Original Article

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Dentistry Section

Comparison of Myofibroblasts Between Solid/Multicystic Ameloblastoma and Unicystic Ameloblastoma: An Immunohistochemical Analysis

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ABSTRACT

Introduction: Microenvironment is crucial for the maintenance of cellular functions and tissue integrity suggesting that cancer-induced changes in the stroma may contribute to cancer invasion and its biological behaviour. One of the major constituent of the tumour stroma is myofibroblasts. Myofibroblasts are differentiated host fibroblasts that express α-Sma as cytoplasmic microfilaments. They are considered as one of the modified stromal component which in recent years have been thought to have a role in the invasion and aggressive behaviour of odontogenic tumours too.

Aim: To detect immunohistochemically the presence of myofibroblasts in solid/multicystic ameloblastoma and in unicystic ameloblastoma and to see if a relationship exists between the frequency and pattern of distribution of myofibroblasts and the behaviour of ameloblastomas.

Materials and Methods: Ten cases each of solid/multicystic ameloblastoma and unicystic ameloblastoma were stained immunohistochemically for vimentin, α-SMA and desmin. The frequency and pattern of distribution of myofibroblasts in the two study groups were analysed and then compared with clinical and radiographic features of pain and cortical perforation respectively.

Results: Immunohistochemical reaction for α-SMA (alpha Smooth Muscle Actin) showed positive cells in the stroma of both solid/multicystic and unicystic ameloblastomas. The mean number of myofibroblasts was more in unicystic ameloblastoma (UA) compared to Solid/Multicystic Ameloblastoma (SMA). Myofibroblasts expression was dense and arranged in the form of fascicles with indistinct cell borders in one case of follicular ameloblastoma, two cases of plexiform ameloblastoma and in a focal area of one case of type 1UA. In all other cases where the expression was noted, the myofibroblasts were spindle in shape with distinct cell boundaries.

Conclusion: The results of the study indicate that myofibroblasts alone may not play a role in the behaviour of ameloblastomas. This calls for determining the role of various other stromal components in the biological behaviour of ameloblastomas. Our study could not establish a correlation between pattern of distribution of myofibroblasts and the behaviour of ameloblastomas.

Keywords: Biological behaviour, Cortical perforation, Pain, Stromal patterns

INTRODUCTION

Odontogenic tumours constitute a group of heterogeneous lesions that range from hamartomatous or non-neoplastic tissue proliferations to malignant neoplasms [1].

Ameloblastoma is the second most frequent type of odontogenic tumour [2]. Based on their clinical behaviour and prognosis, four types of ameloblastomas can be distinguished: Solid/Multicystic Ameloblastoma (SMA), Unicystic Ameloblastoma (UA), peripheral ameloblastoma and desmoplastic ameloblastoma [3]. It is generally believed that solid ameloblastomas show a locally invasive and infiltrative behaviour with frequent recurrence, whereas the unicystic type has a more favourable prognosis [2]. However, UA with mural proliferation is usually considered to have similar biological behaviour as SMA [4]. Due to this, confusion still exists in planning the appropriate treatment for UA's.

Attempts have been made to assess tumour behaviour, by employing immunocytochemical markers of cell activity like PCNA and Ki-67 [5]. Alterations in the stromal compartment resulting from neoplastic changes in the adjacent epithelium have also been seen. This includes, among other phenomenon, the appearance of myofibroblasts (MF) [6]. Earlier it was thought that MF had a protective role against tumour development but recently there is growing evidence that their presence at tumour front are not part of the host defence mechanism but is actually in promoting tumour development [6]. Hence it will be interesting to know whether the stroma of ameloblastomas is modified by MF thus contributing to their invasiveness and aggressiveness.

The presence of MF in odontogenic tumours however has not been thoroughly investigated and till date there is no myofibroblast specific immunocytochemical marker. The characterization of tumour-associated MF is based on a combination of positive markers such as actin isoforms specialized in cellular contraction such as α-SMA; the intermediate filaments vimentin and occasionally desmin [7].

AIM

This study was an attempt to assess and compare immunohistochemically the presence and pattern of distribution of MF in SMA and UA and to correlate it with their behaviour.

