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Expression of CD56 in Human Tooth Germ, Adenomatoid Odontogenic Tumor, and Ameloblastic Fibroma

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Abstract

Background: Odontogenesis is a multistage process involving interaction between ectodermally derived odontogenic epithelium and neural crest–derived ectomesenchyme. CD56 is a neural cell adhesion molecule with sparse information regarding its expression in tooth germs. Therefore, the purpose of this study is to evaluate the immunohistochemical expression of CD56 in adenomatoid odontogenic tumor (AOT), ameloblastic fibroma (AF), and odontogenic myxoma (OMYX) and to compare the staining pattern with the human tooth germs for histogenetic relationship.

Methods: Archival paraffin-embedded tissue blocks of 4 human tooth germs (one in bud stage, 4 in bud-to-cap transition stage, 3 in early bell stage, and 2 in late bell stage), 13 AOT, 4 AF, and 10 OMYX were evaluated by routine sections and immunohistochemistry with the CD56 marker. Immunohistochemical reaction was assessed semi-quantitatively.

Results: Positive CD56 staining was observed on one side of the epithelium of the tooth bud and in the outer enamel epithelium with a rim of positive reaction in the dental follicle. Other components of the tooth germ were negative. Only certain tumor cells in AOT and AF reacted to CD56 while OMYX did not react.

Conclusion: This study demonstrates the expression pattern of CD56 in human tooth germs where the outer enamel epithelium adopts cell-fate decisions early in development. Thus, the histogenetic correlation of cells positive for CD56 in AOT and AF implies that they are linked to the dental lamina and/or outer enamel epithelium while the lack of CD56 expression in the mesenchymal component of AF and OMYX is inconsistent with neural crest–derived mesenchyme participation.

Keywords: Adenomatoid, ectomesenchyme, odontogenic, tooth germ

What is already known on this topic?

- *Expression of CD56 in odontogenic tumors is not clear about its role in histogenesis, diagnosis, and biological behavior or it confers neuroectodermal phenotype.*
- *Expression of CD56 in the tooth germs of mouse and dogs is believed to confer either an undifferentiated state or mark differentiation. However, there is no published study regarding human tooth germs to confirm the state of expression of CD56.*

What does this study add on this topic?

- *This study found a unique pattern of expression of CD56, which is confined to a segment of outer enamel epithelium compared to negligible expression in the ectomesenchyme. This is opposite to the expression of CD56 in mouse tooth germs while the pattern of expression is unreliable in the tooth germs of dogs.*
- *Expression of CD56 in the peripheral cells of the solid nodules of adenomatoid odontogenic tumor indicates an outer enamel epithelium phenotype while expression in the peripheral cells of the epithelial component in ameloblastic fibroma*

INTRODUCTION

Development of human tooth involves a series of interactions between ectodermally derived odontogenic epithelium and neural crest–derived ectomesenchyme.[1] Histologically, odontogenesis is manifested by a sequential morphological expression of various developmental stages, such as dental lamina (DL), bud, cap, and bell, and consequential histodifferentiation of participating tissues to eventually form various tooth-related tissues.[1,2] As odontogenic tumors recapitulate various stages of odontogenesis,[3] they are thought to arise from the odontogenic apparatus such as the remnants and/or DL, enamel organ (EO), reduced enamel epithelium, and the ectomesenchyme.[4]

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may be related to histogenesis from the outer enamel epithelium.

- *Unlike mouse tooth germs, CD56 was not expressed in the ectomesenchyme of the human tooth germs similar to the lack of expression in odontogenic myxoma.*

CD56 is a neural cell adhesion molecule (NCAM) and it has been speculated that the expression pattern of CD56 is similar in human and murine tooth germs.[4] The expression of CD56 in the peripheral columnar cells in ameloblastoma, which is believed to be equivalent to the inner enamel epithelium (IEE) of the EO, is thought to confer neuroectodermal differentiation.[4] However, the significance of CD56 expression in odontogenic tumors is not clear, as to whether it is related to histogenesis or biologic behavior.[3-5] Others have suggested the CD56 expression may serve as a candidate marker for diagnosis of some odontogenic tumors.[3,6]

The literature reveals that information regarding expression of CD56 in tooth germ is sparse,[7-11] and there is no published information regarding immunohistochemical expression in human tooth germs. The odontogenic tumors such as adenomatoid odontogenic tumor (AOT), ameloblastic fibroma (AF), and odontogenic myxoma (OMYX) are believed to exhibit neuroectodermal differentiation in view of the presence of diverse epithelial cells in AOT and participation of ectomesenchymal component in the latter tumors. Therefore, the purpose of the present study is to compare CD56 expression in human tooth germ and odontogenic tumors such as AOT, AF, and OMYX to elucidate histogenetic relationship.

