ORIGINAL ARTICLE

Quantitative evaluation of microvessel density using CD34 in clinical variants of ameloblastoma: An immunohistochemical study

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Received: 15-10-2015
Accepted: 26-03-2016

ABSTRACT

Background: Odontogenic epithelium plays an important role in the histogenesis of odontogenic tumors of the jaws. Ameloblastomas, which arise from odontogenic epithelium, are considered benign with little tendency to metastasize. Tumors require an adequate supply of oxygen and a way to remove their waste products. This can be achieved by angiogenesis. *In situ* quantification of the microvessel density (MVD) is a usual method for assessing angiogenesis. Moreover, angiogenesis may differ in subtypes of ameloblastomas and could play a role in determining the pattern of tumor growth. **Aim:** The aim of the present study was to demonstrate the expression of cluster of differentiation (CD34) in variants of ameloblastomas and to correlate and compare their expression to the aggressive behavior. **Materials and Methods:** A retrospective cross-sectional study which included forty paraffin blocks was conducted after obtaining ethical committee clearance. Ten cases of pyogenic granuloma were used as a positive control and thirty cases were of solid multicystic ameloblastoma (SMA), unicystic ameloblastoma (UA) and desmoplastic ameloblastomas. Angiogenesis was assessed using CD34 antigen and was immunohistochemically localized. Statistical analysis was carried out for comparative analysis with the help of ANOVA test, Kolmogorov–Smirnov test and least significance difference test. **Results:** A significant correlation was obtained between the MVD of all the three variants, i.e., SMA, UA and desmoplastic ameloblastomas which was statistically significant ($P < 0.05$). **Conclusion:** Increased MVD in the three variants, i.e., SMA, UA and desmoplastic ameloblastoma seen in the present study could suggest that the angiogenesis has an important role in tumor progression and aggressiveness of ameloblastomas.

Key words: Ameloblastoma, angiogenesis, CD 34

INTRODUCTION

Odontogenic lesions arising from the tooth producing tissues represent a diverse group of particularly interesting lesions. They are a variety of cysts and tumors with inconsistent clinical and biological behaviors, which are found exclusively in the maxilla, mandible and rarely, the gingiva. Odontogenic tumors have been a subject of great interest to oral pathologists and maxillofacial surgeons for several decades.[1]

Access this article online

Quick Response Code:

Website: www.jomfp.in

DOI: 10.4103/0973-029X.180929

Odontogenic lesions arising from the tooth producing tissues represent a diverse group of particularly interesting lesions. They are a variety of cysts and tumors with inconsistent clinical and biological behaviors.

Among all odontogenic lesions, ameloblastoma has attracted great interest. Ameloblastoma is characterized by a benign

How to cite this article: Pereira T, Dodal S, Tamgadge A, Bhalerao S, Tamgadge S. Quantitative evaluation of microvessel density using CD34 in clinical variants of ameloblastoma: An immunohistochemical study. J Oral Maxillofac Pathol 2016;20:51-8.

but locally invasive behavior with a tendency to recur. It can involve adjacent soft tissues; infiltrate and cause destruction of the bone.

The aggressive clinical behavior of ameloblastomas and their histological features constitute a puzzling paradox. Some of the hypotheses concerning this strange clinico-histological contradiction were analyzed. The additional paradox represented by the neoplastic parenchyma itself, in which a tissue consisting of cells which are normally able to form enamel does not elaborate any of the calcified dental tissues, were also assessed.

"Tumor Microenvironment" implies the total functional and structural constellation of neoplastic and nonneoplastic cells as well as extracellular components, including cytokines, chemokines and growth factors that may be derived from either the neoplastic or the nonneoplastic cells.[2]

Over the past decade, role of the neoplastic microenvironment has become appreciated largely because of the realization that tumors are not merely masses of neoplastic cells but instead are complex tissues composed of both noncellular (matrix proteins) and cellular component (tumor-associated fibroblasts, capillary-associated cells and inflammatory cells), in addition to the ever-evolving neoplastic cells. As a consequence, in tumor tissue, the microenvironment also profoundly influences many steps of tumor development and progression. The growth, invasion and metastatic potential of tumor cells are influenced through tumor-stroma interaction in which tumor cells and mesenchymal cells including inflammatory cells surrounding the tumor cells interact with each other.[3-5]

