

# TO DETERMINE THE EXPRESSION OF E-CADHERIN IN DIFFERENT HISTOLOGICAL SUBTYPES OF AMELOBLASTOMA FOR GAUGING AND PREDICTING THE AGGRESSIVENESS AND BEHAVIOR OF THIS TUMOR IN OUR POPULATION

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## ABSTRACT

**Objective:** The current study is designed to determine the expression of immunohistochemical markers E-Cadherin in different histological subtypes of ameloblastoma for gauging and predicting the aggressiveness and behavior of this tumor in our population.

**Materials and Methods:** A total of 60 cases of ameloblastoma were collected for this study from June 2016 to June 2017, in AFIP Rawalpindi. The tumor was sub-classified histologically on the basis of WHO classification. Final results were analyzed. Mean and standard deviation were calculated for quantitative variables. Frequency and percentages were calculated for qualitative variables. Chi square test was applied to find the association of E-Cadherin with histological variants of ameloblastoma. P-VALUE <0.05 was considered significant.

**Results:** All samples showed positive E-Cadherin expression with moderate to strong positivity. Highest positivity was observed at the level of stellate reticulum like cells, as they are closer to invasive front. Follicular type showed maximum immunoexpression (73.6%) significantly different from 25% of acanthomatous type. P value for E-Cadherin was calculated to be 0.156.

**Conclusion:** E-Cadherin showed positive results in all histological subtypes of ameloblastoma with maximum intensity in follicular type and minimum in acanthomatous type.

**Keywords:** Ameloblastoma, E-Cadherin, Immunohistochemistry, odontogenic tumor

## INTRODUCTION

Ameloblastoma as one of the benign epithelial odontogenic tumors is known for over a century and half. Because of its devastating jaw destruction and local aggressive behavior, it occupies a special place in the list of odontogenic tumors. It has a high recur-

rence rate even after the radical surgery. Its persistent local growth, frequency and marked deformity lead to its early recognition.

Ameloblastoma constitutes 1-3% of cysts and tumors of the jaws. It is more commonly seen in the mandible than in the maxilla and has the tendency to appear in posterior parts of the mandible in different racial groups. The ratio of occurrence of ameloblastoma in mandible to maxilla ranges from 80-20% to 99-1%.

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Clinically, ameloblastomas are divided into three main subtypes which are multicystic, unicystic and peripheral ameloblastomas (86%, 13% and 1% respectively of all the reported ameloblastoma cases). It is considered that ameloblastomas generally develop from odontogenic remnants of dental lamina cells of dental organ, or sometimes arise from the walls of an odontogenic cyst.<sup>1</sup> In 2005, the World Health Organization (WHO) clinical classification of head and neck tumor classified benign ameloblastoma into three variants: Solid or Multicystic, Unicystic, Extraosseous or Peripheral.<sup>2</sup> Histopathologically, it can be further subdivided into following types on the basis of histological patterns: Follicular, Plexiform, Acanthomatous, Granular cell, Desmoplastic and Basal cell.

The recurrence rates account for 55% to 90% in case of solid or multicystic ameloblastomas when treated by conservative approaches like enucleation or curettage

Cadherins find their roots originating from a family of glycosylated calcium-dependent adhesion molecules. They are known as single-pass trans-membrane proteins. These proteins form homophilic cellular interactions by means of several tandemly recurring extracellular cadherin domains. There are more than eighty known members of the cadherin superfamily. E-cadherin enjoys as one of the most important members of this family and is known to be essential in cell-cell adhesion in epithelial tissues. It is localized on the surfaces of epithelial cells in regions of cell-cell contact which are known as adherens junctions.<sup>4</sup> The suppression of E-cadherin expression is viewed as one of the major molecular events responsible for dysfunction in cell-cell adhesion. Loss of function of E-cadherin tumor suppressor protein links with increased invasiveness and metastasis of tumors, bringing about it being alluded to as the “suppressor of invasion” gene.<sup>4</sup>

## MATERIALS AND METHODS

In this descriptive study sixty (60) paraffin embedded blocks of freshly diagnosed ameloblastomas at AFIP, Rawalpindi were collected along with their demographic data and clinical/radiographical information.

