**UNIVERSITY OF MEDICINE AND PHARMACY**

**CRAIOVA**

# DOCTORAL THESIS

# SUMMARY

## *STUDY OF THE PROGNOSTIC FACTORS IN AMELOBLASTOMAS*

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**CRAIOVA 2013**


**Investeşte în oameni!**

**FONDUL SOCIAL EUROPEAN**

**Programul Operaţional Sectorial Dezvoltarea Resurselor Umane 2007-2013**

**Axa Prioritară: 1. Educaţia şi formarea profesională în sprijinul creşterii economice şi dezvoltării societăţii**

**Domeniul Major de Intervenţie: 1.5. Programe doctorale şi postdoctorale în sprijinul cercetării**

**Titlu proiect: "Sprijinirea tinerilor doctoranzi cu frecvenţă prin acordarea de burse doctorale"**

**Cod Proiect: POSDRU/6/1.5/S/8**

**Beneficiar: Universitatea de Medicină şi Farmacie din Craiova**

***DISCIPLINE FUNDAMENTALE – DAM 01***

***MORFOPATOLOGIE – DASc 22***

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## THE STUDY PURPOSE AND OBJECTIVES

The purpose of this study due to the marked variability of pathogenic and risk factors involved in developing of odontogenic tumors and especially of ameloblastomas is to determine the clinical and epidemiological, histological and immunohistochemical analysis of these tumors and identify factors involved in tumorigenesis or prognostic value.

In this respect it will pursue the following objectives:

- Identification of the malignancies origin;
- Evaluation of risk factors involved in the occurrence of tumors;
- Establishment of different histological patterns and the degree of tumor extension;
- Immunohistochemical profile of malignancies;
- The establishment of certain clinico-morphological and immunohistochemical parameters and the degree of ameloblastomas aggressiveness, identifying markers with prognostic value.

**KEYWORDS:** AMELOBLASTOMA, PROGNOSTIC FACTORS, CLINICAL-IMAGISTIC, HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY

## STATE OF KNOWLEDGE

Tumors derived from odontogenic tissues are relatively rare, accounting only about 1-3% of all jaw tumors **[10, 88, 122]**. Ameloblastoma although the most common odontogenic neoplasm **[93]**, represents about half of jaw tumors **[65, 122, 184]**.

Ameloblastoma is a benign epithelial odontogenic tumor morphological similar with enamel organ. According to the WHO definition (1992), ameloblastoma is a benign , polimorphous neoplasm, but locally invasive, consisting of proliferation of odontogenic epithelium, usually with follicular or plexiform appearance, associated with a fibrous stroma. **[101]**.

Currently, the most widely used classification, based on clinical, radiological and morphological criteria divided ameloblastomas into the following categories **[122, 184]**: solid ameloblastoma, unicystic ameloblastoma, desmoplastic ameloblastoma, peripheral ameloblastoma, malignant ameloblastoma. Due to Clinico-pathological diversity and radiological features, diagnosis and correct treatment of ameloblastoamas continues to be a challenge.

Ameloblastomas are a group of tumors considerated as "enigmatic" **[98]** because their etiology is still unknown **[157]**. There are a number of genetic and molecular changes that appear to promote the development and multistage progression of odontogenic tumors **[122]**. Some of these include oncogenes such as the receptor of the fibroblast growing factor, transcription factors (MYC), tumor suppressor genes (retinoblastoma gene, oncoviruses) **[115]**. The mechanisms by which ameloblastoamas grow and evolve include overexpression of anti-apoptotic proteins (Bcl-2, Bcl-xL) and of the interface protein (FGF fibroblast growth factor and matrix metalloproteinases MMPs). Histopathological and Molecular prognostic Factors are quite less investigated both in ameloblastoma and in ameloblastic carcinoma . It is estimated that neoplastic transformation, consisting in an multistage accumulation of adverse genetic events, occurs in a variety of human tumors on regions of the human genome **[59]**. Establishing the histopathological type and subtype of the ameloblastoma play an important role in assessing the prognosis of neoplasias. Most studies focused yet on indirect link between the proteins expressed by the tumor and the biological behavior.

# PERSONAL RESEARCH

## CHAPTER V. MATERIAL AND METHODS

This study was a retrospective and prospective analytical type study, in which we compared the clinical, morphological and biomolecular features of ameloblastoamas diagnosed in the Laboratory of Pathology of the County Emergency Clinical Hospital Craiova (1996-2012). The studied cases included a total of 211 odontogenic tumors, 42 of them being ameloblastomas.