Angiogenesis (neovascularization) is an important hallmark in the development of cancer. It is well known that tumor growth is limited to 1–2 mm^3 in the absence of adequate perfusion, so solid tumors need to develop a blood supply to grow and metastasize. This association of angiogenesis and cancer has been credited to the visionary pioneer Judah Folkman (1933-2008), who stated that tumor growth was directly dependent on blood vessel network development.[6,7]

Angiogenesis is the physiological process involving the growth of new blood vessels from preexisting vessels. Like cancer, it is a complex multistage process including degradation of extracellular matrix, proliferation and migration of endothelial cells, capillary differentiation and anastomosis.[8]

Angiogenesis is driven by a cocktail of growth factors and pro-angiogenic cytokines and is tempered by an equally diverse group of inhibitors of neovascularization and it is tightly controlled by the balance between these pro-angiogenic growth factors and cytokines.[9]

The angiogenic promoters and inhibitors originate from the neoplastic cell and from stromal elements. These are the constituents of tumor microenvironment. Studies have shown that angiogenesis acts like a double-edged sword, valuable in the normal physiologic conditions but uncontrollable and invasive in neoplastic and inflammatory conditions. The epithelial tissue stroma is the major supporting factor. Any changes in epithelium cause some changes in the stroma. The blood vessels in the stroma are one of the essential factors for epithelial growth.[10]

Angiogenesis cannot be measured directly but quantification of microvasculature can be done by assessment of mean microvessel density (MVD). The technique involves immunohistochemical staining of endothelial cells of capillaries using monoclonal antibodies.[11]

Different markers (monoclonal antibodies) such as cluster of differentiation (CD) CD34, CD31, CD105 and antibodies like vascular endothelial growth factor (VEGF), beta fibroblast growth factor are used to measure MVD in each microscopic field.[12]

CD34 is a cell surface 110–120 KD monomeric transmembrane glycoprotein and is a pan-endothelial marker of endothelial cells. CD34 has a major role in the evaluation of MVD in tumors. Expression of CD34 is done for the assessment of MVD, unlike CD31 which in addition to being present on endothelial cells is also localized in macrophages.[13,14]

Hence, we have undertaken this study to determine the MVD using CD34 in variants of ameloblastoma, i.e., solid multicystic ameloblastoma (SMA), unicystic ameloblastoma (UA) and desmoplastic ameloblastoma.

MATERIALS AND METHODS

This retrospective cross-sectional study was carried out in the Oral and Maxillofacial Pathology Department. Institutional Ethical Committee clearance was obtained for the study protocol. The medical records of the patients were retrieved from the archives and reviewed. The samples included the clinical variants of ameloblastoma.

Clinical information including the age, gender and the location of the lesion was extracted from the patient's files and then recorded in tables. After selecting the paraffin blocks, a 3 μm section was prepared, stained using hematoxylin-eosin (H and E) staining and the diagnosis was confirmed by two pathologists. Sections with inflammation, hemorrhage and incisional biopsy with insufficient tissue were excluded from the study. The study was carried out over a period of 6 months.

A total of forty paraffin blocks which included 12 cases of SMA, 10 cases of UA and 8 cases of desmoplastic ameloblastoma

(30 cases) and 10 cases of pyogenic granuloma were selected as internal positive control.

