ORIGINAL RESEARCH

Comparison of CD105 (Endoglin) Expression in Odontogenic Keratocyst and Dentigerous Cyst - An Immunohistochemistry Study

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Abstract

Introduction: Tumors like Odontogenic Keratocyst (OKC), Dentigerous Cyst (DC)and Pyogenic Granuloma are frequently occurring in the oral cavity with each of them having relation to angiogenesis. Higher angiogenesis may be associated with increased tissue metabolism, more aggressive biologic behaviour, and increased recurrence and growth rate. Tumor growth is dependent not only on a rise in the number of blood vessels, but also on factors such as protein molecules produced in endothelial cells. Microvessel density (MVD), Microvessel area (MVA), Microvessel perimeter (MVP) can predict the growth of the tumour, metastasis and patient's survival and this value is related to the aggressiveness of the tumour.

Aims: The aim of the present study was to determine the angiogenic potential of OKC and DCcompared with normal mucosa using CD 105 marker immunohistochemically.

Materials and methods: Immunohistochemical staining was done on 70 paraffin embedded tissue samples. Histopathologically diagnosed cases of OKC, DC and Pyogenic granuloma and healthy gingival tissue samples were retrieved for the study purpose.

Results: There was no statistically significant difference in the mean MVD, MVA, MVP values of OKC, DC and pyogenic granuloma groups.

Conclusion: The angiogenic potential was determined in 3 different cases of OKC, Dentigerous Cyst and Pyogenic granuloma in terms of MVD, MVA and MVP and compared to normal mucosa using CD105 marker immunohistochemically.Though the mean values of MVA, MVD, MVP were statistically not significant but was estimated to be higher than the normal mucosa.

Keywords: Angiogenesis, CD105, Dentigerous cyst, Mean Vessel Density, Odontogenic Keratocyst.

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Introduction

Kysitis, a Greek word from which the term cyst was derived simply means a sac or a bladder. Cysts are more common in the head and neck region explicitly in the jaws. The formation of cyst can be attributed to the opulent number of epithelial remnants which are left inside the bones of the jaws. The embryologic process wherein the ectodermal tissues are trapped along the line of fusion, which later undergoes atrophy is credited to be forming a cyst.[1] These have 2 origins which are either from the tissues that develop into teeth, namely odontogenic cyst and the other termed as non-odontogenic cyst. Among the other cysts that arise in the jaw, Odontogenic Keratocyst (OKC) has been ascribed to be the third most common because of its high recurrence rate (38%) and also their antero posterior growth pattern which extends to the medullary spaces with Radicular and Follicular cysts occupying the first and second place respectively. OKC as defined by WHO (1971) is, "a benign uni- or multicystic, intraosseous tumour of odontogenic origin, with a characteristic lining of parakeratinized stratified squamous epithelium and potential for aggressive, infiltrative behaviour". Even though OKC was reclassified and renamed by WHO (2005)

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as Keratocystic Odontogenic Tumor due to its neoplastic nature, it gained its pioneer name as OKC in the classification proposed by WHO of Head and Neck Pathology (2017). Dentigerous Cyst (DC) on the other hand, is a developmental cyst with fluid accumulation between the reduced enamel epithelium and an unerupted crown of a tooth preferably a third molar. With an equal sex predilection, it is mostly seen among first to third decade of life. Being a cyst commonly seen in the mandible than in maxilla, most authors favor the development of this cyst from the tooth follicle.

Firstly, interpreted by Philipsen in 1956, the histological characteristics were described by Pindborg and Hansen in 1963[23]. The incidence of OKC has been observed at any age with peak incidence in the 2nd and 4th decade of life, and with a slight male predilection. Also, the mandible constitutes for the formation of cyst two times more than the maxilla. According to Toiler, after impeded eruption the breakdown of proliferating cells is the main reason for the development of Dentigerous cyst[4] whereas in 1928, Bloch-Jorgensen was the researcher who propounded that the overlying necrotic primary tooth is the origin for the development of cyst[5].

