Int.j.pathol.2022;20(3): 121-126

Original Article

Quantitative Evaluation of Mean Vascular Density using CD34 as Immunohistochemical Marker in Variants of Ameloblastoma

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ABSTRACT

Introduction: CD34 (Q-BEND 10) is a pan endothelial marker, monomeric glycoprotein and the cell surface trans membrane which is found in blood vessels of normal and neoplastic endothelial cells and use as a vascular marker for quantitative evaluation of angiogenesis. We determined the mean vascular density using CD34 as immunohistochemical marker in variants of Ameloblastoma.

Material and Method: This is a descriptive cross-sectional study of 6 months duration from June 2018-dec 2018. Sample was collected using consecutive sampling technique (non-probability sampling) and 30 Formalin fixed paraffin embedded (FFPE) blocks of Ameloblastoma were collected. The mean vascular density (MVD) in ameloblastoma was evaluated using method described by Nielsen and McNagny. Three areas with the highest amount of vascularization (known as the hot spot) were selected under low magnification (×10) and micro vessels were counted in each specimen at ×40 magnification. The mean in three selected regions was considered as the MVD.

Results: The study consisted of already diagnosed 30 cases of ameloblastoma. The age range was between 20 - 40 years followed by 50 to 60 years with mean age 36.13 $\pm$ 16.0. CD34 shows positive expression for all 30 cases. Among them 14 (46.7%) were males and 16 (53.3%) were females. The most common site of tumor was mandible in 83.3% cases followed by maxilla in 16.7%. The most common type was follicular ameloblastoma comprising of 16 (53.3%) cases, followed by plexiform 8 (26.7%), unicystic 5 (16.7%) while only 1 (3.3%) was of mixed type. The MVD range from 9.33 - 26.3. MVD of all cases was found to be 19.12 $\pm$ 7.8.

Conclusion: It was concluded from this study that, CD34 can be used as a reliable endothelial marker to access MVD in different types of ameloblastoma.

Key Words: Ameloblastoma, Follicular ameloblastoma, Angiogenesis, CD34

Introduction

Ameloblastoma is a benign epithelial odontogenic tumor that morphologically resembles an enamel organ. According to the World Health Organization (1992) definition, ameloblastoma is a benign polymorphic tumor characterized by an increase in local invasive and odontogenic epithelium, the appearance of which is usually associated with follicular or plexiform and fibrous stroma.

The ratio of the mandible to the upper jaw varies from 80 to 20% and 99 to 1%. Most ameloblastomas of the mandible are located in the area of the molar ramus. [1]

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Annually, about 0.5% cases of ameloblastoma are diagnosed per million people worldwide[2] and odontogenic tumors are more common in Africa and China. [3] Ameloblastoma is the second most common odontogenic tumor in the Western Hemisphere after squamous cell carcinoma. Therefore, African Americans are five times more likely than Caucasians to develop ameloblastoma than Caucasians with any other. [4] Ameloblastomas account for 47.4 percent of all odontogenic tumors in Nigeria, according to the National Cancer Registry [5]. According to an Iranian study, ameloblastomas account for 42.5 percent of all odontogenic tumors[6]. Paediatric cases accounts for approximately 10 percent to 15 percent of all ameloblastoma cases in India, while in Africa and Asia, this percentage can reach as high as 25 percent of all cases [7]. A study conducted in Karachi, Pakistan, in

2014 discovered that ameloblastoma accounted for 24.8 percent of all odontogenic tumors [8].

The most commonly used classification system classifies ameloblastoma into the following categories based on clinical, radiological, and morphological criteria [9].

- Solid/multicystic.
- Unicystic.
- Desmoplastic.
- Peripheral.
- Malignant.

The most common type of ameloblastoma is solid/multiple cystic and traditional, accounting for approximately 91% of all ameloblastoma cases. It grows slowly and takes benign path. Ameloblastoma is divided into two separate histological pattern: follicular and plexiform. Odontogenic cells of the follicular epithelium are grouped in islands, and plexiform epithelial cells are grouped in a continuous anastomosis row. Ameloblastoma does not often show all histological types.

The follicular type shows proliferating epithelial odontogenic cells organized in islands, while the plexiform type shows epithelial cells arranged in continuous anastomosing row. It is not rare for an ameloblastoma to exhibit all histological trends. In addition to these two histological forms, cystic, granular, acanthomatous, spindle cell, basal cell, clear cell and other microscopic subtypes have been identified. Unicystic ameloblastoma is the second most prevalent ameloblastoma and accounts for approximately 5–15 percent of all cases[10]. Unicystic Ameloblastoma have a relatively benign biologic behavior and better response to conservative treatment. Predominantantly it is located in mandible, radiologically unilocular, and histologically it is characterized as a cystic lesion lined by an ameloblastomatous epithelial lining[11] and the more recent recommendations recognize two major histopathological forms of luminal and mural unicystic ameloblastoma[12]. The luminal version reveals a cystic pattern lined with an ameloblastomatous epithelium protruding into the lumen as plexiform proliferations that appear like an intraluminal subtype. The wall variant reveals either the follicular or the plexiform configuration of the epithelial ameloblastomatous cells within the cyst wall. It is not rare for both variants to be found in the same ameloblastoma lesion.