The methods used in this study included the clinical, morphological and immunohistochemical study. For each case has drawn up a tracking sheet (Appendix 1).

• For the the clinico-epidemiological study, investigation of the actual and retrospective observation sheets, provided data concerning: year of diagnosis, sex and age of patients, symptoms, objective changes and imagistic aspects.

•Ameloblastomas morphological study sought to identify the microscopic main parameters of tumors. Surgical excision pieces were fixed in 10% buffered formalin, processed by the usual technique of paraffin embedding, followed by standard Hemalaum eosin staining and special stains (stain Gomory and periodic acid Schiff staining). Histopathological analysis included the following assessment criteria: architectural and / or cytologic type and subtype and tumor invasion into adjacent structures.

• Immunohistochemical study was of enzymatic detection type method using the LSAB technique work (Labelled Streptavidin-Biotin2 System). Antibodies used in this study were for:

- Assessment of tumor aggressiveness by: investigation of the rate of proliferation of tumoral cell using Ki67 marker, investigation of the potential of invasion using the expression of markers MMP-9 and TIMP-2 in tumoral cells, investigation the potential of epithelial-mesenchymal transition of tumoral cells with E -cadherin and vimentin markers.

- Assessment of tumorigenic potential by investigating the expression of BCL2, p16 and p53 in tumoral cells.

• For the statistical analysis were used averages values, standard deviations and confidence intervals, and comparison tests (uni factorial ANOVA, chi square, Pearson) for the batches , made with SPSS10 software.

## CHAPTER VI. RESULTS

### VI.A. CLINICO-EPIDEMIOLOGICAL AND IMAGISTIC STUDY OF AMELOBLASTOMAS

• The 42 ameloblastomas clinical assesed, were selected in a period of 18 years between the years 1998 to 2012. The number of cases diagnosed in a calendar year ranged between 1 and 5 cases / year. Analysis of selected cases by age group indicated that most of them were diagnosed in the fourth and the fifth decade of life. We noticed that ameloblastoamas showed a slight predominance in males compared to females. Another parameter investigated was the topography of ameloblastomas. Location of tumors was predominantly in the jaw with a report mandible / maxilla of 6/1. Analysis of objective signs from local clinical examination revealed in majority of cases the presence of an endooral growth with varying sizes, tough, which distorts the skeletal plan.

• Of the 42 ameloblastomas, 34 were imagistic investigated by simple radiography in different incidences in all 34 cases, in 18 cases was added and computed tomography. Routine radiological investigations revealed various aspects. The most common appearance corresponded to a round, multilocular, cysts like radiolucent image with clearly defined edges. CT scan revealed also cystic, sometimes large tumoral growth, in the ascending mandibular ramus that determine important deformation of the contours of gonion and ascending mandibular ramus

## VI.B. HISTOPATHOLOGICAL STUDY OF AMELOBLASTOMAS

Histopathological study of the 42 studied ameloblastomas intended to establish the histological type and subtype of malignancy and the damage of the adjacent structures. We noted that solid ameloblastomas were mostly of casuistry investigated (28 cases), followed in order of frequency of unicystic ameloblastomas (13 cases) and desmoplastic ameloblastomas (2 cases).

► Solid ameloblastoma was present in 28 cases, which constituted 66.7% of all ameloblastomas and 19.9% of the investigated tumors, diagnosed in patients from the third and eighth decades of life, predominantly male. Neoplasms had two distinct histopathological patterns, typically follicular in 23 cases and plexiform in 7 cases, associated with some tumoral subtypes.

► Unicystic ameloblastoma was present in 13 cases, representing 31% of the studied ameloblastomas. All tumors had mandibular posterior localization and were diagnosed in patients younger than the one with solid or desmoplastic ameloblastoma (III-VI decades of life), predominantly in males. Histopathologically we found that in 6 cases the tumors were luminal, intraluminal type in 2 cases and mural type in 3 cases.

► Desmoplastic ameloblastoma was present in one case belonged to a male patient, aged 53, with mandibular location, which was characterized by the absence of fibrous capsule, tumor infiltrating spongious bone.

Another issue was related to the extension of ameloblastomas in adjacent tissues. For the investigated casuistry ameloblastomas extension was done in 6 cases in the bone, of which 2 cases were associated with tumoral extension to the oral mucosa.