Immunohistochemistry

A 3 μm section of the tissue was cut and transferred to aminopropyl triethoxy silane (Sigma-Aldrich Chemical Co., USA) coated slides and incubated overnight at room temperature. After warming in a slide warmer for 15 min, the sections were deparaffinized in three changes of fresh xylene each for 5 min followed by dehydration in a series of 100% absolute alcohol each for 5 min. Endogenous peroxidases were blocked with peroxide block (Biogenex life sciences Pvt. Ltd., CA, USA) for 15 min at room temperature and washed with distilled water followed by citrate buffer (pH 6.0) wash for 10 min. Antigen retrieval was undertaken with a help of Biogenex antigen retrieval system. The sections were immersed in citrate buffer solution and placed into the Biogenex antigen retrieval system and heated for 15 min. The system was allowed to cool to room temperature by placing it under running tap water and later the slides were washed with distilled water for 5 min. With an intention to block endogenous biotin, the sections were incubated with a blocking agent (Biogenex life sciences Pvt. Ltd., CA, USA) for 15 min. Excess power block solution was drained and the sections were incubated with primary monoclonal antibody of CD34 (Biogenex life Sciences Pvt. Ltd., CA, USA) for 1 h and later thoroughly washed with citrate buffer. For further enhancement of the staining, the sections were then incubated with the antimouse secondary antibody (super enhancer) (Biogenex life sciences Pvt. Ltd., CA, USA) for 30 min followed by two consecutive buffer washes; each for 5 min. Horseradish peroxide (Biogenex life sciences Pvt. Ltd., CA, USA) was added to the sections and incubated for 30 min. The chromogen diaminobenzidine was prepared just prior to use by mixing one drop of chromogen to one ml of buffer in a mixing vial and later added over the sections. After 5 min, the sections were washed in buffer followed by water and counterstained with Harris hematoxylin, air dried, cleared and mounted with dibutylpthalate xylene. Pyogenic granuloma tissue was used as positive control.

Interpretation of staining

Blood vessels were counted using a Leica Research microscope with provision for photomicrograph (Model no DM 1000 LED, Germany). The stained sections were first screened at low power (×10) to determine the areas of most intense staining for CD34. Blood vessel counting was then performed under × 40 magnification. The area of each field was almost 0.2 mm^2. The blood vessel density was recorded as a mean ± standard deviation (SD). Those endothelial cells colored with brown CD34 (CD34-positive) that formed a cluster of endothelial cells with a lumen were considered as blood vessels. Single CD34-positive endothelial cells were also included in the count. Blood vessels with muscle wall were excluded. Three high-power fields (HPF) with the highest number of blood vessels (hot spots) were chosen. The representative areas were carefully scanned from left to right of every slide to avoid recounting of same areas. The endothelial cells for each case were the average number of blood vessels in these three chosen HPFs and expressed as the number of endothelial cells per HPF (endothelial cells/HPF) [Figures 1-3]. The mean of three values was calculated and expressed as mean ± SD. All IHC-stained slides along with the corresponding H and E slides were evaluated by three qualified observers to minimize the subjective bias.

Statistical methods

All the findings were compiled and analyzed for comparative analyses with the help of ANOVA test, Kolmogorov–Smirnov test and least significance difference (LSD) Test.

Data were presented using frequency, percentage and descriptive statistics such as mean, SD and standard error. Further analysis was done using one-way ANOVA followed by LSD test. $P \leq 0.05$ was considered to be statistically significant. IBM SPSS 20.0 (Chicago, IL, USA) software was used for analysis.

OBSERVATIONS AND RESULTS

The study comprised of 30 cases of which 12 cases were of SMA, 10 cases of UA and 8 cases were of desmoplastic ameloblastoma. They were analyzed for correlation of MVD using a monoclonal antibody against CD34 for visualizing the microvessel and calculating the MVD. Ten cases of pyogenic granuloma were used as a positive control.

Demographic results have been summarized in Table 1. In the present study, the CD34 positive microvessels in SMA and UA showed aberrant morphology, tortuous and dilated lumen near

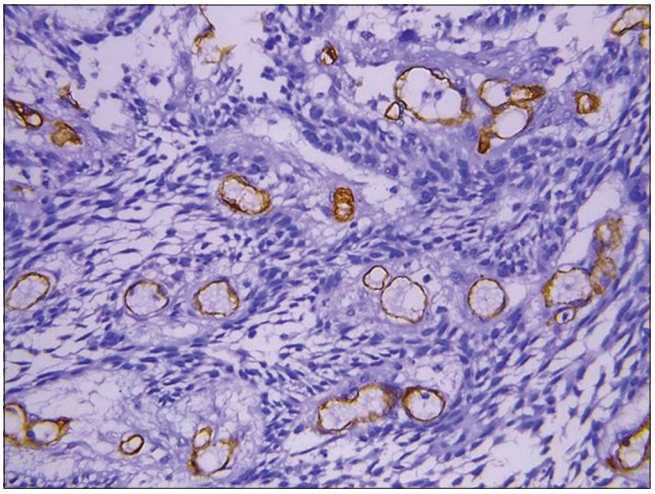


Figure 1: Photomicrograph showing CD34 positive endothelial cells in solid multicystic ameloblastoma (plexiform ameloblastoma) (IHC stain, ×400)