MATERIALS AND METHODS

This study involved the use of formalin fixed, paraffin embedded tissues of histopathologically diagnosed cases of solid/multicystic ameloblastomas and unicystic ameloblastoma between September 2003- February 2012 retrieved from the archives of Department of Oral and Maxillofacial Pathology, The Oxford Dental College, Hospital and Research Centre, Bangalore. Cases which fulfilled Vickers and Gorlin (V&G) criteria [8] and those devoid of considerable inflammation were selected as it has been suggested in a study by Mashhadiabbas F et al., that inflammatory infiltrate may stop MF differentiation. Cases not fulfilling V&G criteria

Serial no.	Variant	Clinical feature-Pain	Radiographic feature-Perforation
1	Plexiform	No (group 1a)	Yes (group 1)
2	Plexiform	No (group 1a)	Yes (group 1)
3	Follicular+cystic degeneration	No (group 1a)	Yes (group 1)
4	Plexiform	Yes (group 1)	No (group 1a)
5	Plexiform	Yes (group 1)	No (group 1a)
6	Follicular+cystic degeneration	Yes (group 1)	No (group 1a)
7	Granular	No (group 1a)	No (group 1a)
8	Follicular+cystic degeneration	Yes (group 1)	No (group 1a)
9	Plexiform	Yes (group 1)	No (group 1a)
10	Plexiform	Yes (group 1)	No (group 1a)

[Table/Fig-1]: Cases diagnosed histopathologically as SMA- group I.
Group 1—cases showing pain and perforation
Group 1a—cases showing no pain and no perforation

Serial no.	Variant	Clinical feature-Pain	Radiographic feature-Perforation
1	Type III	Yes (group 2)	No (group 2a)
2	Type III	No (group 2a)	No (group 2a)
3	Type III	Yes (group 2)	No (group 2a)
4	Type III	No (group 2a)	Yes (group 2)
5	Type III	Yes (group 2)	No (group 2a)
6	Type II	No (group 2a)	No (group 2a)
7	Type II	Yes (group 2)	No (group 2a)
8	Type II	No (group 2a)	No (group 2a)
9	Type I	No (group 2a)	No (group 2a)
10	Type I	No (group 2a)	No (group 2a)

[Table/Fig-2]: Cases diagnosed histopathologically as UA- group 2.
Group 2—cases showing pain and perforation
Group 2a---cases showing no pain and no perforation

and those which had considerable inflammatory infiltrate were excluded [9].

A total of 20 cases were evaluated histopathologically and immunohistochemically for alpha Smooth Muscle Actin (α-SMA), vimentin and desmin markers (BIOGENEX, USA).These included10 cases each of Solid /multicystic ameloblastomas (group 1—Six plexiform, three follicular and one granular cell) and unicystic Ameloblastoma (group 2—five type 3, three type 2 and two type1). Five cases of moderately differentiated oral squamous cell carcinoma served as control. Sections of each case were stained with Haematoxylin and Eosin stain and also for immunohistochemical staining. The clinical and histopathological details of the samples are given in [Table/Fig-1,2]. The immunohistochemical procedure followed here was based on instructions provided by the manufacturer (BIOGENEX, USA).

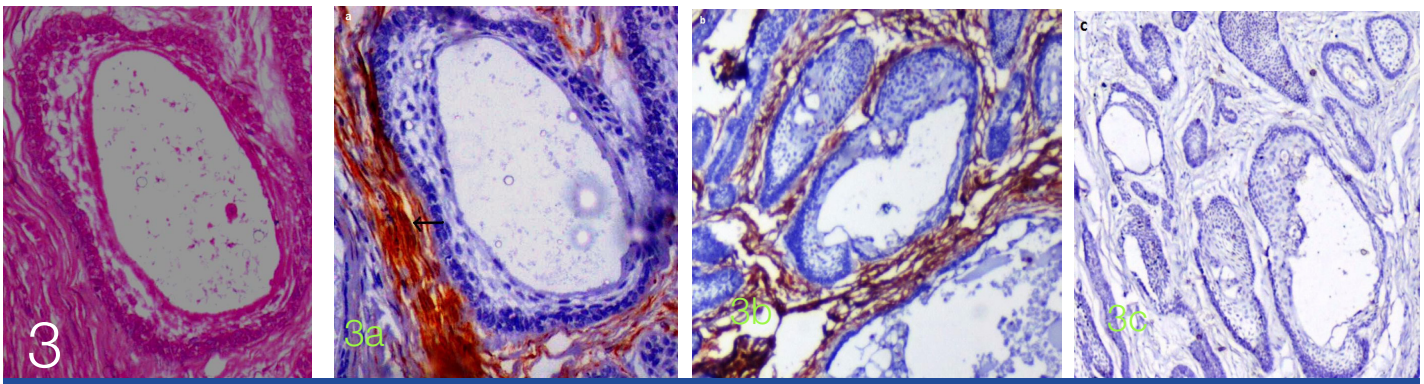
One section each of 5μm was cut for H&E staining and three sections each of 3μm were cut for immunohistochemical staining with vimentin, desmin and alpha smooth muscle actin using a microtome (LEICA RM 2145). For immunohistochemical staining, the 3μm cut sections were mounted onto silane coated slides and incubated overnight in an incubator (AVILAB) at 55-60^0 Celsius, one day before the staining. Following day the slides were dewaxed, hydrated in graded alcohol and rinsed in distilled water.