## VI.C. IMMUNOHISTOCHEMICAL STUDY OF AMELOBLASTOMAS

### VI.C1 Immunohistochemical study of tumorigenic potential of ameloblastomas

• Immunoreactivity for p53 was present in 11 of the 19 cases of ameloblastoma investigated (57.9%). Of the 8 cases with no reactivity 5 were follicular ameloblastomas two acanthomatous ameloblastomas and a case of granular cell ameloblastoma. Statistical analysis showed specific reactivity to p53 for peripheral columnar cells ($p < 0.001$, ANOVA), marking distribution being characteristic (Pearson index <0.3). Test of comparison of medium of the positivity indices showed no significant differences in p53 expression compared with other markers in the stellate reticulum ($p > 0.05$, ANOVA).

• Analysis of bcl-2 protein imunoexpression indicated the presence of the immunomarker in 15 (89.5%) of the investigated ameloblastoamas. Immunoexpression was cytoplasmic, constant and intense, in the columnar cells from the periphery of tumoral islands. Statistical analysis showed specific reactivity for bcl-2 in peripheral columnar cells ($p < 0.001$, ANOVA), with characteristic distribution of the immunostaining (Pearson index <0.3). Test of comparison of the positivity indices showed no significant differences in the expression of bcl-2 in relation to other markers in the stellate reticulum ($p > 0.05$, ANOVA).

• Analysis of p16 protein imunoexpression indicated its presence in 18 of ameloblastomas. Imunostaining pattern was mixed, cytoplasmic and nuclear, maximum of reactivity is observed in stellate reticulum cells. Statistical analysis showed specific reactivity to p16 for stellate reticulum cells ($p < 0.001$, ANOVA). We noticed relationships betwwen the index of positivity and the histologic type of ameloblastoma.

### VI.C2 Immunohistochemical study of the tumor aggressiveness in ameloblastomas

• Ki-67 index investigation showed positivity in 17 cases (89.5%). Cells showed nuclear marking and were located predominantly in the peripheral area of the tumor islands, and stellate reticulum cells in the central area. Basal cells from the periphery of neoplastic islands had the highest

reactivity. Statistical analysis of Ki-67 showed specific reactivity for peripheral columnar cells ($p < 0.001$, ANOVA).

• Imunoexpresion of MMP-9 was found in 15 of the 19 investigated cases (78.95%). The table below showed reactivity scores whichever ameloblastom histological investigation.

• Imunoexpresia TIMP-2 was detected in all cases investigated both in the epithelial component and the stromal level. Statistical analysis found no significant differences in scores of TIMP-2 in relation to histological type.

• Imunoexpresia E-cadherin was observed in all cases investigated is restricted to the epithelial component. Statistical analysis showed that the scores of immunoreactivity for E-cadherin and MMP-9 was no variation inverse relationship ($p = 0.019$) and between scores imunoreactivității for E-cadherin and vimentin was also found an inverse relationship ( $p = 0.043$).

• Vimentin stromal imunoexpresssion was constant, while in tumoral parenchyma the reaction was absent or with low intensity in peripheral cubico-cylindrical cells. Chi-square test indicated a relationship of inverse variation between the immunoreactivity scores to vimentin and TIMP-2 ($p = 0.279$) and between scores on vimentin and E-cadherin ($p = 0.043$).

## CHAPTER VII. DISCUSSIONS

Ameloblastomas are odontogenic tumors with ambiguous behavior due to contradictory, paradoxical and discordant clinical and histological characteristics. Thus, if the histologic aspect of tumor is benign, clinical behavior is invasive and destructive, being reported and rare cases of pulmonary metastases.

Termenul de ameloblastom include mai multe tipuri clinico-radiologice şi histologice. Pe baza comportamentului clinic şi prognostic, se distind trei tipuri de ameloblastoame **[161]**: ameloblastomul convențional sau clasic, ameloblastomul intraosos, solid sau multichistic; ameloblastomul unichistic; ameloblastomul periferic. Studii recente indică ameloblastomul desmoplastic datorită comportamentului său biologic, aspectului radiografic, şi histologic, ar putea fi de asemenea considerat ca al patrulea subtip de ameloblastom **[174]**.