The molecular basis of such cysts has to be evaluated and compared with others in order to prevent its malignant transformation. The connective tissue stroma hosts various epithelial alterations such as angiogenesis[5,6]. Parameters such as Microvessel density (MVD), Microvessel area (MVA), Microvessel perimeter (MVP) are measured with respect to its normal gingiva counterpart aside from the Endoglein CD105 levels. The current review focussed upon these issues to add to the writing proof for a superior pathologic comprehension of the lesions and extra separation of the equivalent.

The present study was aimed to determine the angiogenic potential of OKC and DC, as compared with normal oral mucosa using CD 105 marker immunohistochemically. The objectives were to evaluate the expression and also to study parameters like MVD, MVA, MVP in OKC and DC using CD105 marker immunohistochemically.

Table 1: Distribution of the samples across the 4 different groups based upon their mean scores and standard deviation for MVD, MVA and MVP-

	Group 1 (OKC)	Group 2 (DC)	Group 3 (PG)	Group 4 (control)
MVD	13.25±2.94	12.87±2.4	13.02±1.07	8.01± 4.01
MVA	113.89±24.89	115.5±24.6	113.5±18.7	54.7±21.03
MVP	50.7±12.45	49.43±14.56	49.3±12.46	32.04±5.07

Table 2: Comparison of MVD mean values between the 4 groups (Mann Whitney U test)-

MVD		
Groups	P value	Interpretation
Group 1 Vs Group 2	0.76	No significant difference
Group 1 Vs Group 3	0.87	No significant difference
Group 1 Vs Group 4	0.004	Statistically significant difference
Group 2 Vs Group 3	0.56	No significant difference
Group 2 Vs Group 4	0.00	Statistically significant difference
Group 3 Vs Group 4	0.0032	Statistically significant difference

Materials and Methods

Ethical clearance was obtained from the Institutional Ethics Committee. The study was conducted in the department of Oral and Maxillofacial Pathology of D.Y Patil Deemed to be University, School of Dentistry, Nerul, Navi Mumbai. 70 paraffin embedded tissue samples were retrieved for the study purpose. Of the 60, group 1 comprised 25 samples of OKC, group 2 comprised 25 samples of DC, group 3 comprised 10 samples of pyogenic granuloma and group 4 comprised 10 samples of healthy gingiva. The inclusion criteria were histopathologically diagnosed cases of OKC, DC and Pyogenic granuloma and healthy gingival tissue samples.Medically compromised patients like hypertension, diabetes or Cancer were excluded from this study.

Tissue harvest and formalin fixation was done, followed by tissue processing and paraffin embedding. The paraffin embedded tissue blocks were cut into sections of 3-4 µ thickness and taken on positively charged poly-L-lysine coated slides. The slide was warmed on a hot plate at 600C for 1 hour prior to staining. The slides were then deparaffinized, using 3 changes of xylene. Then the slides were hydrated using 3 changes of decreasing grades of alcohol ie. 95%,

Table 3: Comparison of MVA mean values between the 4 groups (Mann Whitney U test)-

MVA		
Groups	P value	Interpretation
Group 1 Vs Group 2	0.57	No statistically significant difference
Group 1 Vs Group 3	0.67	No statistically significant difference
Group 1 Vs Group 4	0.001	Statistically significant difference
Group 2 Vs Group 3	0.46	No statistically significant difference
Group 2 Vs Group 4	0.001	Statistically significant difference
Group 3 Vs Group 4	0.001	Statistically significant difference

Table 4: Comparison of MVP mean values between the 4 groups (MannWhitney U test)-

MVP		
Groups	P value	Interpretation
Group 1 Vs Group 2	0.068	No statistically significant difference
Group 1 Vs Group 3	0.843	No statistically significant difference
Group 1 Vs Group 4	0.004	Statistically significant difference
Group 2 Vs Group 3	0.08	No statistically significant difference
Group 2 Vs Group 4	0.003	Statistically significant difference
Group 3 Vs Group 4	0.002	Statistically significant difference