After confirmation of diagnosis, classification according to the World Health Organization (WHO)

and histopathological subtyping was done on freshly prepared slides. 2E-Cadherin was applied on the tissue according to standard protocol.

Necrosed, scarce and poorly oriented tissues were excluded. The intensity of the stain was measured using criteria described in Immunohistochemical Staining and Scoring section. Final results were analyzed. Immunoreactivity was evaluated and its association with histopathological subtypes was carried out. Immunoreactivity of E-Cadherin was evaluated on the criteria described by Alves Pereira (Alves et al., 2010) using a semi-quantitative analysis of immune stained cells using the following scores: 0 (without any reactivity in parenchyma component), 1 (<10% of positive cells), and 2 (>10% of positive cells)

For statistical analysis, cases that showed a positive labelling pattern were considered as normal, whereas the other 2 patterns (negative and reduced) were classified as altered. Chi-square test was used to find out the association of E-cadherin with different grades of tumor. P value  $\geq 0.05$  was taken as significant. The data collected on specifically designed proforma was analyzed using (SPSS) version 20.0. Descriptive statistics was used to describe the parameters like age, gender, site and histopathological subtype. Chi Square Test/ Fischer Exact Test was used to compare the histological types with expression of immune marker E-Cadherin. P value  $\leq 0.05$  was considered statistically significant. Non-probability convenience sampling was carried out.

## RESULTS

In the present study clinicopathologically out of the total 60 patients, 32 were males and 28 were females. The mean age range of patients was 34.63 +/-12.6 years. Histologically 38 cases were follicular ameloblastomas, 14 were diagnosed as plexiform and 8 were acanthomatous. The mean age range of patients was 34.63 +/-12.6 years. In accordance with Immunohistochemical Labelling Pattern E-Cadherin immunostain was restricted to the neoplastic epithelial component of ameloblastomas and was evident in all investigated cases. The most reactivity was observed in stellate reticulum like cells, the intensity reduced in the peripheral columnar cells especially to the invasive front.

The E-Cadherin reactivity was predominant

on the membrane at cell to cell boundary but cytoplasmic staining was also noted in a few peripheral columnar cells

All histological subtypes of ameloblastomas showed 100% positivity with E-Cadherin immunostain. Among all these positive cases, 38 cases revealed score 2 (63.3%) and 22 cases were with score 1 (36.7%).

According to statistical analysis criteria pro-

posed for E-Cadherin, amongst the 8 cases of acanthomatous ameloblastoma, 2 cases scored 2 (25%) and 6 cases scored 1 (75%).

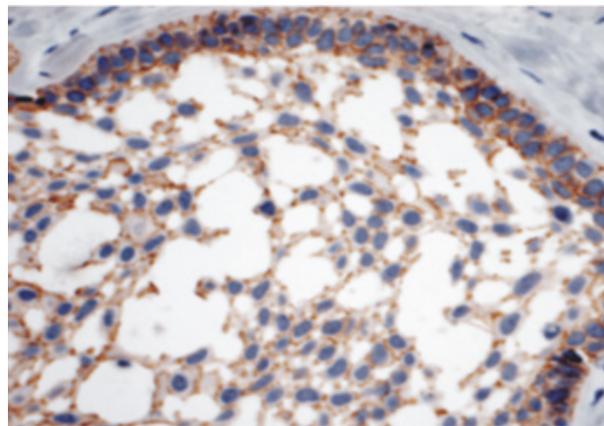
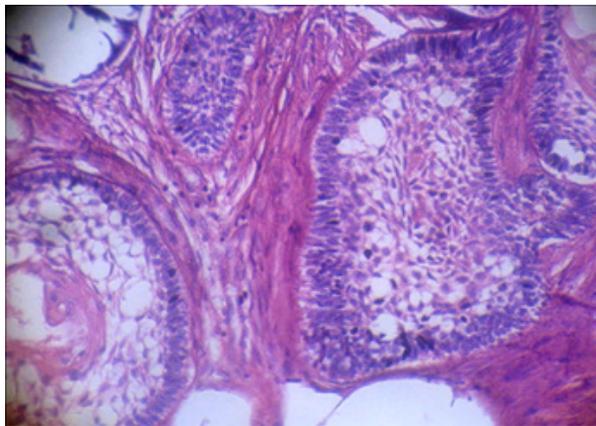
The 14 plexiform cases were all positive with 6 cases scoring 1 (42.8%) while 8 cases showing score 2 (56.14%).