MATERIALS AND METHODS

The study was based on the dissertation (2018–2021), which was submitted to Tamil Nadu Government Dental College and Hospital. The study protocol was approved by the Institutional Review Board (Approval no: 4– 2019; Date: August 22, 2019) of Tamil Nadu Government Dental College and Hospital. For a comprehensive evaluation of histogenesis, tumors derived from benign odontogenic epithelium (AOT), mixed epithelial and mesenchyme (AF), and mesenchyme (OMYX) were included, ensuring representation from each category which are believed to exhibit neuroectodermal differentiation. Pertinent cases of odontogenic tumors were selected from archival formalin-fixed paraffin-embedded tissue blocks and reviewed by the investigator and the guide for selection of representative cases. Hybrid odontogenic tumors, specimens with insufficient tumor tissue, and severely hardened paraffin embedded tissue block that hinder proper sectioning and histological assessment were omitted and unequivocal cases of 13 AOT, 4 AF and 10 OMYX were included in this study. In addition, 4 archival formalin-fixed paraffin-embedded tissue blocks of human tooth germs were included from previous research in the author's institution (Sharief et al,[12] 2018, Selvam et al,[13] 2018). The sample size was determined based on practical constraints such as the availability of specimens, feasibility of slide preparation, and logistical factors rather than strict statistical calculations. The tissue blocks of human tooth germ consisted of unclaimed, spontaneously aborted fetuses with a gestational age ranging from 20 to 26 weeks, a critical period during which critical tooth development events take place and for which permission was previously obtained from the Institutional Ethical Committee (vide Ref. No. 0430/DE/2010, dated February 21, 2014 and Ref. No. 0420/DE/2016, dated November 25, 2016) Tamil Nadu Government Dental College and Hospital. The study included tissue blocks which were well preserved with intact dental structures, including the EO, dental papilla (DP), dental follicle (DF), developing hard tissues and odontogenic tumor cells ensuring clear histological sectioning and staining without excessive artefacts or tissue degradation. Informed consent was not applicable as this study utilizes tissue blocks of previously approved studies.

Serial sections (4 µm thickness) were prepared from all the included samples for both hematoxylin and eosin (H & E) staining and immunohistochemistry. For the latter, the sections were transferred to 3-aminopropyltriethoxysilane-coated slides, which were deparaffinized and rehydrated, and antigen retrieval was performed with Tris-EDTA buffer at pH 8.8 to 9.2 at temperature of 100°C for 5 minutes followed by 130°C for 15 to 20 minutes using the pressure cooker. The sections were incubated with peroxide

blocker and power blocker (Super Sensitive™ Polymer-HRP IHC Detection System, BioGenex, Fremont, CA) for 10 minutes each to avoid endogenous peroxidase activity and other non-specific reactions. The sections were incubated with mouse monoclonal anti-CD56 antibody (Clone-NKH-1 ready to use with BioGenex Detection System) for an hour and 15 minutes. This was followed by super-enhancer for 20 minutes and horse radish peroxidase for 30 minutes. The sections were then covered with diaminobenzidine chromogen for 15 minutes, which was followed by counterstaining with Mayer's haematoxylin for 2 minutes. The sections were thoroughly washed in Tris-buffered saline (pH 7.5-7.7) between each step of the procedure except for incubating with power block. Finally, the sections were dehydrated and mounted. Negative controls were performed as described earlier, but the primary antibody was replaced with Tris-buffered saline. Sections of schwannoma were used as an external positive control.

Immunohistochemical reaction was assessed semi-quantitatively, and the sections were examined independently by the investigator and the guide using conventional light microscopy for intensity of staining as follows: 0, complete lack of expression; 1+, less intense or weak; 2+, moderately intense; 3+, strongly intense. The extent of immunoreactivity of CD56 within the section was assessed as diffuse when there is >50% of cells expressing CD56 and focal when there is 1%-50%. To maintain reproducibility, each observer examined the stained sections separately under identical magnification and lighting conditions. Once the independent assessments were completed, the results were compared to evaluate inter-observer agreement. In cases where discrepancies arose, the final results were determined through discussion and slide re-evaluation. Additionally, intra-observer reliability was verified by reassessing a randomly chosen subset of slides after a specific interval, ensuring consistency in individual evaluations over time. The study sections were evaluated using Olympus Microscope (BX43), and the photographs were captured by infinity camera attached to the microscope or by smartphone using the free-hand technique.

The study did not include statistical and power analysis because the study aimed to provide a qualitative or semi-quantitative assessment of staining intensity and extend solely on visual scoring rather than a fully inferential statistical evaluation.

RESULTS

CD56 Expression in External and Internal Controls

Sectioning of the 4 archival paraffin-embedded tissue blocks of human tooth germs revealed 1 in bud stage, 4 in bud-to-cap transition stage, 3 in early bell stage, and 2 in late bell stage. In addition, the DL and cell rests were noted in the sections. As it was not possible to differentiate between incisor and canine tooth germs, they were regarded as incisor tooth germs.

Sections of schwannoma were used as external control, in which the CD56 immunostaining was strong and diffuse. In general, apart from the immunostaining reaction described below in the tooth germ and odontogenic tumors, positive CD56 staining reaction was also evident in the human tooth germ sections in the nerve fibers, hair follicles, skeletal muscle fibers, and variable reactions in osteoblasts and osteoid matrix while negative reaction was evident in the skin and oral epithelium, cartilage, and salivary gland acini. These structural elements served as positive and negative internal controls.