Angiogenesis is triggered by a cocktail of growth factors and pro-angiogenic cytokines that modulates another group of molecules blocking neovascularization. It is strictly regulated by balancing these pro-angiogenic growth factors and cytokines[13].

Although Angiogenesis cannot be directly quantified, microvascularity can be determined by measuring MVD. The procedure involves immunohistochemical staining (IHC) of capillary endothelial cells with monoclonal antibodies[14]. Thus, MVD is useful for predicting metastasis and tumor recurrence, and angiogenesis which is critical for tumor growth, differentiation, development, and progression [15, 16].

The MVD differentiation group (CD) is evaluated microscopically using various markers (monoclonal antibodies) such as "antibodies to CD105, CD34 and vascular endothelial growth factor (VEGF) and beta fibroblast growth factor [17].

CD34 is found in endothelial cells of normal and malignant blood vessels. It is used as a specific vascular marker to detect angiogenesis in various lesions, depending on its availability and ease of use [18-20].

Materials and Methods

This is descriptive cross-sectional study of six months duration from june 2018 to dec 2018. The study consisted of already diagnosed formalin fixed paraffin embedded tissue (FFPE) sections of 30 cases of the ameloblastoma of different age and gender group. Formalin Fixed Paraffin Embedded (FFPE) representative tissue, blocks with the adequate tissue were included. FFPE with processing artifacts, blocks of true cut biopsy and non-representative tissues were excluded.

H&E slides of available blocks were reviewed by the pathologist. Five slides from representative blocks, 4 to 5 micron thin sections were made. Two of them were stained with H&E and two kept for IHC and one as reserve. Additional slides of the tonsil tissues was mounted for positive control.

IHC were carried out with the use of with the use of Mouse Anti-Human CD 34 Monoclonal Antibody (Clone: Q Bend 10, Product code: M7165 A/S, Glostrup, and DAKO, Denmark)."

The IHC staining of blood vessel in ameloblastoma was assessed by using technique described by (Nielsen & McNagny, 2008). The stained sections were first inspected at a low magnification[10] in order to identify the locations with the strongest CD34 staining. Following that, blood vessel counting was carried out with a magnification of 40x. A cluster of endothelial cells with a lumen is termed a blood vessel those

endothelial cells are brown in color and positive for CD34. Three regions were chosen because they had the greatest amount of vascularization (known as hot spot). The mean of three selected areas was considered to be the MVD.

The objects which originate from one blood vessel were counted if it is completely separated from it .Blood vessels with muscular walls were eliminated. This was done by two pathologists blindly. In case of any disagreement opinion from third pathologist was taken.

After approval from the institutional Ethical committee of Peshawar Medical college and formal permission from Lab Administration was obtained for collecting blocks from the laboratories.

The data was analysed using the Statistical Package for Social Sciences (SPSS) version 22. Independent t-test was used to compare MVDs. Probability value ($p \leq 0.05$) was considered statistically significant.

Results

Majority of patients diagnosed with ameloblastoma were between the ages of 20 and 40 years, followed by those between 50 and 60 years, with a mean age of 36.13 ±16.0 years.

Among these 30 cases 14 (46.7%) were males, 16 (53.3%) were females (Figure 1). Male to female ratio was1:1.1. The most common site of the tumor was mandible in 25 (83.3%) cases while maxilla in only 5 (16.7%) cases.

The age range is between 12 and 80 years, with 36.13 + 16.0.

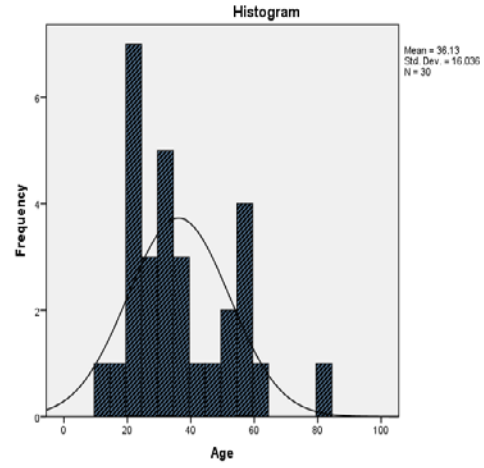

Figure 1 A histogram showing the mean age of the cases (n=30)