In 38 follicular ameloblastomas, 10 cases scored 1 (26.3%) while 28 cases scored 2 (73.6%) with E-cadherin immunostain. (Table 1).

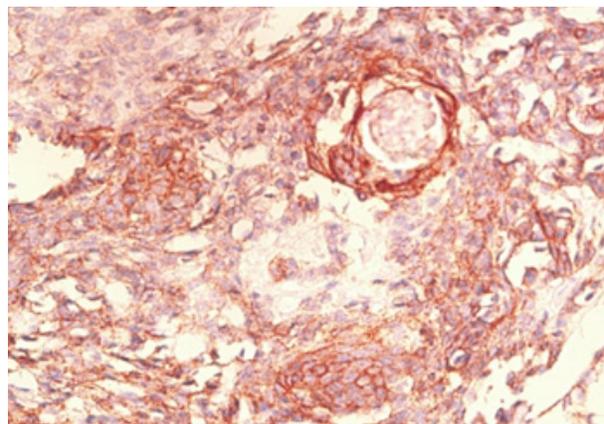
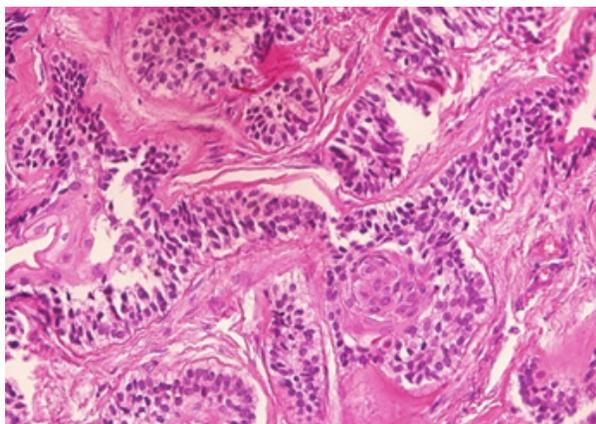
**Table: 1 E-Cadherin Expression in Different Subtypes of Ameloblastoma**

E-Cadherin Expression					
S. No	Histological Sub types of Ameloblastoma	Score 0	Score 1	Score 2	Total
1	Acanthomatous Ameloblastoma	0	3 (75%)	1 (25%)	4
2	Plexiform Ameloblastoma	0	3 (42.8%)	4 (57.14%)	7
3	Follicular Ameloblastoma	0	5 (26.3%)	14 (73.6%)	19
Total		0	11	19	30

P=0.1564 (insignificant)



**Fig 1: (a) Follicular Ameloblastoma H&E Stain (400x magnification) (Showing islands demonstrating peripheral columns and central stellate reticulum like cells showing cystic degeneration). (b) Follicular Ameloblastoma E-Cadherin (400x magnification) (Showing mild positive expression in the cell membrane of columnar cells and stellate reticulum like cells)**



**Fig 2: (a) Acanthomatous Ameloblastoma H&E (400x magnification) (Showing ameloblastic follicles exhibiting squamous metaplasia and keratin formation). (b) Acanthomatous Ameloblastoma E-Cadherin (400x magnification) (Showing mild positive expression in the acanthomatous area)**