CD56 Expression in Human Tooth Germ

Dental Lamina

The DL is made up of cords of epithelial cells with an inner and outer layer enclosing sparse inner cell (Figure 1A). A moderate positive CD56 staining occurred in a mosaic pattern in both the incisor and molar tooth germs (Figure 1B).

Bud and Bud-to-Cap Transition Stage

During the bud (data not shown) and bud-to-cap transition stages, strongly intense CD56 expression was characteristically polarized to one side of the epithelial structure that presumably represents the prospective outer enamel epithelium (OEE), while the prospective IEE assumed a tall columnar shape but remained unstained. The organization of ectomesenchyme into DP and DF was clearly evident, but there was no positive staining in the ectomesenchyme (Figure 1C-F).

Early Bell Stage

During early bell stage (EBS), positive CD56 staining reaction was evident in the OEE of both incisor (Figure 2A) and molar tooth germs (data not shown). The positive reaction in the DF involved the entire circumference of incisor tooth germ (Figure 2B) but was restricted to the DF in relation to the apical or basal part of the DP of molar tooth germ (data not shown).

Late Bell Stage

During late bell stage (LBS) in the incisor tooth germ, strongly intense CD56 expression was restricted to the OEE and the DF, which was confined to the cervical loop region. The expression in the DF also involved the apical or basal part of the DP (Figure 2C-F).

CD56 Expression in Adenomatoid Odontogenic Tumor, Ameloblastic Fibroma, and Odontogenic Myxoma

Adenomatoid Odontogenic Tumor

Of the 13 cases of AOT, the intensity of staining reaction to CD56 ranged from moderate to strongly intense and the extent varied from focal to diffuse in 9 cases while in 4 cases it was negative. The positive CD56 staining was limited to the cuboidal-to-round tumor cells, which were often arranged in a cribriform pattern, with a high nuclear

