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Diagnostic Efficacy of Calretinin Expression in Various Histological Types of Ameloblastoma

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Abstract

Objective: This study was conducted to evaluate the usefulness of calretinin as a confirmatory Marker for ameloblastic tissue.
Study Design: Prospective study
Place and duration: study was conducted in the Dental Section Allied Hospital, Faislabad and Sharif Medical and Dental College from October 2017 to October 2018.
Methodology: A total of 50 patients was included in the study, main variables assessed in this study were positive predictive value neative predictive value, sensitivity, specificity and accuracy of calretinin in diagnosis of ameloblastoma. SPSS version 23 was used to analyzed the data. P value less than or equal to 0.05 was taken as significant. Study was started after permission from hospital ethical committee and patients were informed in detail about disease and procedure to be done. Non probability consecutive sampling was used.
Results: Estimated sensitivity was 78.9%, it means that of the patients with ameloblastoma on calretinin, 78.9% were diagnosed correctly. The estimated specificity was 16%. Positive predictive value was 60% and negative predictive value was 16%. The overall accuracy was 38% for diagnosing calretinin**.**
Conclusion: Ameloblastoma found to be an important immunohistochemical marker for ameloblastoma of ameloblastic epithelium and strong diagnostic tool for differential diagnosis of ameloblastoma.
Keywords: Ameloblastoma, Calretinin, Histopathology, immunohistochemical marker, Maxillofacial.

Introduction:

For regulation of many biological systems like contraction, metabolism, secretion, cell growth, memory storage and cell division require calcium ions directly or indirectly. In many tumor cells elevated cytoplasmic calcium was found which may increase the invasiveness and motility of cells. Intracellular response is also a result of calcium signals through interaction with number of intracellular Ca binding proteins which are the part of many activities inside the cells. Among these proteins EF hand contributes a common calcium binding motive which has about 200 different calcium binding proteins.

One of these proteins is 29kDa calretinine which is clone from the retina of a chick. In preferal and central neural tissues calretinine widely found especially in the retina and sensory neuron pathways. Beyond the Central nervous system it is found in pylar infundibulum, lining of cerosal mesothelial membrane, convoluted tubules in kidneys, eccring glands , sertoli and leydig cells in testes , adrenal cortex, endometrial of stromal cells , adipocytes and thymus epithelial cells. In normal human tissues calretinine has been found in many varieties of tumor.

Recently calretinine emerged as immunohistochemicalmarker which is a great diagnostic tool for adenocorsinoma and mesothelioma of lungs. It's sensitivity for mesothelioma diagnosis is about 100%. Ameloblastoma on its histological presentation can be mistaken for cretosisticodonto genic tumors which can be diagnosed by overlapping radiographically and clinical presentations. Light microscopy is a better diagnostic tool for eratosisticadonto genic tumors but beyond the light microscopy some more refined diagnostic tool were required for these both entities which are different in biological nature and acquired different surgical protocols.

Many immunological markers proved as a significant and some are under investigation. The role calretinine is not well known but it is a known calcium buffer which regulates the appoptosis. In benign and malignant tumors role of calretinine is highly sensitive and specific which is well established about 100%.We conducted this study to establish the role of calretinine as a marker for ameloglastoma.

Methodology

This study was conducted in the study was conducted in Dental Section Allied Hospital, Faislabad and Sharif Medical and Dental College from October 2017 to October 2018.. Study was started after permission from hospital ethical committee and patients were informed in detail about disease and procedure to be done. Non probability consecutive sampling was used.

Chemical staining was done by using immunohistochemical for primary antibody detection on calretinin. Polymer HRP supersensitive one step technique was used and section was than deparafinnized through xylene, same technique was used for rehydration. Whole procedure was named as antigen retrieval was carried out using commercial microwave antigen retrieval system. Tris buffer was used for rinsing and section was incubated in water for 15 minutes with hydrogen peroxide 3%. After that section was settled at room temperature for 30 minutes. Section again incubated in rabbit polyclonal antibody (an optimally prediluted solution) against calretinin antibody. Section once again washed in tris buffer for 10 minutes and then incubated in super enhancer and 20 minutes at room temperature. Second step antibody incubation was done with same polymer HRP one step method. Freshly prepared 3,3-diaminobenzidine hydrochloride (DAB) substrate was used for 5 min to visualized the system. Mayer's hematoxylin for 3min was used to counterstain the slides. Positive and negative staining was done at the same time on study specimens. Human mesothelioma which is known to have a high level of calretinin expression served as the positive control. after that evaluation of slides was done with binocular research microscope and under mahnification of 100x and 400x. this evaluation was done for stained presence, distribution, localization and intensity of immunoreactive cells. Positivity and negativity of stain was evaluated from presence of stain, localization for its nuclear status, distribution was assessed for flicular and diffused nature. Number of stained cells was seen for intensity. Zero cell no staining, one cell weak staining, 2 cells moderate staining and 4 cell intense staining.
SPSS version 23 was used for analysis of data, mean standard deviation and frequency percentages were calculated for numerical and qualitative data respectively. Chi square and t test was applied to see association among variables. P value ≤ 0.05 was taken as significant.