## DISCUSSION

Cadherins find their roots originating from a family of glycosylated calcium dependent adhesion molecule. E- Cadherin is one of the most important members of this family and is located at the cell junction and binds the tissue cells together.<sup>4</sup> It also plays an important role in maintaining cell differentiation and morphology.<sup>3</sup> The suppression of E- Cadherin expression is also known as a fundamental event in EMT. The invasion is enabled by EMT which is required for metastasis with suppression of E-cadherin, primary tumor cells loose cell-cell adhesion and break through the basement membrane and cause invasion. Therefore, EMT forms the initiation of invasion leading to metastasis.<sup>6</sup> E-cadherin suppression can be done by many EMT transcription factors like SNAIL1, SNAIL 2, HZEB 1, ZEB 2 and Twist.<sup>7,8</sup> Loss of E-Cadherin expression links with increased invasiveness and metastasis. That is why it is regarded as the “suppressor of invasion” gene.<sup>4</sup> Loss of E-Cadherin is noted in a number of human malignancies like squamous cell carcinoma, soft tissue sarcomas, odontogenic tumors, mucoepidermoid carcinoma, breast cancers and colorectal cancers.

In current study, 60 cases of ameloblastoma were taken in total. After applying E-cadherin, immunoreactivity was observed in all cases ranging from mild positivity (36%) to strong positivity (63.3%). It was restricted to the neoplastic epithelial component of ameloblastoma. Highest reactivity was observed in stellate reticulum like cells and central angular cells. The intensity decreased in the peripheral columnar cells especially towards the invasive front. In acanthomatous ameloblastoma, E-Cadherin showed a weak or even lost expression in squamous metaplasia areas and keratinized areas of acanthomatous ameloblastomas.

The immunohistochemical assessment revealed that the most prevalent score observed was follicular type (28 out of 38 cases, 73.6%). In plexiform variant (8 out of 14 cases, 42.8%) and acanthomatous type (6 out of 8 cases, 75%), the percentage of immunostained cells was < 10% (score 1).

Very few studies are available concerning the expression of E-cadherin on different subtypes of ameloblastoma. In a study by Kumamoto, both qualitative and quantitative expression of E-cadherin was high in central stellate reticulum like cells of

ameloblastoma.<sup>9</sup> Another study by Miyake showed high expression in the central angular cells which is in accordance to our result.<sup>10</sup>

The high expression of E-Cadherin in the stellate reticulum suggested high concentration of this protein at this specific site promoting the adhesion between these cells.<sup>5</sup>

The weak E-cadherin dependent cell-cell adhesion can be related to a combination of genetic, epigenetic, transcriptional and post transcriptional mechanisms and thus promote invasion.<sup>11</sup>

On the other hand, related E-Cadherin expression decreasing in the peripheral columnar cells in follicular type and in the keratinized areas from the acanthomatous ameloblastoma to the terminal differentiation of tumor cells including maturation or degenerative changes and was not thought to reflect tumor progression.<sup>12</sup>

A recent study by Florescu on E-cadherin expression on various histological subtypes of ameloblastoma showed similar results to our study with highest expressions of E-cadherin in stellate reticulum like cells in ameloblastoma in follicular type, and weakest or lost in keratinized area of acanthomatous and granular cells of granular cell ameloblastoma.<sup>13</sup>

Although our study included 3 histological variants of ameloblastoma showing different immunoscore with E-cadherin, it did not reveal any significant differences between the E-Cadherin scores of immunoreactivity and the histological subtypes ( $p > 0.05$ ).

## CONCLUSION

Expression of E-cadherin is found positive in all 3 histological variants of ameloblastoma with maximum intensity in follicular type and minimum in acanthomatous. It is therefore, concluded that E-Cadherin is an important regulator of intercellular adhesion and is also linked to the process of ameloblastoma morphogenesis. But unlike the malignant lesions in other parts of the body it may not be a very significant prognostic marker for ameloblastoma. This study was the first step in understanding the role of E-Cadherin in ameloblastoma and should be considered as a starting point. More research with larger sample size over longer time period is required for getting a better insight of this relationship.

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