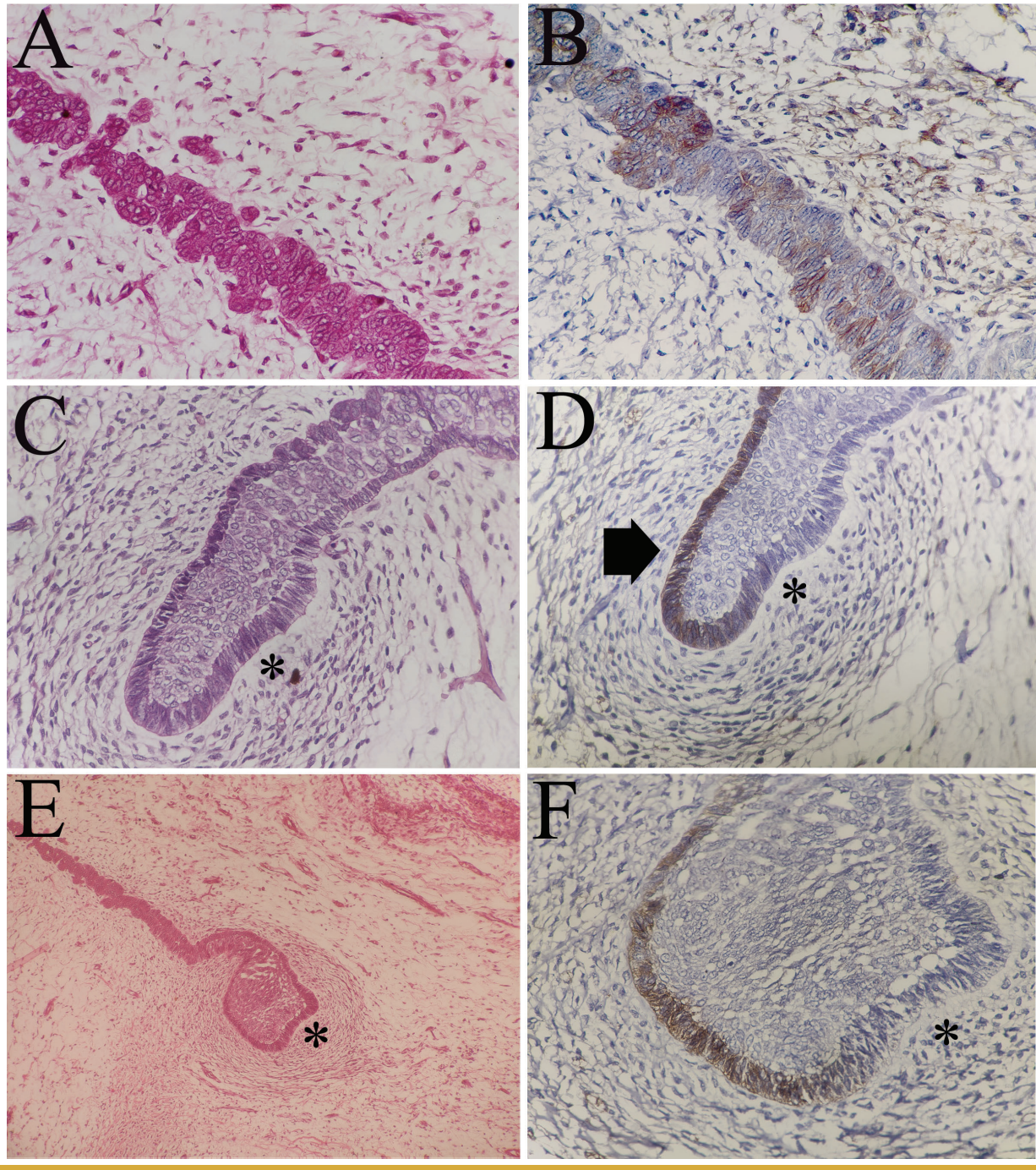

Figure 1. A & B, routine and CD56-stained sections showing the strand of dental lamina and undifferentiated ectomesenchymal cells with a patchy mosaic, moderately positive staining in the epithelial cells accompanied by sporadic expression in the undifferentiated ectomesenchymal cells. C, routine section during the bud-to-cap transition stage characterized by indentation on the prospective (asterisk) and hugged by ectomesenchymal condensation, and the corresponding serial sections in D show a positive CD56 staining pattern restricted to one side of the peripheral layer of the epithelium or the prospective outer enamel epithelium (OEE) (arrowhead). The ectomesenchyme is negative for CD56 staining. E, routine section of a bud-to-cap transition stage with visible organization of dental papilla (DP) and dental follicle (DF) and the corresponding serial section illustrates positive CD56 staining polarized to one side of the prospective OEE in F. The DP and DF did not react to CD56. ×100, ×400.