the odontogenic epithelium. Numerous clusters of endothelial cells were also observed in the stroma showing evidence of lumen formation. Results from the tests for normality of data showed that the data were found to be normally distributed at 5% level of significance ($P > 0.05$) [Table 2]. When a comparison of MVD in SMA, UA and desmoplastic ameloblastoma was done using the ANOVA test, the results indicated that there was a significant difference between the three groups ($P < 0.05$) [Table 3]. When multiple comparisons were done using the LSD test, the results indicated that there was a significant difference in the MVD of solid multicystic and desmoplastic; and unicystic and desmoplastic ameloblastomas ($P < 0.05$). Moreover, there was no significant difference in MVD of solid multicystic and UAs ($P > 0.05$) [Table 4].

DISCUSSION

Folkman's hypothesis stated that tumors secrete a diffusible substance that could stimulate endothelial cell proliferation in host capillary blood vessels. The development of new microvessels in tumors, i.e. angiogenesis, is a complex multistep process that involves extracellular matrix remodeling, migration and proliferation of endothelial cells and morphogenesis of new microvessels. Angiogenesis is often a significant, independent prognostic indicator for both the overall and disease-free survival.[15]

The rate of tumor progression is associated with increased MVD, a histomorphological measure of tumor angiogenesis. Measurement of MVD is a widely regarded predictor of tumor growth, metastasis and patient survival and it correlates with tumor aggressiveness.[6] The growth of tumor includes not only the increase in number of blood vessels but also a change of protein molecules in the composition of endothelial cells. Angiogenesis is one of the best known stromal factors participating in tumor progression.[15] This has been extensively investigated in various tumors such as breast carcinoma,[16] hepatocellular carcinoma,[17] astrocytoma,[18] cervical carcinoma[19] and ovarian carcinoma.[20]

Angiogenesis have also been studied in various oral lesions such as oral squamous cell carcinoma,[21] oral lichen planus,[22] oral submucous fibrosis,[11] odontogenic tumors[23] and mucoepidermoid carcinoma of salivary glands.[24]

Ameloblastoma is a tumor arising from odontogenic epithelial cells. It is slow-growing, locally invasive tumor that has an explicit biologic behavior with a high recurrence rate.[25,26] Various stromal factors such as growth and angiogenic factors, extracellular matrix components and proteinases play an important role for the invasion, growth and progression of these tumors. Several authors have shown elevated expression of MMP 2, MMP 9,[27,28] transforming growth factor β[29] fibronectin, tenascin[30] and stromal myofibroblasts[31] which are related to the aggressive behavior of ameloblastoma. This could indicate an increased metabolic activity in the connective tissue of ameloblastoma.

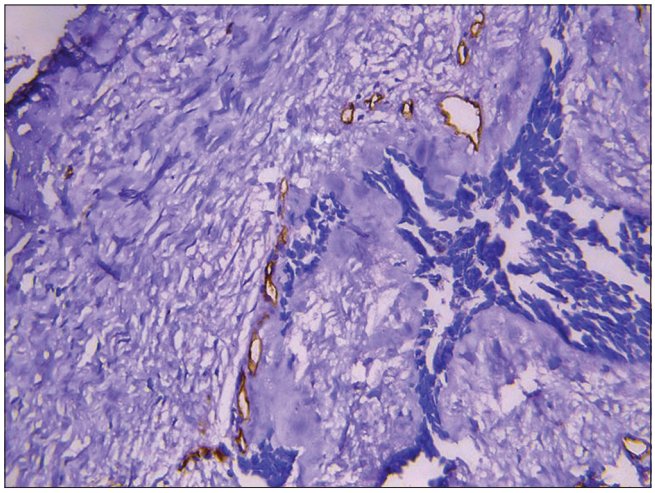


Figure 2: Photomicrograph of the section showing CD34 positive endothelial cells in desmoplastic ameloblastoma (IHC stain, × 400)