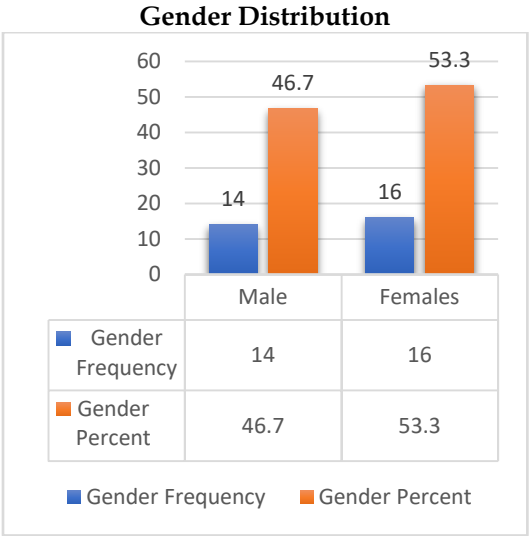

Figure 2 Gender distribution of Ameloblastoma cases (n=30)

Site of the Tumor:

The site of the tumor was mandible in 25 (83.3%) cases while maxilla in 5 (16.7%) cases

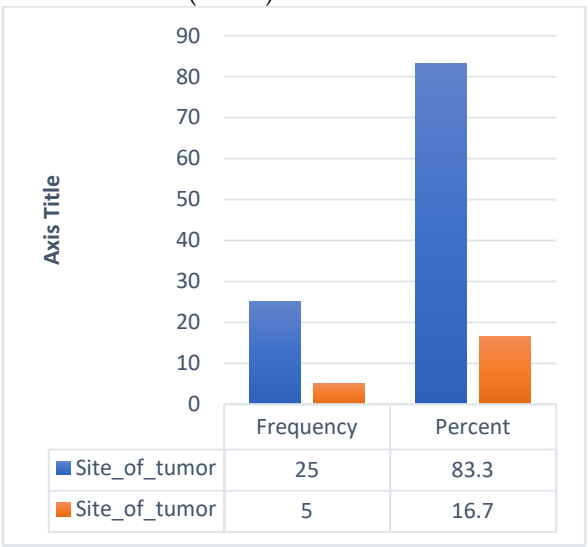

Figure 3 Frequency of the site of the tumor in ameloblastoma cases (n=30)

Among the 30 samples included in this study, 16 (53.3%) were follicular, 8 (26.7%) were plexiform, 5 (16.7%) were unicystic, while only 1 (3.3%) was of mixed type.

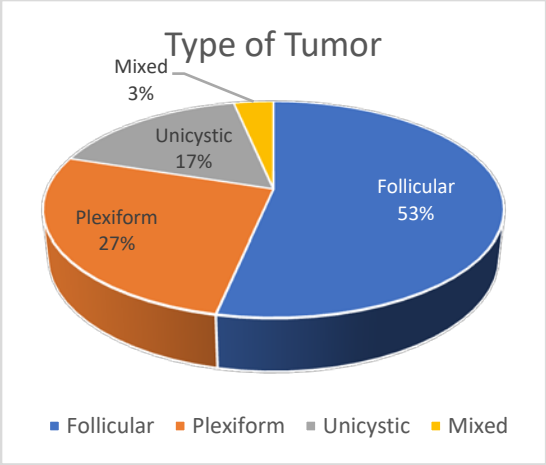

Figure 4. Frequency of the types of ameloblastoma (n=30)

The MVD in ameloblastoma ranged from 52 to 9.33 with a mean of 19.1 ± 7.8.

Table-1*: Mean Vascular Density*:

The MVD in ameloblastoma ranged from 52 to 9.33 with a mean of 19.12 ± 7.8.

S.NO	Vascular Density 1	Vascular Density 2	Vascular Density 3	MVD
1	26	24	20	23.33
2	36	16	27	26.33
3	15	11	7	11
4	21	25	16	20.66
5	19	12	13	14.66
6	13	15	11	13
7	52	46	58	52
8	14	17	12	14.33
9	26	18	17	20.33
10	17	15	19	17
11	18	26	22	22
12	20	23	18	20.33
13	13	12	19	14.66
14	20	31	33	28
15	21	16	22	19.66
16	16	11	18	15
17	26	33	19	26
18	14	24	13	17
19	22	38	18	26
20	11	9	8	9.33
21	16	25	18	19.66
22	16	13	11	13.33
23	17	15	24	18.66
24	16	13	14	14.33
25	15	10	16	13.66
26	18	18	13	16.33
27	10	13	9	10.66
28	12	17	18	15.66
29	20	20	20	20
30	19	25	18	20.66

Table 2: Relationship of MVD to ameloblastoma variants

Variants	No. of cases	Minimum MVD	Maximum MVD	Means ± standard deviations
Follicular	16	13	52	20.89 ± 4.42
Plexiform	8	11	22	15.74 ± 2.53
Unicystic	5	9.33	26.33	18.59 ± 5.38
Mixed	1	16	25	19.66 ± 4.36

Discussion

The result of the present study, shows staining positive for CD34 as in internationally published studies [21-22] and a study published in Iran [23].