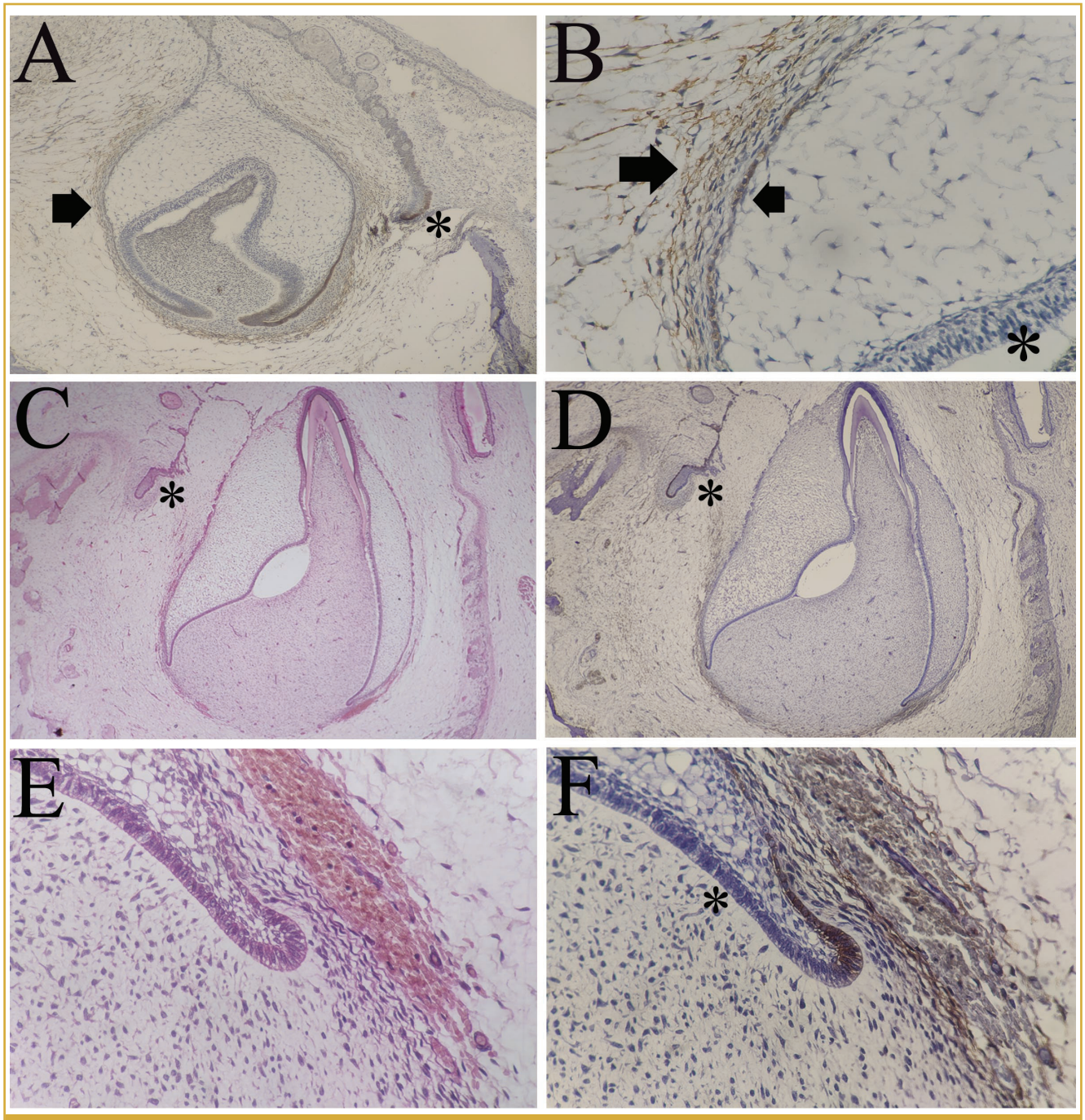

Figure 2. A shows a bud stage with a positive CD56 reaction on one side of the peripheral layer of epithelium (asterisk) and an early bell stage with a positive reaction in the outer enamel epithelium (OEE) and dental follicle (DF) while dental papilla and other cellular layers of the enamel organ are negative. B shows a higher magnification of the arrowhead in A to illustrate dental follicle (arrow), OEE (arrowhead), and inner enamel epithelium (asterisk). C shows the routine section of the tooth germ during the bud stage and late bell stage (LBS). D shows positive CD56 staining restricted to the prospective OEE of the bud stage and the OEE and DF in LBS. E & F show higher magnification of the routine and CD56 stained sections corresponding to the cervical loop region of the LBS in C & D. ×20, ×400.

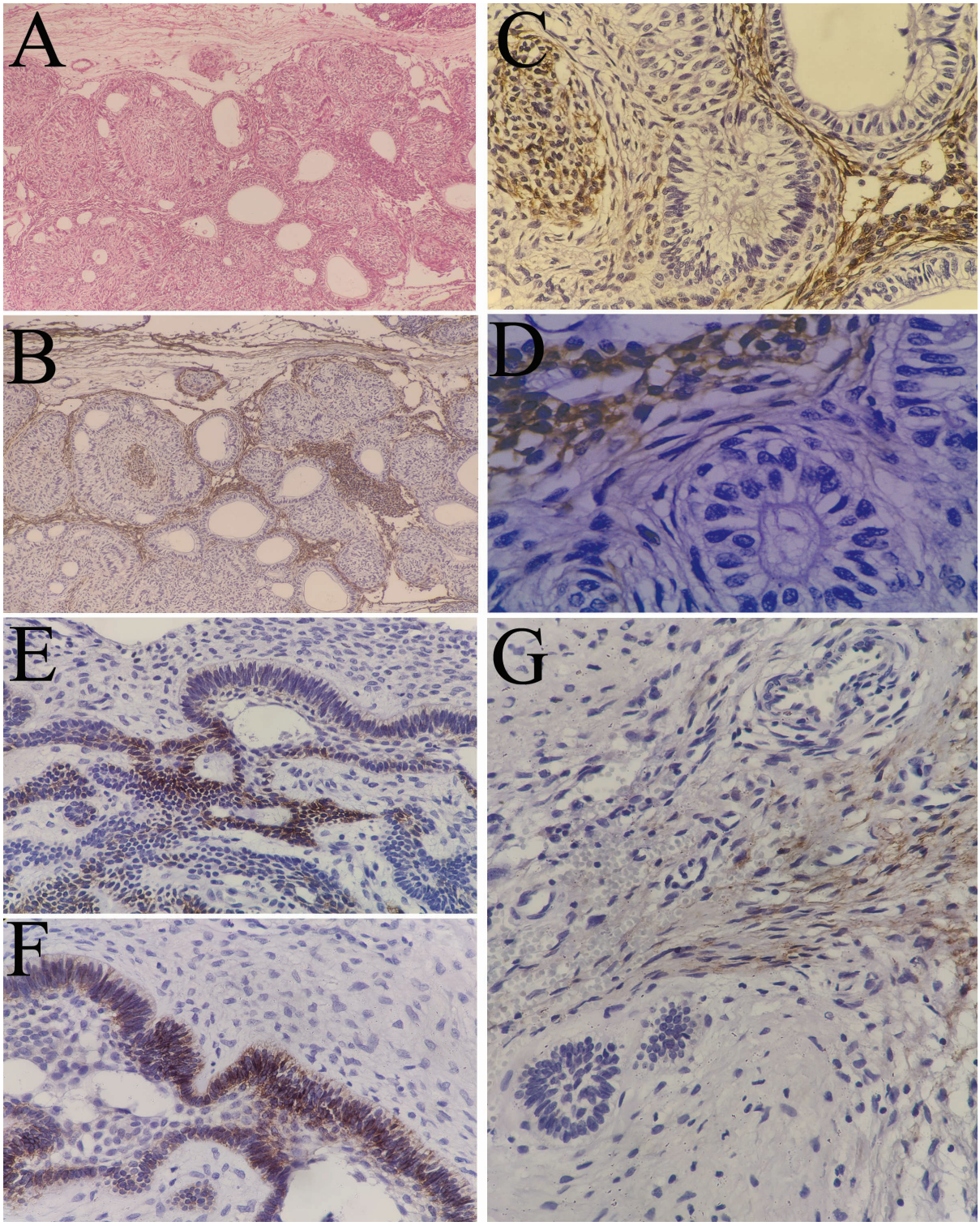

Figure 3. A, routine section of a representative case of adenomatoid odontogenic tumor with various growth patterns and the corresponding CD56 stained sections illustrates a positive staining reaction restricted to the round epithelial cells in relation to the various growth patterns in B-D. In E and F, ameloblastic fibroma, moderate-to-intense CD56 staining reaction is evident in the epithelial strand and follicles, with a negative reaction in the dental papilla–like stroma in G. ×40, ×100, x 400.