80%and 60% and distilled water. Slides were washed in running tap water for 4-5 minutes. Then they were placed in the decloaking chamber in a container containing Tris -EDTA as an antigen retrieval solution.Antigen retrieval program was selected and the cycle was started. After the completion of this cycle the slide container was removed from the decloaking chamber and allowed to cool down for 20 minutes. Then it was washed in 2-3 chambers of distilled water for 5 minutes each. The slides were marked with PAP pen. After that it was washed with a TBS wash buffer for 5mins. The slides were incubated with Horseradish peroxidase block for 10 minutes. After that again the slides were washed in 2 changes of TBS wash buffer 5 minutes each. The slides were arranged in the humidity chamber. The protein block was poured on the slides and kept for 5 minutes at room temperature. Slides were again washed with TBS wash buffer for 5 minutes. 1-2 drops of the primary antibody, CD 105-EP 274-Rabbit monoclonal PR 188 antibody, was poured on each slide for 30-60 minutes and incubated at room temperature. Again, the slides were washed with a TBS buffer for 5 minutes each. Diluted Betazoid DAB chromogen in DAB substrate buffer was poured on the slides and kept for 1-3 minutes at room temperature. The slides were checked under the microscope to confirm color development in the test and control slides. The slides were rinsed with distilled water. It was later counter stained with haematoxylin for 20-30 seconds and washed with distilled water. The slides were later dipped in a TBS wash buffer for bluing, for approximately 5 minutes. The slides were dehydrated, cleared and mounted using DPX and labelled accordingly.

Quantification of MVD, MVA, MVP-Two investigators determined MVD, MVA, and MVP in each slide independently. In brief, the microvessels were highlighted by immunostaining with CD105 monoclonal antibodies. Any single brown-stained cell or cluster of endothelial cells that were clearly separated from adjacent microvessels, histiocytes, was counted as a single vessel unless there was a discontinuity in the structure. Slides were screened at 40X magnification on a research microscope and 3 areas with the highest number of stained microvessels were identified ("hot spots"). Then, the MVA, MVD, MVP of the blood vessels were counted in the 3 spot areas using LES-4 software in a Leica research microscope. Microvessel density was expressed as the mean number of vessels in these areas. Microvessel area was expressed as the mean vessel areas in µm2. Microvessel perimeter was expressed as the mean vessel perimeter in µm.

Results

The present study is a cross sectional study of archived samples comprising of 4 groups; group 1- 25 samples of OKC, group 2- 25 samples of DC, group 3- 10 samples of pyogenic granuloma and group 4- 10 samples of healthy gingiva. The data was coded and entered in Microsoft Excel 2007. SPSS (Statistical Package for Social Studies) 24.0 (IBM, Analytics, New York, U.S.A) was used to carry out the statistical analysis. All p values less than 0.05 was considered to be statistically significant. Since the data was not normally distributed, Mann Whitney U test was performed. The mean MVD of the OKC group was 13.25±2.94, of the DC group was 12.87±2.4, of the pyogenic granuloma group was 13.02±1.07 and of the normal gingiva group was 8.01±4.01. The mean MVA of the OKC group was 113.89±24.89, of the DC group was 115.5±24.6, of the pyogenic granuloma group was 113.5±18.7 and of the normal gingiva group was 54.7±21.03. The mean MVP of the OKC group was 50.7±12.45, of the DC group was 49.43±14.56, of the pyogenic granuloma group was 49.3±12.46 and of the normal gingiva group was 32.04±5.07 (Table 01). There was no statistically significant difference in the mean MVD values of OKC, DC and pyogenic granuloma groups. The mean values of OKC, DC and pyogenic granuloma were significantly higher than that of the healthy gingiva ($p< 0.05$) (Table 02).There was no statistically significant difference between the mean MVA values of OKC, DC and pyogenic granuloma groups. The mean values of OKC, DC and pyogenic granuloma was significantly higher than that of the healthy gingiva ($p< 0.05$) (Table 03). There was no statistically significant difference in the mean MVP values of OKC, DC and pyogenic granuloma groups. The mean values of OKC, DC and pyogenic granuloma were significantly higher than that of the healthy gingiva ($p< 0.05$) (Table 04).