In the present study the common age group for occurrence of the ameloblastoma was second and third decade of life, with the mean age 36.13± 16.0 years. The results were similar to other reported studies[24,25], Santos et al, 2014 in their review of 112 cases of ameloblastoma reported a mean age of 35.1 years.

Male to female ratio of the disease is in correlation with study from Iran [23]. The ratio of male to female was slightly higher than our study performed in Nigeria i.e 1.2: 1, [24] while in another study reported from Bangladesh male to female ratio was higher i.e 1.4:1 29.

In our study mandible was the commonest site of ameloblastoma (83.3%) followed by maxilla (16.7%) cases. The results are comparable with an international study. A study in Libya conducted showed comparable results with our study i,e Almost 92,8% of ameloblastomas were located in the mandible, with a very high mandible to maxilla ratio 13:1 [26].

In current study follicular ameloblastoma was the most common histological variant followed by plexiform. These findings are consistent with the internationally published study in which follicular ameloblastoma was the common histological type (64.9%), plexiform (13.0%), desmoplastic ameloblastoma accounting for (5.2%), acanthomatous ameloblastoma shows squamous metaplasia accounted for (3.9%), and unicystic ameloblastoma was diagnosed in (1.3%) cases [27].

Like other tumors ameloblastoma in our study showed a classic blood vessel-dependent pathology. The MVD of our cases was 19.12 ± 7.8 which was comparable with study done by Chen who found it to be 20.63 ± 7.30 [28].

Among multicystic ameloblastomas follicular showed higher MVD as compared to plexiform, Unicystic showed lesser MVD as compared to multicystic ameloblastoma. These findings were consistent with

the findings of the majority of studies, namely that the more aggressive the tumor that emerges from a certain tissue, the higher the MVD value of that tissue. Ameloblastomas possessed a dense vascular network, and the number of microvascular complications (MVDs) increased with recurrence and malignant transformation [29]. In most odontogenic lesions, the distribution of blood vessels throughout all zones of the disease is not consistently the same. When comparing intratumoral MVD of multicystic ameloblastomas to peritumoral MVD, the intratumoral MVD exhibits the highest angiogenesis rate. An increase in MVD in the intratumoral region implies that odontogenic epithelial cells are responsible for angiogenesis in that region. It seems that the accumulation of blood vessels close by odontogenic epithelium for the provision of nutritional substances and oxygen is necessary for essential growth of odontogenic cysts and tumor [22].

Angiogenesis has a prognostic significance and MVD is a useful marker to identify patients with ameloblastoma and aggressive ameloblastoma.

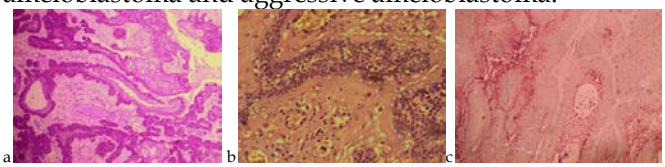

Figure 5.Follicular ameloblastoma a. (H &E 10X) b.(H&E 40x) arrow shows the vessel. c.Microvessels positive for immunohistochemical staining of CD 34 antigen for follicular ameloblastoma (10X)

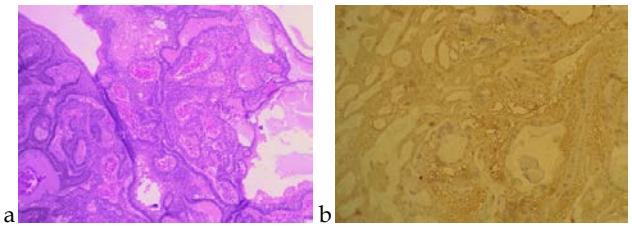

Figure 6 Plexiform ameloblastoma (H & E 10 X) b.Microvessels positive for immunohistochemical staining of CD 34 antigen for plexiform ameloblastoma (10X).

Conclusion

The study concluded thatCD34 is a useful marker to assess angiogenesis and MVD. Follicular ameloblastoma showed a higher MVD as compared to other pointing at an aggressive behavior and close follow up for recurrence.

Research limitations

1. Small sample size
2. Financial constraint

Conflict of interest: None
Grant Support & Financial Disclosures: None

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HISTORY	
Date received:	18-8-2022
Date sent for review:	22-8-2022
Date received reviewers comments:	30-8-2022
Date received revised manuscript:	12-11-2022
Date accepted:	24-12-2022

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B. Active Participation in Active Methodology
C. Interpretation/ Analysis and Discussion