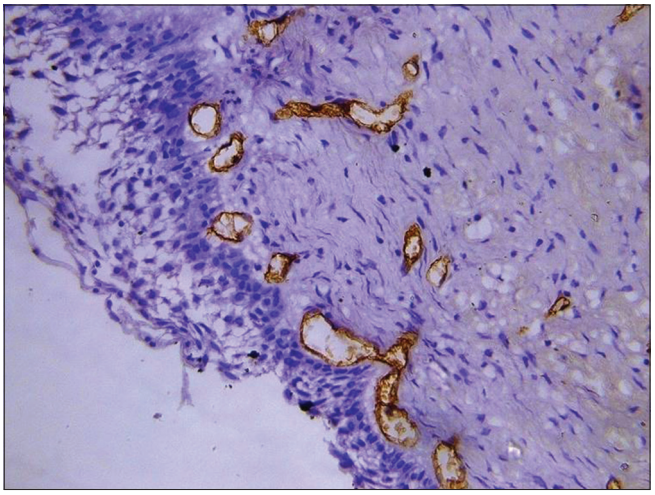


Figure 3: Photomicrograph of the section showing CD34 positive endothelial cells in unicystic ameloblastoma (IHC stain, × 400)

Table 1: Demographic data

Lesion	Number	Sex		Average	Jaw affected		Site	
		Male	Female		Mandible	Maxilla	Anterior	Posterior
Solid multicystic	12	10	2	32.3±18.7	11	1	10	2
Desmoplastic	8	4	4	32.5±4.8	8	0	6	2
Unicystic	10	6	4	29.1±15.3	9	1	7	3

Table 2: Test for normality of data

Test of normality: The normality of data was tested using Kolmogorov–Smirnov test						
	Kolmogorov–Smirnov			Shapiro–Wilk		
	Statistic	df	Significance	Statistic	df	Significance
Observer 1	0.138	30	0.153*	0.957	30	0.254
Observer 2	0.121	30	0.200*	0.980	30	0.815
Observer 3	0.082	30	0.200*	0.971	30	0.563
Overall average	0.098	30	0.200*	0.969	30	0.502

*:*P*>0.05. df: Degree of freedom

Table 3: Comparison of microvessel density in solid multicystic, desmoplastic and unicystic ameloblastoma using the analysis of variance test

	Sum of squares	df	Mean square	*F*	Significance
Observer 1					
Between groups	226.793	2	113.396	4.302	0.024*
Within groups	711.690	27	26.359		
Total	938.482	29			
Observer 2					
Between groups	173.633	2	86.817	3.752	0.036*
Within groups	624.705	27	23.137		
Total	798.338	29			
Observer 3					
Between groups	243.798	2	121.899	7.391	0.003*
Within groups	445.288	27	16.492		
Total	689.086	29			
Overall					
Between groups	213.575	2	106.788	5.326	0.011*
Within groups	541.371	27	20.051		
Total	754.947	29			

*statistically significant. df: Degree of freedom

Hence, we had undertaken this study to determine the immunohistochemical expression of CD34 for MVD in the three variants of ameloblastoma, i.e., SMA, UA and desmoplastic ameloblastoma.

In the present study, the CD34 positive microvessels in SMA and UA showed aberrant morphology, tortuous and dilated lumen near the odontogenic epithelium. Numerous clusters of endothelial cells were also observed in the stroma showing evidence of lumen formation.