Discussion

Tumorigenesis is marked by invasion and metastasis of tumors with an increase in myofibroblasts (MF), blood vessels and inflammatory cells in the tumor stromaregulated by various molecules such as CD31, CD34, Factor VIII and CD105 (also known as pan-endothelial markers) which are expressed differentially in angiogenic and normal vessel endothelial cells as propounded by Legan[7,8]. OKCs and DCs, though cystic lesions, exhibit invasiveness into the connective tissue as some suggested unknown factor consolidated in the epithelium might be responsible for the same. For the survival of the neoplastic tissues, nutrition is essential which happens by recruiting new microvessels as suggested by Folkman[9].

In the present study, the mean MVD was highest in OKC followed by DC and pyogenic granuloma. The mean MVD in the present study for OKC was 13.25±2.94 apparently due to the level of inflammation present while collecting the tissue samples in the present study that raised the MVD mean values. This was much higher than that reported by Kumar DV et al, where the mean MVD was 6.25± 2.88 and in Jamshidi et al where the values were 9.6±2.9[10]. Another study by Sefi et al also reported that mean MVD in keratocystic odontogenic tumors was higher than follicular cyst ($p<0.001$) but it was much lower when compared to that of the Ameloblastoma[11]. The mean MVD value for DC in the present study was 12.87±2.4 while it was 3.75±1.72 in the study by Kumar DV et al. Also, the value for PG was 13.02±1.07, which was lower than the value reported by Kumar DV et al.which was 15.02±0.64 and Vasconcelos MG et al who reported a value of 20.22[10,12]. The Vasconcelos MG et al study reported that the mean MVD values were significantly higher with OKC as compared to DC, which was in contrast to our results[12].

We observed that the mean MVA was higher with DC than with OKC and pyogenic granuloma. But there was no statistically significant difference. This was in complete contrast to the findings of another Indian study where OKC values were significantly higher than that of DC and normal mucosa[13]. We also observed that there was no significant difference between MVP of the 3 groups but was higher as compared to the normal mucosa. Since tissues require oxygen and nutrients to continue their growth and development, they induce neovascularization. On the other hand, angiogenesis is not only necessary for the growth of the tumor, it is also necessary for cellular metastasis[14–16].

Conclusion

The present study was a retrospective analysis of the tissue samples carried out with the aim of determining the angiogenic potential of OKC, DC as well as pyogenic granuloma when compared

with normal mucosa, using CD 105 marker immunohistochemically. MVD, MVA and MVP were used as the parameters. There was no statistically significant difference in the mean MVD, MVP and MVA values of OKC, DC and pyogenic granuloma groups but it was definitely higher than that of the normal mucosa. MVD, MVP and MVA can predict the growth of the tumor, metastasis and patient's survival and this value is related to the aggressiveness of the tumor. CD105 expression is a prominent feature of newly formed blood vessels and could reflect as the primary indicator for an increased potency towards recurrence and further expansion among the odontogenic cysts.

Nevertheless, these parameters cannot be considered as the gold standard for a definitive conclusion since a mere inflammation also can raise up CD105 counts. Hence carefully coordinated and systematic evaluation of the lesion and its correlation both clinically and histopathologically should go hand in hand for a better treatment plan. We were able to better understand the expression of CD105 with the different conditions when compared to normal mucosa. But the study had certain limitations-

1. Most of the literature reports the use of other markers like CD34, which was not assessed in the present study.

2. The level of inflammation was not compared. There is definitely a chance of more inflamed tissue specimens in the sample that gave higher mean values compared to the other previous literature evidence.