Results:

Fifty patients were included in the present study. It was observed that 15 patients with ameloblastomaon calretinin as well as on histopathology, labeled as true positive. 10 patients with ameloblastoma on calretininbut absent on histopathology, labeled as false positive. 4 patients had no ameloblastoma on calretinin but on histopathology, labeled as true negative. 21 Patients had no ameloblastoma on calretininbut absent on histopathology, labeled as false negative.The difference was statistically significant (p=0.000). (Table. I).

Therefore, the estimated sensitivity was 78.9%, it means that of the patients with ameloblastoma on calretinin, 78.9% were diagnosed correctly. The estimated specificity was 16%. Positive predictive value was 60% and negative predictive value was 16%. The overall accuracy was 38% for diagnosing calretinin.(Table. II).

Table. I
Comparison of calretinin presence in ameloblastomaon calretinin and histopathology
(n = 50)

Histopathology			**Total**	**p-value**
	Ameloblastoma on Calretinin	**No**		
Ameloblastoma on histopathology	True positive 15	True negative4	**19**	0.000
No	False positive 10	False negative 21	**31**	
Total	**25**	**25**	**50**	

Table. II
Diagnostic Accuracy

Diagnostic Measures	Value
Sensitivity	**78.9%**
Specificity	**16%**
Positive Predictive Value (PPV)	**60%**
Negative Predictive Value (PPV)	**16%**
Accuracy	**38%**

Discussion:

In our study we found that there were more males than females and a wide age limit was observed. In a study conducted by Neville et al[11] in 2002 and reported that there was equal presipitation of both genders in ameloblastoma. He also reported a wide age limit as in our study and mandible was the most frequent sight of ameloblastoma. Our study demonstrated presence of calretinine in stellate reticulum which is located in Central core of tumor location.

Another study was conducted by Altini et al[12] in 2000 and reported 29 ameloblastoma patients out of 31 who were positive for calretinine in intense stellate staining reticulum cells. De villier et al[13] also conducted a study in 2008 and reported 100% calretinine positive staining in ameloblastomapatients. In his study 19 cases of ameloblastoma were reportedcalretinine positive. These two studies were aso comparable with our study as we also observed mostly ameloblastoma cases as calretinine positive.

Another study was conducted by Piattelliet al[14] in 2003 and reported the non involvement of calretinine in many tumors and different types of cysts. In his study 24 patients have radicular cysts, 22 have odonto genic crestosistic tumors and 24 patients have follicular cysts and reported negative expression of calretinine.

Another study was conducted by Coleman et al[15] in 2001 on uni cystic ameloblastoma which was present on the epitheal in which the four ameloblastoma cases were multi cystic and solid. He reported that no case of positive staining of calretinine expression. He also recommended calretinine as a special immunohistochemical marker in cases of neoplastic ameloblastic tissues and it is an important diagnostic tool for diagnosis of uni cystic ameloblastoma and its difference from cystic odontogenic lesions.

In 2008 a study was conducted by Alaeddini et al[16] on Iranian population and compares the expression of calretinine in odontogenic tumors. In his study 55 patients of tumors were explode for express of calretinine and concluded 20 cases of ameloblastoma and 10 cases of ameloblastic fibromas. In his study all cases of ameloblastoma staining work calretinine positive expression and other odontogenic tumors.

A Pakistani study was conducted by Kalsoom F et al[17] and reported that calretinine is a useful marker for diagnosis of ameloblastoma and it can be used as differential diagnostic tools from other types of tumors. Results of our study reveal a similar conclusion as in study by Kalsoom F[17] and other previous studies. Another study was conducted by D'silva S et al[18] and reported that calretinin is specific marker for ameloblastoma. If any suspicion of ameloblastoma than immunohistochemical marker can be used as early marker diagnosis. These two studies were also supportive for our results.

In a study by Scharfetter et al[19] and reported that also reported similar finding that calretinin is first indicator of diagnosis of ameloblastoma at any stage. It is an important indicator of cystic odontogenic lesions. In study he shows out of 100% about 40% patients shows calretinin positivity. Our study also shows positivity of calretinin and confirmed on histopathology, so that we can compare these results with previous studies.

Anandani C et al[20] also conducted a si ilar study on this topic and concluded that calretinin found to be an important factor for diagnosis of ameloblastoma. It is specific for ameloblastoma epithelium cells and plays an important role in diagnosis of tumors of maxilla.

Conclusion: Ameloblastoma found to be an important immunohistochemical marker for ameloblastoma of ameloblastic epithelium and strong diagnostic tool for differential diagnosis of ameloblastoma.

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