The distribution of blood vessels across all the zones of an odontogenic lesion is not the same. Intratumoral MVD of multicystic ameloblastomas showed the highest rate of angiogenesis as compared to peritumoral MVD. Higher MVD in intratumoral areas suggests the activity of odontogenic epithelial cells inducing angiogenesis. It seems that accumulation of blood vessels around odontogenic epithelium in order to provide oxygen and nutritional substances is critically important for the growth of odontogenic cysts and tumors.[23]

Table 4: The pair-wise significance of difference in solid multicystic, desmoplastic and unicystic according to different observers tested using least significance difference test

Multiple comparisons					
Dependent variable	Mean difference	SE	*P*	95% CI	
				Lower bound	Upper bound
Observer 1					
Solid multicystic					
Desmoplastic	6.87375*	2.34338	0.007	2.0655	11.6820
Unicystic	2.74850	2.19829	0.222	−1.7620	7.2590
Desmoplastic					
Solid multicystic	−6.87375*	2.34338	0.007	−11.682	−2.0655
Unicystic	−4.12525	2.43531	0.102	−9.1221	0.8716
Unicystic					
Solid multicystic	−2.74850	2.19829	0.222	−7.2590	1.7620
Desmoplastic	4.12525	2.43531	0.046	−0.8716	9.1221
Observer 2					
Solid multicystic					
Desmoplastic	6.01417*	2.19551	0.011	1.5094	10.5190
Unicystic	2.35617	2.05957	0.263	−1.8697	6.5821
Desmoplastic					
Solid multicystic	−6.01417*	2.19551	0.011	−10.519	−1.5094
Unicystic	−3.65800	2.28164	0.121	−8.3395	1.0235
Unicystic					
Solid multicystic	−2.35617	2.05957	0.263	−6.5821	1.8697
Desmoplastic	3.65800	2.28164	0.049	−1.0235	8.3395
Observer 3					
Solid multicystic					
Desmoplastic	7.12583*	1.85361	0.001	3.3225	10.9291
Unicystic	2.94983	1.73884	0.101	−0.6180	6.5176
Desmoplastic					
Solid multicystic	−7.12583*	1.85361	0.001	−10.929	−3.3225
Unicystic	−4.17600*	1.92633	0.039	−8.1285	−0.2235
Unicystic					
Solid multicystic	−2.94983	1.73884	0.101	−6.5176	0.6180
Desmoplastic	4.17600*	1.92633	0.039	0.2235	8.1285
Overall average					
Solid multicystic					
Desmoplastic	6.67042*	2.04383	0.003	2.4768	10.8640
Unicystic	2.68567	1.91728	0.173	−1.2483	6.6196
Desmoplastic					
Solid multicystic	−6.67042*	2.04383	0.003	−10.864	−2.4768
Unicystic	−3.98475	2.12401	0.041	−8.3429	0.3734
Unicystic					
Solid multicystic	−2.68567	1.91728	0.173	−6.6196	1.2483
Desmoplastic	3.98475	2.12401	0.041	−0.3734	8.3429

*The mean difference is significant at the 0.05 level. SE: Standard error, CI: Confidence interval

Margaritescu *et al*. suggested that a number of blood vessels in peritumoral and intratumoral zones is the same and they do not believe that it can be used for prediction of aggressive behavior and recurrence.[32] However, the present study did not compare the intratumoral and peritumoral MVD of SMA, UA and desmoplastic ameloblastomas.

Ackermann *et al*.,[33] Reichart *et al*.[1] and Lau and Samman[34] studied the average age of presentation of ameloblastoma which was approximately 33–39 years. The results of the present study are also in accordance with the above studies. They also concluded that UA occurs more commonly among those aged 22–26 years.

In the present study, the mean age of patients with SMA, UA and desmoplastic ameloblastoma were 32.3, 29.1 and 32.5 years, respectively, indicating that all the three variants occur in the second and third decade of life.

In 2004, Koizumi *et al*. assessed angiogenesis in ameloblastoma using CD34 antibody based on age of patient and they found that plexiform type of ameloblastoma was seen in a younger age group, while follicular type of ameloblastoma was seen in older age groups which suggested that angiogenesis could affect the pattern of tumor growth in ameloblastoma, which could be additionally modulated by the patient's age.[35] In the present study, the three variants of ameloblastoma, i.e. SMA, UA and desmoplastic, were compared for age which showed that the three variants occur in the late second decade and early third decade of life [Graph 1].