These hydrated sections were then blocked for endogenous activity by incubating in 3% hydrogen peroxide solution for 5-10mins and then rinsed in Tris Buffer Solution (TBS). The sections were later immersed in citrate buffer solution (pH-6) in a microwave oven for three cycles for antigen retrieval. The first two cycles were done at 800W for six & four minutes respectively and the third cycle was done at 200W for 14 minutes. The sections were allowed to cool back to room temperature. The sections were then washed in TBS and again incubated with protein block to eliminate background staining.

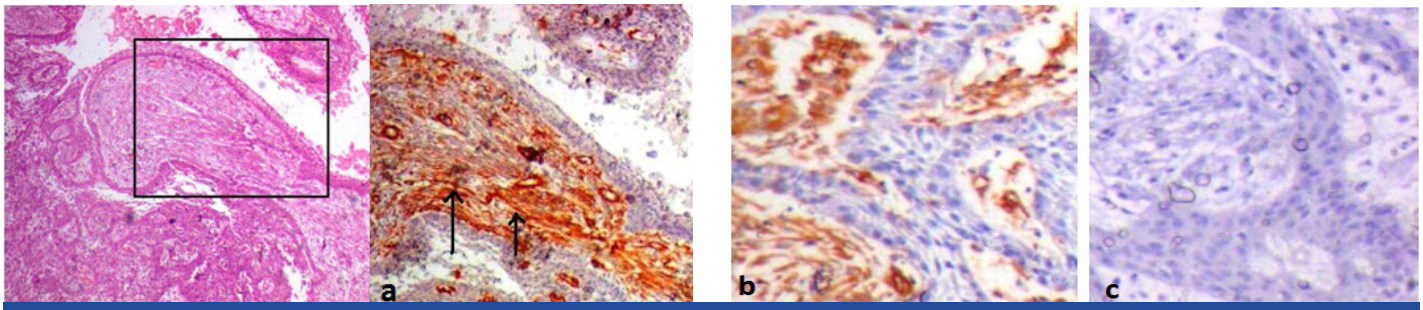
Next they were incubated with the three markers vimentin, desmin and primary alpha smooth muscle actin (α-SMA) monoclonal mouse antihuman antibody, 6 ml ready to use respectively for 60 minutes, then washed with TBS twice for 5 minutes each. Then for antibody detection universal Poly HRP- Anti-mouse secondary antibody kit, 6ml ready to use was used for 30 minutes and then washed in TBS as before. Reaction was developed with Diaminobenzidinechromogen (DAB) and counter stained with haematoxylin provided in the kit for one minute, rinsed, dehydrated and mounted using DPX. With each set of slides for α-SMA, vimentin, desmin, one positive control slide of moderately differentiated squamous cell carcinoma were stained for each of the primary antibody.

Assessment of α-SMA Expression Using Grid: All samples demonstrated positivity for α-SMA in the smooth muscle of the blood vessel walls in the stroma of SMA and type 3 UA and also in the smooth muscle of blood vessel walls in the stroma of intraluminal areas of type 2 & 3 UA which served as internal positive control [Table/Fig-3-9]. All cells classified as a MF in our study were vimentin [Table/Fig-3b,4b,5b,6b,7b,8b&9b] and α-SMA [Table/Fig-3a,4a,5a,6a,7a,8a&9a] positive but desmin negative [Table/Fig-3c,4c,5c,6c,7c,8c&9c]. Immunohistochemical reaction for α-SMA showed positive cell in the stroma of ameloblastoma. These were quantitatively assessed for α-SMA positive cells by counting them using a counting grid in a graticulated eyepiece containing 400 squares that determined the perimeter of the chosen field. Five fields were selected randomly for each slide at magnification of 40X.

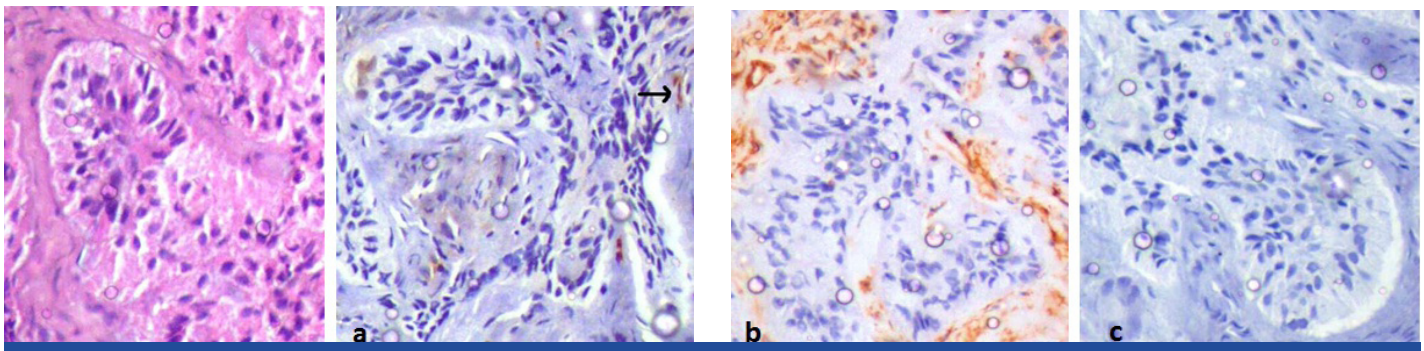
The expression of vimentin and desmin was checked for positivity and then the pattern of of α-SMA was studied by counting α-SMA positive cells just around the follicles in follicular ameloblastoma [Table/Fig-3a] and granular cell ameloblastoma [Table/Fig-5a], in the connective tissue cores of plexiform pattern of SMA [Table/