cytoplasmic ratio along the periphery of the tumor epithelial nodules (Figure 3A-D). One of the 9 positive cases demonstrated a non-specific thin epithelial lining and solid sheets of round tumor cells in some microscopic fields (data not shown).

Ameloblastic Fibroma

Of the 4 cases employed in this study, only 2 showed a positive reaction to CD56. The intensity of staining reaction to CD56 ranged from moderate to strongly intense, but the extent was only focal in the positive cases. The CD56 staining reaction marked the tumor cells in the epithelial strands and the peripheral cells in the follicles or islands. The latter staining reaction was variable even within the same follicles or islands, with abrupt negative reaction. The ectomesenchymal cells were completely negative in all cases except for rare positive staining in some spindle cells collaring the DP-like stromal cells in one case (Figure 3E-G)

Odontogenic Myxoma

All the 10 cases of OMYX did not react to CD56.

DISCUSSION

The present study compared the expression of CD56 in human tooth germs with AOT, AF, and OMYX in order to determine the histogenetic relationship based on the pattern of immunohistochemical expression. In general, this study found that the expression of CD56 staining reactions in the external and internal controls is in agreement with the published literature.[14-17]

In the present study, a characteristic polarized pattern of positive CD56 staining was first observed, being limited to the prospective OEE during early stages and to a restricted segment of OEE during the bell stage of crown development. Although CD56-positive undifferentiated mesenchymal cells were noticed adjacent to the strand of DL (Figure 1B), the dental follicle (DF) exhibited CD56 expression only during the bell stage while it was negative in the DP. The expression of CD56 in AOT specifically marked the cells at the periphery of tumor nodules, while the expression is heterogeneous in the peripheral cells of the epithelial component in AF. Based on the expression of CD56 in both human EO and the studied tumor entities, it is likely that the CD56-positive tumor cells are derived from the DL and/or OEE.

Expression of CD56 in Human Tooth Germ

The organization of the ectomesenchyme and the EO becomes evident during the transition from bud-to-cap stage of development.[2] The EO comprises an OEE, stellate reticulum (SR), stratum intermedium (SI), and IEE, while the ectomesenchyme distinctly segregates into DP and DF. The cervical loop is regarded as a mitotically active region of the EO that delimitates the DP and DF.[2] The cervical loop is formed by the IEE and OEE,[2] but the separation between them is not clear.[2] In this context, the observation of a distinct polarized CD56 expression pattern in the present study is significant, which likely indicates the regional distribution of IEE and OEE in the EO. In addition, it also marks the possible onset of acquisition of the neuroectodermal phenotype of the prospective OEE, even at the early developmental stages, while the surface epithelium from which the tooth primordia arose remained negative.

The available literature indicates that the expression of NCAM (CD56) is primarily observed in the DF with varying intensity during the progress of odontogenesis, right from the bud-to-bell stage, which is believed to maintain them in an undifferentiated state or is related to cell differentiation.[7-10] In the present study, the CD56 expression in the ectomesenchyme (DF) was unequivocally evident only during the bell stage of human tooth germs (Figure 2). However, the expression in the DF was limited to the apical or basal part of the DP in molar (during EBS) and incisor tooth germs (during LBS). The findings of the present study differ from the expression pattern of NCAM (CD56) in mouse incisor and molar tooth germs, where the expression marked the entire DF.[7-9]

In the mouse molar tooth germ, in contrast to the DF, the expression of NCAM (CD56) in the epithelial component was not a prominent feature.[7,8,10] Obara et al[7-10] in their series have observed NCAM (CD56) expression in the DL,[7,8,10] inner cells of the bud stage, and in the SI of the bell stage.[10] In the present study, the expression of CD56 marked the inner cells of the DL. However, the positive CD56 staining was restricted to one side of the outer layer of the tooth bud (prospective OEE), which continued in the bud-to-cap transition stage and in a short segment of the OEE during the bell stage, while other cells of the EO remained negative. Although human incisor and molar tooth germs are comparable in terms of their basic structure to the mouse molar tooth germ, the consistent pattern of expression of CD56 in the aforementioned epithelial component of both human incisor and molar tooth germs observed in this study is at variance compared to the mouse tooth germs. In the latter, the expression of NCAM (CD56) was not a consistent feature in the epithelial component except the DL.[7-10]

The expression of CD56 in dog tooth germs has been documented, differing from observations in mouse tooth germs and the results of the present study.[7-11] In the dog tooth germs,[11] though the peripheral epithelial cells of the tooth bud had a prominent polarized staining pattern similar to the present study, CD56 was virtually positive in the tooth germ during all the stages of development and as well as in the adjacent tissues. The discrepant results between human, mouse, and dog tooth germs likely indicate either species or immunomarker variations.

