manuscript.

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Declarations

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