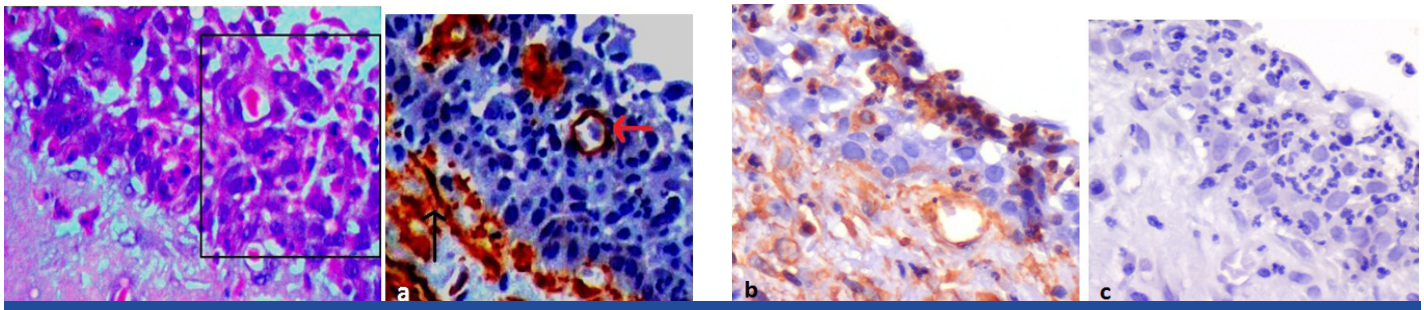
[Table/Fig-3]: Follicular variant of SMA with cystic degeneration x200 H&E. **[Table/Fig-3a]:** x200 α- SMA expression (black arrow represents myofibroblasts in fascicle pattern. **[Table/Fig-3b]:** x40 Vimentin. **[Table/Fig-3c]:** x40 Desmin (-ve for myofibroblasts).



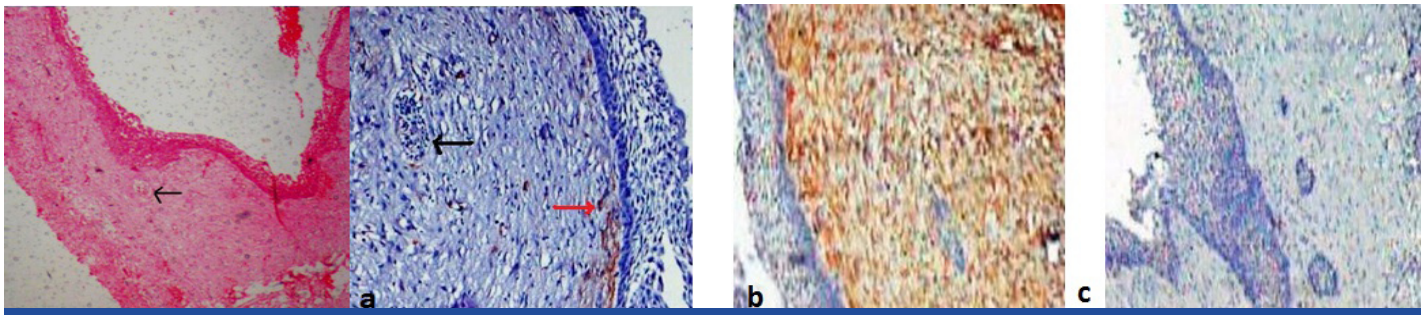
[Table/Fig-4]: Plexiform variant of SMA x40, H&E. **[Table/Fig-4a]:** α- SMA expression (represents area of the inlet in H&E, black arrow represents myofibroblasts in fascicle pattern). **[Table/Fig-4b]:** x200 Vimentin. **[Table/Fig-4c]:** x200 Desmin (-ve for myofibroblasts).