In the human EO, ultrastructural findings reveal that SI, SR, and OEE have many free ribosomes, well-developed Golgi

complexes, numerous desmosomes, and gap junctions, but there is a scarcity of rough endoplasmic reticulum, indicating common functional roles in transport and support due to their ability to produce acid mucopolysaccharides.[18] However, the specific function of OEE is poorly understood in all the studied species.[19] The OEE is made up of undifferentiated cells in mouse incisors and is thought to function as an epithelial stem cell niche due to its high regenerative potential.[19,20] However, in the rabbit incisor tooth germs, it was found that cells from the OEE do not migrate to the IEE to provide progenitor cells for the subsequent differentiation.[21] Thus, in the present study, the consistent and characteristic distribution of CD56 restricted to the cells of OEE right through the developmental stages examined in this study indicates that the expression is distinct and may distinguish OEE not only from the IEE but also from the stellate reticulum and stratum intermedium cells.

Expression of CD56 in Odontogenic Tumors (Adenomatoid Odontogenic Tumor, Ameloblastic Fibroma, and Odontogenic Myxoma)

The expression of CD56 in certain odontogenic cysts and tumors has been previously documented in the literature.[3-6,22] Nevertheless, the significance of CD56 staining in odontogenic tumors is not clear,[3,5,6,22] but interestingly, the expression of CD56 is thought to confer a neuroectodermal phenotype in ameloblastoma and ameloblastic differentiation in odontogenic keratocyst.[5]

The literature reveals that in odontoma, which is the closet correlate to the pattern of development in normal human teeth, only rare cells adjacent to ameloblasts are reactive to CD56.[3] The authors believe that cells reactive to CD56 are likely to represent SI.[3] Although expression of CD56 has been transiently observed in the SI of mouse molar tooth germs,[10] in the present study, CD56 did not react with any of the cellular components of the human EO except for the positive staining reaction in the OEE. This indicates a variable expression pattern between mouse and human tooth germs.

CD56 Expression in Adenomatoid Odontogenic Tumor

Adenomatoid odontogenic tumor comprises more than one population of tumor cells that are organized similar to the EO of tooth germs.[23] The polygonal and columnar cells that make up the solid tumor nodules, ducts, and rosettes are thought to belong to the IEE lineage,[24-26] whereas the flattened or spindle and cuboidal to round epithelial cells with high nuclear cytoplasmic ratio envelope the solid nodules in a circumferential manner to simulate the organization of SI, SR, and OEE seen in the EO.[23,25,26] Previous immunohistochemical observations in AOT revealed variable reaction to CD56 with 3 negative cases among the total 7 cases from the two published studies.[3,6] In the present study, though the lack of CD56 staining in the 4 cases are unlikely to the differences in fixation of the archival paraffin-embedded tissue blocks, age of the blocks, and immunohistochemistry procedure, and there is no explanation to offer.

The previous studies clearly demonstrated that the tall columnar cells of the ducts and rosettes within the solid nodules of AOT reacts to amelogenin and cytokeratin 19 (CK19), while both are uniformly negative in the peripheral cuboidal to round cells that make up the cribriform growth pattern.[27,28] The expression of amelogenin and CK19 was not a significant feature of SR and SI in human tooth germs,[12,29] but CK19 marks both OEE and IEE and amelogenin reacts only with the IEE.[29] By implication, these observations suggest that expression of amelogenin and CK19 in the columnar cells of AOT indicates functional and cytodifferentiation similar to the IEE lineage of the EO. Therefore, by analogy, the negative CD56 reaction in the solid nodules (polygonal to columnar cells) and the surrounding cells (flattened or spindle cells) of the AOT observed in the present study is consistent with the pattern of negative CD56 reaction in the equivalent cells of the human EO (Figure 3A-D). Nevertheless, the previously observed positive expression of CK19 in both the OEE and the differentiated lineage of IEE in human EO would warrant an alternative interpretation.[12] Interestingly, it may be inferred that positive or negative expression of CK19 in the EO implies that either the cells are already committed to the differentiation pathway or remain undifferentiated to replenish the proliferative pool.[12,30] Thus, as in the present study, CD56 expression has also been reported specifically in the cuboidal-to-round epithelial cells at the periphery that constitute the cribriform pattern in AOT.[6] This indicates that the negative CD56 staining reaction in the flattened or spindle-shaped epithelial cells observed in this study, which are thought to represent SI or SR,[25,26] might represent another distinct cell type from the cuboidal-to-round epithelial cells (Figure 3B-D). In addition, though previous ultrastructural observations in cat and human tooth germs indicates common cytoplasmic features among the SR, SI, and OEE,[18,31] the presence of myelin was only demonstrated in the cytoplasm of certain cells located within the OEE in proximity to the SR.[18] Therefore, in this contextual background, it is prudent to suggest that the CD56-positive cuboidal-to-round tumor epithelial cells in AOT may be histogenetically related to the OEE of the EO.