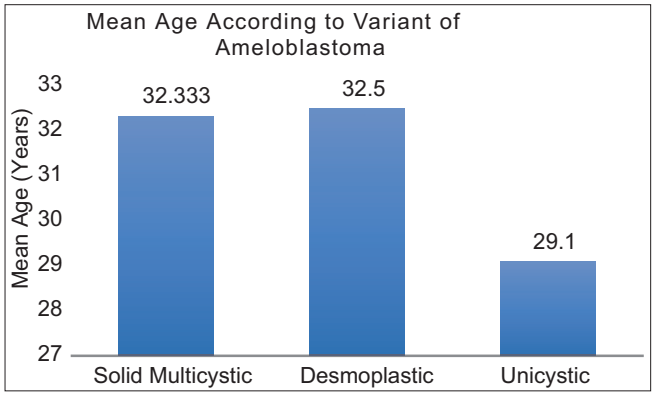
In the present study, males were more commonly affected than females (males - 66.67%, females - 33.33%). Among males, 33.3% had SMA, 13.33% had desmoplastic and 20% had UA. Among females, 6.67% had SMA, 13.33% had UA and 13.33% had desmoplastic ameloblastoma. However, Barnes *et al*. in their study in 2005[36] concluded that males and females are equally affected.

Mandible was the most commonly affected jaw seen in 93.33% (36.67% in SMA, 26.67% in desmoplastic and 30% in UA) of subjects, while maxilla was affected in 6.67% (3.33% each in SMA and UA, desmoplastic was not seen in maxilla) of subjects [Graph 2]. Posterior region of both jaws was affected the most in 76.67% while the anterior region was affected in 23.33%. These results show that posterior mandible is the most common site affected in all the three variants, i.e. SMA, UA and desmoplastic ameloblastoma. This was in line with the results from the study by Barnes *et al*.[36] and Riechart *et al*.[1]

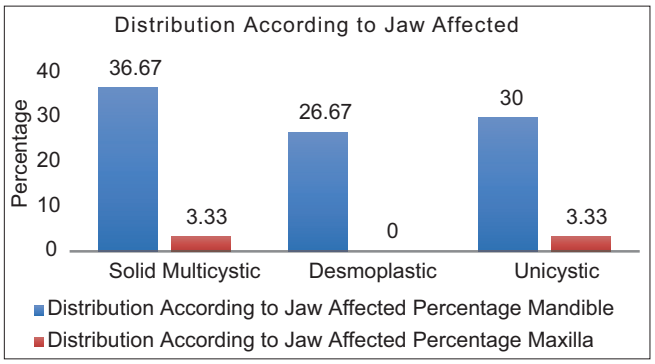
Results from the present study showed significantly increased MVD in SMA, UA and the desmoplastic variant of ameloblastoma. Moreover, on doing a pairwise comparison among them, there was an overall significant difference between SMA and desmoplastic ameloblastoma ($P = 0.003$) and between UA and desmoplastic ameloblastoma ($P = 0.041$). However, there was no significant difference between SMA and UA ($P = 0.173$) [Table 4]. This was in accordance with the study conducted by Hande *et al*.[15] who found no significant difference in MVD, total vascular area and mean vascular area between SMA and UA. This may reflect the fact that though clinical behavior, histopathological presentation and prognosis of SMA and UA differ, the process of angiogenesis is not different. This suggests that the angiogenesis has an important role in tumor progression and aggressiveness of ameloblastoma.

Thus, there is increased angiogenesis in SMA followed by UA and desmoplastic ameloblastoma suggesting a more aggressive behavior for SMA and a higher incidence of recurrence.