[Table/Fig-5]: Granular Cell Variant of SMA (x200) x200, H&E. **[Table/Fig-5a]:** x200 α- SMA expression (black arrow represents myofibroblasts in spindle pattern). **[Table/Fig-5b]:** x200 Vimentin. **[Table/Fig-5c]:** x200 Desmin(-ve for myofibroblasts).



[Table/Fig-6]: Type 2 UA (less expression in intraluminal area of α- SMA expression) H&Ex200. **[Table/Fig-6a]:** α- SMA expression (represents area of the inlet in H&E, black arrow spindle shape myofibroblasts, red arrow-internal +ve control). **[Table/Fig-6b]:** x400 Vimentin. **[Table/Fig-6c]:** x400 Desmin (-ve for myofibroblasts).



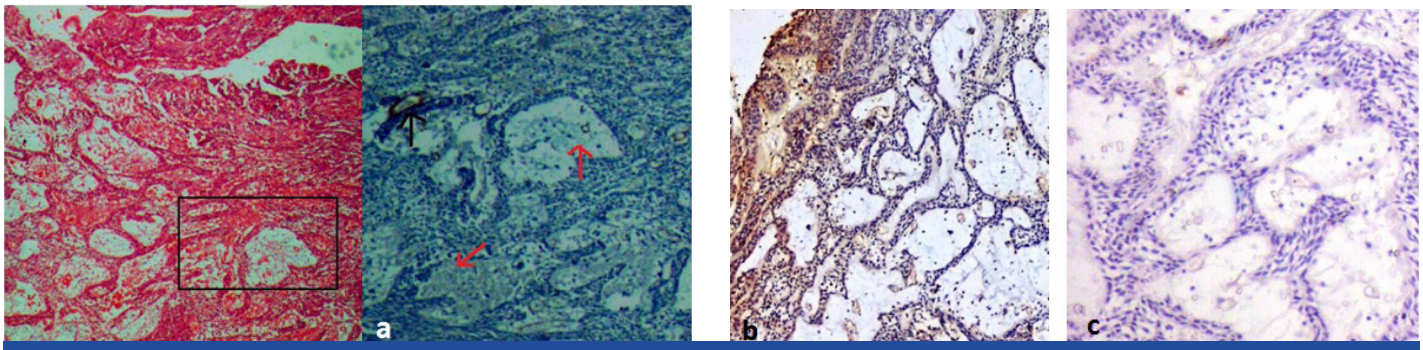
[Table/Fig-7]: Type 3 UA α-SMA expression H&Ex40. **[Table/Fig-7a]:** x100, Intra Mural- α-SMA Expression not evident- black arrow, red arrow spindle pattern adjacent to cyst lining). **[Table/Fig-7b]:** x40Vimentin. **[Table/Fig-7c]:** x40 Desmin (-ve for myofibroblasts).

Fig-4a], below the cystic epithelial lining of all the three types of UA in the connective tissue cores of type 2 [Table/Fig-6a] and around follicles in type 3 of UA [Table/Fig-7a]. A comparative study of expression patterns of α-SMA was made between SMA and UA cases. Each α-SMA positive cell, excluding those in the walls of the blood vessels, was counted and the total number of positive cells for all five examined fields per case was calculated. Then the mean number of α-SMA positive cells per field was taken. The results were presented as the mean number of α-SMA positive cells per field for each type of lesion.

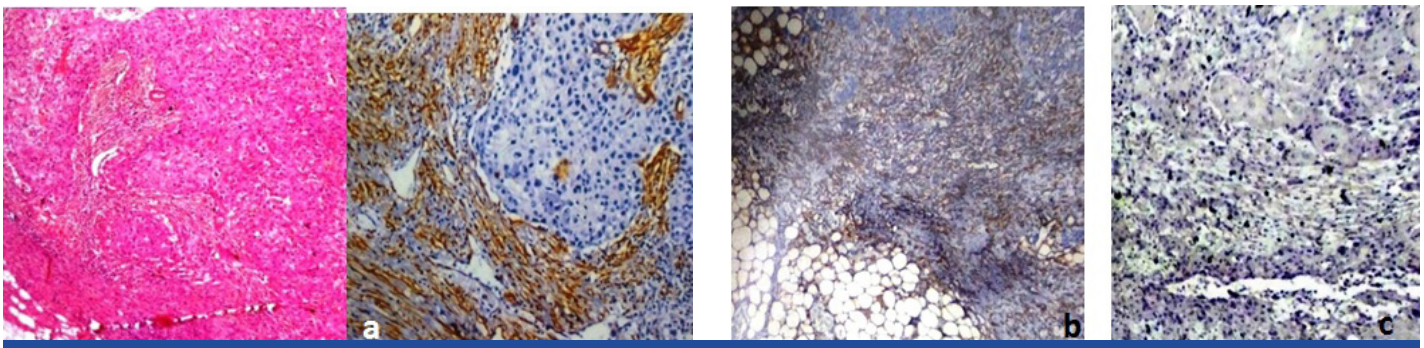
Vimentin which is a general connective tissue marker stained all the connective tissue components and thus indicating myofibroblast is a connective tissue component. Desmin being a skeletal muscle marker stained negative indicating the characteristic myofibroblast feature which is negative for the marker. Hence only those cells which showed α-SMA and vimentin positivity and desmin negativity were considered as MF and quantified for the same.