CD56 Expression in Ameloblastic Fibroma

The previous research in AF found that the tumor cells are unreactive to the neural markers like S100, neuronspecific enolase, and glial fibrillary acidic protein.[32] In contrast, the peripheral columnar shaped cells of the epithelial follicles in AF reacted to CD56 in a manner similar to ameloblastoma.[3,4,22] However, the expression of CD56 staining reactions in AF has been reported to be heterogenous, which varied from intense staining with an abrupt change to no expression,[3] a finding also observed in the present study. Research on ameloblastomas has shown prominent CD56

expression, particularly in the outer columnar cells, whereas dentigerous cysts exhibit no expression, and keratocystic odontogenic tumors (KCOT) display only partial positivity.[4] A similar pattern has been observed in comparisons with adenomatoid odontogenic tumors (AOT) and odontogenic keratocysts (OKC), where ameloblastomas demonstrate the highest CD56 expression levels.[6] Furthermore, studies have linked CD56 expression in ameloblastomas and odontogenic keratocysts to clinical features such as root resorption, tooth displacement, and bone perforation. These findings highlight the potential diagnostic relevance of CD56 and suggest that it may serve as a prognostic marker for evaluating the aggressiveness of odontogenic lesions, identifying high-risk cases, and informing clinical management strategies.[33] However, its diagnostic and prognostic reliability should be further evaluated through larger sample sizes and comparative studies incorporating additional odontogenic lesions. Nevertheless, our study does not primarily focus on the diagnostic or prognostic utility of CD56 but rather on its role in odontogenic tumor histogenesis.

Pertinent published sources demonstrate that the cytodifferentiation (such as CK19) and functional (such as ameloblastin) markers are expressed heterogeneously in the tumor cells of ameloblastoma compared to the expression in the equivalent cells of human EO.[12,13] The corollary to this observation is that not all tumor cells of ameloblastoma attain the same level of cytodifferentiation as those in the EO. Thus, as previously reported for ameloblastoma,[4,22] the positive CD56 reaction pattern in the epithelial strands in AF and in the DL of human tooth germs in the present study may well indicate acquisition of neuroectodermal phenotype in AF. Alternatively, in the present study, the positive staining reaction in the peripheral tall columnar cells of the larger epithelial follicles in AF and negative reaction in the IEE of human EO may well imply that the epithelial cells in both the strands and follicles are derived from the same source. In other words, the epithelial component in AF is likely derived from earlier stages of tooth development or OEE rather than from the differentiated phenotype of IEE lineage of EO.

CD56 Expression in Odontogenic Myxoma

Odontogenic myxoma is a mesenchymal odontogenic tumor that exhibit spindle and stellate shaped cells suspended in an abundant myxoid matrix. Based on the consistent immunoreactivity to proteoglycan M/versican in both DP and the myxoid matrix of OMYX, it was thought that the tumor is derived from DP than DF.[34] Still, others regard that OMYX is not derived from odontogenic apparatus because the glycosaminoglycans in OMYX are quite different from the dental tissues.[35] However, Obara et al[7-10] believe that NCAM (CD56) expression in ectomesenchyme of mouse tooth germs is related to differentiation of DP and DF.[7-10] In contrast, S100 has been reported to be unreactive in the ectomesenchyme component of human tooth germs and primordial odontogenic tumor.[36]

In view of the aforementioned information, an attempt was made to clarify whether the mesenchymal component of AF and OMYX would react to neuroectodermal marker like CD56 and thereby provide answers to the histogenesis. The negative reaction in the mesenchymal components of both AF and OMYX indicates that CD56 is not a useful marker to determine origin from the equivalent tissues in the tooth germ while the rare positive reaction observed in the spindle cells bordering DP—like stromal cells in AF require further studies to confirm (Figure 3G).

The limitation of this study is the lesser numbers of human tooth germs and AF employed. Therefore, similar studies with a greater number of human tooth germs involving various developmental stages are required to avoid possible bias.

In conclusion, the expression of CD56 was demonstrated in the epithelial component of human tooth germs adopts certain cell-fate decisions early in odontogenesis. Thus, it is believed that a subset of tumor cells in AOT and AF that express CD56 are presumably related to histogenesis from the DL and/or OEE while lack of CD56 expression in the mesenchymal component of AF and OMYX needs further study with appropriate markers to ascertain participation of neural crest-derived mesenchyme.

Data Availability Statement: The data that support the findings of this study are available on request from the corresponding author.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of Tamil Nadu Government Dental College and Hospital University (Approval no: 4- 2019; Date: August 22, 2019).

Informed Consent: Informed consent was not applicable as this study utilizes tissue blocks of previously approved studies.

Peer-review: Externally peer-reviewed.

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