3. Factors contributing to inflammation and angiogenesis were not considered into the study.

References

1. Vij H, Vij R, Gupta V, Sengupta S. Odontogenic keratocyst: a peripheral variant. Niger J Clin Pract. 2011;14(4):504–7.
2. Gaitán-Cepeda LA, Quezada-Rivera D, Tenorio-Rocha F, Leyva-Huerta ER. Reclassification of odontogenic keratocyst as tumour. Impact on the odontogenic tumours prevalence [Internet]. Vol. 16, Oral Diseases. 2010. p. 185–7. Available from: http://dx.doi.org/10.1111/j.1601-0825.2009.01625.x
3. Pindborg JJ, Hansen J. Studies on odontogenic cyst epithelium. 2. Clinical and roentgenologic aspects of odontogenic keratocysts. Acta Pathol Microbiol Scand. 1963; 58:283–94.
4. Toller P. Origin and growth of cysts of the jaws. Ann R Coll Surg Engl. 1967;40(5):306–36.
5. Gillette R, Weinmann JP. Extrafollicular stages in dentigerous cyst development. Oral Surg Oral Med Oral Pathol. 1958;11(6):638–45.
6. Khot K, Deshmukh SB, Alex S. Comparative analysis of the immunohistochemical expression of vascular endothelial growth factor and matrix metalloproteinase-9 in keratocystic odontogenic tumor, dentigerous cyst and radicular cyst. J Cancer Res Ther. 2015;11(3):635–40.
7. Raouâa B, Samèh S, Dorsaf T, Rahma A, Jamil S. Recurrence challenge in odontogenic keratocyst variants, two clinical cases [Internet]. MédecineBuccaleChirurgieBuccale. 2014;20: 203–8. DOI:10.1051/mbcb/2014008
8. Zerrin E, Peruze C, Husniye D. Dentigerous cysts of the jaws: Clinical and radiological findings of 18 cases [Internet]. Vol. 2, Journal of Oral and Maxillofacial Radiology. 2014. p. 77. Available from: http://dx.doi.org/10.4103/2321-3841.144673
9. Mohan KR, Natarajan B, Mani S, Sahuthullah YA, Kannan AV, Doraiswamy H. An infected dentigerous cyst associated with an impacted permanent maxillary canine, inverted mesiodens and impacted supernumerary teeth. J Pharm BioalliedSci. 2013;5(Suppl 2):S135–8.
10. Kumar DV, Hemavathy S, Kulkarni D, Rudraiah PM, SidramayyaMathpati SK, Priya S. Expression of CD105 in tumor angiogenesis a comparative study (ameloblastoma, keratocystic odontogenic tumor and dentigerous cyst). J Int Oral Health. 2015;7(6):23–7.
11. Seifi S, Shafaie S, Ghadiri S. Microvessel density in follicular cysts, keratocystic odontogenic tumours and ameloblastomas. Asian Pac J Cancer Prev. 2011;12(2):351–6.
12. Vasconcelos MG, Alves PM, Vasconcelos RG, da Silveira ÉJD, Medeiros AMC, de Queiroz LMG. Expression of CD34 and CD105 as markers for angiogenesis in oral vascular malformations and pyogenic granulomas. Eur Arch Otorhinolaryngol. 2011;268(8):1213–7.
13. Miyata Y, Sagara Y, Watanabe S-I, Asai A, Matsuo T, Ohba K, et al. CD105 is a more appropriate marker for evaluating angiogenesis in urothelial cancer of the upper urinary tract than CD31 or CD34 [Internet]. VirchowsArchiv. 2013; 463:673–9. DOI:10.1007/s00428-013-1463-8
14. Acharya S, Goyal A, Rattan V, Vaiphei K, Kaur Bhatia S. Dentigerous cyst or adenomatoid odontogenic tumor: clinical radiological and histopathological dilemma. Case Rep Med. 2014; 514-720.
15. Gupta P, Jawanda M, Narula R, Singh J. Inflammatory dentigerous cyst mimicking a periapical cyst [Internet]. Journal of the International Clinical Dental Research Organization. 2016;8: 63. DOI:10.4103/2231-0754.176258
16. Khajuria N, Metgud R, Naik S, Lerra S, Tiwari P, Mamta, et al. Immunohistochemical expression of vascular endothelial growth factor in keratocystic odontogenic tumor, dentigerous cyst, and radicular cyst: A comparative study. Indian J Dent Res. 2016;7(1):17–22.