STATISTICAL ANALYSIS

Descriptive statistical analysis was performed in the present study; results on continuous measurements were presented as Mean±SD of the α-SMA positive cells per field among the ameloblastoma cases selected in the study and a comparative analysis of SMA with UA were carried out using one-way Student t-test. The Statistical Software namely SPSS 11 was used for the analysis of the data and Microsoft Word and Excel were used to generate tables etc.



[Table/Fig-8]: Plexiform variant of SMA (absence of myofibroblasts) x40, H&E. **[Table/Fig-8a]:** Represents area of the inlet in H&E, Absence of α- SMA expression -red arrow, black arrow internal +ve control). **[Table/Fig-8b]:** x40 Vimentin. **[Table/Fig-8c]:** x100 Desmin(-ve for myofibroblasts).



[Table/Fig-9]: Moderately differentiated squamous cell carcinoma (Control slide) x40, H&E. **[Table/Fig-9a]:** x100, Myofibroblasts expressing smooth muscle actin (alpha SMA). **[Table/Fig-9b]:** x40 Vimentin. **[Table/Fig-9c]:** x40 Desmin (-ve for myofibroblasts).

Ameloblastoma	Mean	Std. dev.	Mean difference	T	p-value
Solid	15.50	12.08	-6.380	-1.309	0.207
Unicystic	21.88	9.57			

[Table/Fig-10]: Correlation of the mean MF counts between Group I and Group II.

Group	n	Mean	Std. dev.	Mean difference	T	p-value
Group1(Pain)	6	16.33	14.75	2.083	0.253	0.807
Group 1a(No Pain)	4	14.25	8.47			

[Table/Fig-11]: Correlation of the mean MF counts in SMA showing Pain and No-Pain.

RESULTS

Study samples were divided into two groups based on their clinical and histopathological diagnosis as mentioned in methodology [Table/Fig-1,2] and the correlation of MF between the two groups (group I & II) are shown in [Table/Fig-10].

Higher mean myofibroblast counts were recorded in unicystic ameloblastoma (group-II) compared to solid multicystic ameloblastoma (group-I). The difference in mean myofibroblast counts between the two groups was found to be statistically insignificant (p = 0.207).

Correlation of the mean MF counts in SMA showing pain and no-pain is shown in [Table/Fig-11]. There was no difference in the mean number of myofibroblasts in cases of SMA associated with pain and those not associated with pain (p = 0.807).

Correlation of the mean MF counts in UA showing pain and no-pain is shown in [Table/Fig-12]. There is no difference in the mean number of myofibroblasts in cases of UA associated with pain and those not associated with pain (p = 0.805).

Mean MF count correlated in SMA showing perforation and non-perforation is shown in [Table/Fig-13]. Higher mean myofibroblast counts were recorded in cases showing Perforation (Group 1) compared to Non-Perforation (Group 1a) cases of solid multicystic ameloblastoma but the difference in mean myofibroblast counts between the two groups was found to be statistically insignificant (p = 0.440).

Group	n	Mean	Std. dev.	Mean difference	T	p-value
Group2(Pain)	4	20.75	6.95	1.430	0.256	0.805
Group 2a(No-Pain)	6	19.32	9.23			

[Table/Fig-12]: Correlation of the mean MF counts in UA showing Pain and No-Pain.

Group	n	Mean	Std. dev.	Mean difference	T	p-value
Group 1 (Perforation)	3	20.33	2.91	-6.930	-0.810	0.440
Group 1a (Non-Perforation)	7	13.40	14.10			

[Table/Fig-13]: Mean MF count correlated in SMA showing Perforation and Non-Perforation.

Group	n	Mean count
Group 1(Perforation)	1	33.6
Group 2(Non-Perforation)	9	18.25

[Table/Fig-14]: Mean MF count in UA cases showing Perforation and Non-Perforation.

Mean MF count in UA cases showing perforation and non-perforation is shown in [Table/Fig-14]. Statistical analysis was not done as only 1 case showed perforation.