Alaeddini *et al*. evaluated and compared angiogenesis in keratocystic odontogenic tumors, dentigerous cysts and ameloblastomas using a monoclonal antibody against CD34 and found a statistically significant difference in the mean MVD. It was suggested that angiogenesis may be one of the mechanisms possibly contributing to the different biological behaviors of keratocystic odontogenic tumors, dentigerous cysts and solid ameloblastomas.[37] Kumamoto *et al*.[8] observed that increased MVD was associated with the elevated expression of VEGF in benign and malignant ameloblastomas as compared to tooth germs. They suggested that role of VEGF was associated with neoplastic changes,



Graph 1: The mean distribution of subject group according to age



Graph 2: The distribution of subject group according to the jaw affected

malignant transformation or both. In 2011, Seifi *et al*. studied MVD in follicular cysts, keratocystic odontogenic tumors and ameloblastomas using CD34 antibody. Their results showed that there was an increase in mean MVD in multicystic ameloblastoma as compared to the keratocystic odontogenic tumor and follicular cyst. Thus, they concluded that angiogenesis is one of the main factors responsible for aggressive behavior of multicystic ameloblastoma.[23] The results of the present study also showed highest MVD in SMA followed by UA and then desmoplastic ameloblastomas. Thus, it can be concluded that SMA has a greater aggressive behavior as compared to UA and desmoplastic ameloblastoma.

In 2012, Guzmán-Medrano *et al*. used CD68 and CD34 antibodies to evaluate the density of macrophages and microvessels associated with ameloblastomas. In SMA, they observed significantly higher densities of both macrophages and microvessels than in UA and desmoplastic ameloblastomas. Likewise, higher densities of macrophages and microvessels were found in UA than in desmoplastic ameloblastoma. Results suggested that these two tumor microenvironmental elements could have an important role during ameloblastoma progression.[38] The results from the present study are also in accordance with the above results showing higher MVD in SMA followed by UA and desmoplastic variant.

Jamshidi *et al*. evaluated the expression of CD34 and CD105 in ameloblastoma and odontogenic keratocyst. They found that MVD was significantly higher in ameloblastomas than odontogenic keratocyst and MVD with CD34 was significantly higher than MVD with CD105 in ameloblastomas. They suggested that angiogenesis might be one of the mechanisms that would possibly contribute to the aggressive biological behavior in ameloblastoma rather than odontogenic keratocyst.[26]

Angiogenesis is of prognostic significance and MVD is a useful marker to identify those patients with a more aggressive tumor, for whom a better therapeutic approach should be considered. Thus, results from the present study conclude that there is increased angiogenesis in SMA followed by UA and desmoplastic ameloblastoma suggesting a more aggressive behavior for SMA and a higher incidence of recurrence.

The role of angiogenesis and its effect on the behavior of the lesions is also significant. Histomorphometry method can be used to study and measure the vessel area and diameter of vessels to evaluate the role of vessel area affecting the aggressive behavior of the lesion. This method is used by Hande *et al*.[15] in their study. The present study did not assess the measurements of the vascular area and its role in aggressive behavior of the lesion.

Identification of proliferating and invasive activities in tumors may be useful to predict their biological behavior. It has significant implications in treatment decisions and the incidence of recurrence.

CONCLUSION

The present study has provided us with valuable information regarding the role of angiogenesis in the three variants of ameloblastoma, i.e., SMA, UA and desmoplastic ameloblastoma and also the role of CD34 as a marker for estimating the MVD for assessing angiogenesis.

The study results show that all the three variants occurred at a young age, males being commonly affected than females and the mandibular posterior region was the most commonly affected site.

In the present study, a significant correlation was obtained between the MVD of all the three variants, i.e. SMA, UA and desmoplastic ameloblastoma. In addition, on doing the pairwise comparison among the three variants, a significant correlation was obtained between SMA and desmoplastic ameloblastoma and UA and desmoplastic ameloblastoma. However, no significant correlation was obtained between SMA and UA. This may reflect the fact that though clinical behavior, histopathological presentation and prognosis of SMA and UA differ, the process of angiogenesis is not different.

Thus, increased MVD in the three variants, i.e. SMA, UA and desmoplastic ameloblastoma seen in the present study could suggest that the angiogenesis has an important role in tumor progression and aggressiveness of ameloblastoma though neither as a strong arbiter nor as a distinct diagnostic test. Thus, the present study forms a nidus for further research to be conducted to affirm the absolute utility of angiogenesis in ameloblastomas using CD34 as an immunohistochemical marker. Above all, studies including the follow-up of patients are necessary; to understand the true value of the MVD as a prognostic parameter in ameloblastomas.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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