Pattern of distribution of myofibroblast: In the SMA, MF expression was seen adjacent to follicles in follicular and granular cell variants, and within the connective tissue cores of plexiform variants. Out of 10 cases of SMA, in one case of follicular and two cases of plexiform variants the MF expression was in the form of thick bands and fascicles with indistinct cell boundaries [Table/Fig-3a&4a] respectively, in most of the areas and in form of spindle shaped cells with distinct cell boundaries in other areas. Whereas in all other cases (one case of follicular, three cases of plexiform and all cases of granular cell ameloblastoma variant [Table/Fig-5a] the MF cells were placed farther apart, spindle in shape with distinct cell boundaries with the exception of one case of plexiform variant which was negative for MF expression ([Table/Fig- 8a] represented by red arrow).

In UA MF expression was seen adjacent to the epithelial lining of type 1, 2 [Table/Fig-6a] and 3 ([Table/Fig-7a] represented by red

arrow); within the stroma of intraluminal component of type 2 UA and no expression around the mural component ([Table/Fig-7a] represented by black arrow) of type 3 UA. In two cases of type 1, three cases of type 2 and five cases of type 3 the MF were spindle in shape placed farther apart with distinct cell boundaries adjacent to the epithelial lining. Only in one case of type 1UA a focal area showed expression in form of thick band. In the type 2 all of the three cases the intraluminal component showed plexiform arrangement of odontogenic cells with minimum stroma where the number of MF were few, spindle in shape ([Table/Fig-6a] represented by black arrow) with distinct cell boundaries. In type 3 the mural component in the sections we had one to two follicles and surrounding these follicles there was no MF expression ([Table/Fig-7a] represented by black arrow).

DISCUSSION

Odontogenic cysts and tumours share the same sources of odontogenic epithelium yet exhibit different degrees of aggressiveness in their biological behaviour. This discrepancy has been attributed to differences in the specific features that the epithelial component acquires during the development of the lesion [6]. But, within the group of odontogenic tumours too, from a biological point of view [10], behavioural differences exist. Vered M et al., have tried to find reasons for the variation in the biological behaviour of solid/multicystic ameloblastoma and unicystic ameloblastoma [6]. In general, it has been seen that stroma is essential for maintenance of the epithelial tissues [6]. Over the past decade; microenvironment or stroma of neoplastic tissues has been found to play an active role in tumour progression [11]. However, only a few studies have investigated these stromal factors that could contribute to the variable biological behaviour in odontogenic tumour [6,12].

Alterations in the stromal compartment resulting from neoplastic changes in the adjacent epithelium include, among other phenomenon, the appearance of MF [6]. The presence of MF in these odontogenic tumours (SMA and UA) however has not been thoroughly investigated.

Till date there is no myofibroblast specific immunocytochemical marker. Therefore, characterization of tumour-associated MF is based on a combination of positive markers such as actin isoforms specialized in cellular contraction such as α-SMA; the intermediate filaments vimentin and occasionally desmin [7]. MF in normal tissue, granulation tissue and pathologic tissues disclose five phenotype:

i) Phenotype V represented by cells expressing only Vimentin.

ii) Phenotype VA –cells expressing vimentin and α-SMA.

iii) Phenotype VAD-cells expressing vimentin α-SMA and Desmin.

iv) Phenotype VD represented by cells expressing Vimentin and Desmin.

v) Phenotype VA (D) M, representing those MF expressing Vimentin, α-SMA, Smooth Muscle Myosin Heavy Chain with and without Desmin [13].

In the present study immunohistochemical analysis was carried out in both the groups using vimentin, α-SMA and desmin primary antibodies to check for the presence of MF. Myofibroblasts found in this study stained consistently for the VA phenotype (vimentin +, actin +, desmin –). The VA phenotype has also been found to be the predominant phenotype in carcinoma of the breast and colon, and in Hodgkin's disease (nodular sclerosis).

Based on the propensity and the location of MF next to epithelial or parenchymal cells, the term "juxtaparenchymal cells" has been suggested for them [14]. In our study too, MF were distributed just adjacent to the epithelial component i.e. in the stroma adjacent to the follicles in the follicular ameloblastomas [Table/Fig-3], in the connective tissue stroma of plexiform ameloblastoma [Table/Fig-4] and type 2 unicystic ameloblastoma [Table/Fig-6] and in the stroma just subjacent to the cystic lining in type 1 and type 3 [Table/Fig-7] unicystic ameloblastomas. The exception being the stroma adjacent to the follicles in type 3 unicystic ameloblastoma. The grid was placed adjacent to the above mentioned areas for the respective cases and the number of MF counted. The absence of MF in the tumour-free stroma of the samples, suggest that a close contact is needed for ameloblastoma cells induction of MF transdifferentiation probably by epithelial mesenchymal interaction between ameloblastoma cells and stromal fibroblasts [15]. Fregnami ER et al., have suggested that through expression and secretion of cytokines, chemokines, growth factors and extracellular matrix molecules myofibroblasts promote epithelial mesenchymal interactions including neoplastic growth and invasion [15].

Presence of MF in odontogenic tumours has been reported in very few studies. However, those were isolated case reports or studies comparing the presence of MF in aggressive and non-aggressive odontogenic tumours. In an earlier study it was seen that the number of MF in solid ameloblastomas was significantly higher than in unicystic variants, suggesting that MF can contribute to the biological behavior of aggressive lesions [15]. In contrast, in our study, the mean number of MF in SMA and UA were 15.50 and 21.88 with a standard deviation of 12.08 and 9.57 respectively and there was no statistical difference seen in the two groups ($p = 0.207$). The reasons for the reduced number of MF in SMA can be speculated to be due to two reasons. One is that 5/10 cases of SMA were plexiform type where the stromal component was very minimal and hence the reduced number of MF in these cases contributing to the overall decrease in mean number of MF in SMA. The second reason could be the pattern of distribution of MF in these cases. MF in tumour stroma may be distributed in various patterns and have found that the reticular pattern is predominantly observed in the non-invasive carcinoma of urothelial carcinoma and the fascicular pattern is chiefly seen in the invasive carcinoma of the urothelial carcinoma [16,17].

Researchers have made an observation that MF are known to be present in the stroma of OSCC in two dominant patterns, 'spindle' and 'network' as described by Vered M et al., In the 'network' pattern, MF are exceptionally abundant and occupy almost the entire tumour stroma [18]. The 'spindle' pattern is characterized by stromal MF that has spindle-shaped morphology and is located at the periphery of carcinomas as 1–3 concentric layers, a pattern that can also be found adjacent to a few or many tumour islands/nests. But they did not correlate the patterns of myofibroblast expression with any of the prognostic parameters such as radiographic pattern and histological type [19].

We noted that the distribution of MF in unicystic ameloblastoma cases was discrete, with distinct cell boundaries allowing proper identification and counting of each individual cell but in SMA cases, there seemed to be bunching of MF with indistinct cell boundaries making it difficult to identify each MF discretely. This may have contributed to erroneous counting of the MF and thus leading to a reduced number of MF in the SMA cases. One case of plexiform ameloblastoma and another case of follicular ameloblastoma with cystic degeneration did not show the presence of any MF.

In this study, we also attempted to correlate the expression of MF with clinical and radiological features of pain and cortical perforation respectively [Table/Fig-11-14]. No statistical significance was observed when we tried to correlate the expression of MF with cases showing pain. Correlation of cortical perforation and MF could be done only in SMA cases and not in UA cases as only 1/10 cases of UA showed lingual cortical perforation. Among the 10 cases of SMA two showed perforations of lingual cortical wall and one showed buccal cortical perforation. However, no statistical significance was observed between the number of MF and cortical perforation [Table/Fig-13,14]. This is in contrast to a study where

presence of MF was found to be an independent factor for bone infiltration when correlated with clinical parameters such as size of tumour and time of disease evolution which are actually known to influence rupture of the bone cortex [15]. The same study had found a strong correlation with the presence of MF and expression of MMP-2 in the stroma of ameloblastomas indicating that growth factors, including TGF-β1 and Tumour Necrosis Factor Alpha (TNF-α), which are produced in elevated amounts by MF, induce MMP secretion contributing to bone resorption [15]. The present study was a retrospective one where the data available was not uniform in most of cases as the pattern of recording clinical and radiographic features was not uniform. In contrast to our study a recent study showed spindle patterns to be more dominant followed by network pattern of MF arrangement in ameloblastoma, however they did not correlate the pattern with the quantification of myofibroblasts expression [20].

Though myofibroblastic expression has been seen in these lesions, whether they decide the biological behaviour is still not clear. To best of our knowledge in odontogenic tumors the present study was the first to correlate the pattern of myofibroblast expression and the biological behaviour of ameloblastomas and also to have made an attempt to quantify the myofibroblast expression to correlate with the behaviour of ameloblastomas. A study by Vered et al., [18] have reported about the pattern of distribution of MF expression in Oral Squamous cell carcinoma in tongue and premalignant lesions by immunomorphometry.

LIMITATION

In this study, the sub histological variants of SMA and UA were less. So, future studies could be designed, where equal and higher number of subtypes between the 2 groups along with full clinical, treatment and follow up details can be considered for a definite correlation of MF expression and their biological behaviour.

CONCLUSION

To conclude MF alone may not have a role in the aggressive behavior of ameloblastomas and that various other epithelial/ stromal factors or both may play a role. This calls for further research in this direction. Future studies may be targeted on the frequency and pattern of distribution of myofibroblasts considering equal number of subtypes of ameloblastomas. Such studies may help to clarify how and to what extent these cells and their pattern of distribution contribute to the behavior of these ameloblastomas.

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