Ameloblastoma term includes several clinico-radiological and histological types. Based on clinical behavior and prognosis they are three types of ameloblastomas **[225]**: conventional or classic ameloblastoma, intraosseous solid or multicystic ameloblastoma, unicystic ameloblastoma; peripheral ameloblastoma. Recent studies indicate that, due to its biological behavior, radiological and histological appearance, desmoplastic ameloblastoma could also be considered as a fourth subtype of ameloblastoma **[132]**.

The main biological aspect of ameloblastoma is the locally invasive behavior, which is responsible for the high rate of postoperative recidive even under conditions of radical surgical therapies. However, some aspects of both pathogenesis and invasive growth remains to be elucidated.

Kumamoto et al. (2004) find that aberrations in the p53 cell cycle control system pathway are correlated with neoplastic transformation. Although classical and metastasant ameloblastomas nuclear expression of p53 gene are very similar, ameloblastic carcinomas had an increased p53 reactivity suggesting malignant transformation of odontogenic epithelium.

Soluk et al. have shown that 20-50% of the stromal cells of ameloblastoamas had bcl-2 reactivity, which would justify their aggressive growth pattern **[105]**. Regarding variants of solid ameloblastoma, Luo et al. showed that the plexiform type presented reactivity in 58.3% of cases, compared to only 48.1% in follicular ameloblastoma **[132]**.

Kumamoto et al noted p16 overexpression in the vast majority of neoplastic cells of ameloblastoma, concluding that odontogenic epithelium could be under the control of this oncoprotein **[105]**. On the other hand, Artese and col suggests that p16 may control the odontogenic epithelium and, furthermore, p16expression and localization would influence the biological behavior, explaining in part infiltrative growth of some of them **[15]**.

Reported strictly to the parenchymatous cell compartments of ameloblastoma, most authors have shown the prevalence of Ki-67 expression mainly in basal cells, proliferation index was significantly higher in peripheral than in the central cellular compartment **[58, 118, 125, 139, 166, 193, 201]**.

The expression of MMP-9 in relation to that of MMP-2 in neoplastic cells reflects their differentiation potential of the cells, cubic and columnar cells from the periphery of ameloblastic proliferation recalling themselfs the function of ameloblastic cell, but without reaching full maturity in order to produce enamel **[192, 247]**. The immunohistochemical study conducted by Kumamoto et al. observed increased expression of TIMP-1 and TIMP-2 in the neoplastic cells of ameloblastoma, while the reactivity of the MMP-9 was reduced **[117]**, authors suggesting that these proteins help the to suppress the progression of ameloblastomas, by the inhibition of the MMP-9 activity.

Very few studies have dealt with the investigation of the reactivity of tumor cells for vimentin in ameloblastomas. Kumamoto and Ooya investigating apoptosis in ameloblastomas with granular cells found no reaction for vimentin in granular tumoral cells **[113]**. The study conducted by Wang et al. showed a weak reactivity for vimentin in the ameloblastoma cells grown in vitro **[262]**.

## CHAPTER VIII. CONCLUSIONS

The study, which included a total of 42 ameloblastomas selected within 18 years (1998-2012) revealed the following:

• *Clinical and epidemiological* analysis indicated a random distribution of lesions in calendar years, the age group distribution showed a peak incidence in the fourth and fifth decades of life, with slight predominance in males (M / F=1,21/1) and topography preferentially in the posterior mandible;

• analysis of objective signs after local clinical examination showed in most cases the presence of an endooral proliferation, and the most common aspect found on routine radiological investigations was of round, multilocular radiolucency, while CT scan showed cystic, sometimes large tumoral formations;

• *histopathological* study with usual and special stains showed that solid ameloblastomas represented the majority of the investigated cases (66,7%), followed in order of frequency by unicystic ameloblastomas (31%) and desmoplastic ameloblastoma (2,3%) solid ameloblastoma had two distinct patterns: typical follicular in 23 cases and plexiform in 7 cases; unicystic ameloblastomas corresponding in 6 cases to luminal tumors, in 2 cases to intraluminal tumors and in 3 cases to the mural type; desmoplastic ameloblastoma was present in one case characterized by the absence of fibrous capsule and infiltration of the spongious bone;

• the extension of ameloblastoamas was done in 6 cases to the bone tissue, in 2 cases tumors were associated with extension to the oral mucosa;

•*immunohistochemical* study aimed distinguish tumorigenic immunophenotype of ameloblastomas (p53, bcl-2 and p16), the proliferative potential of tumors and tumoral aggressiveness by evaluating imunoexpresion (MMP9, TIMP2 and E-cadherin);

•Tumorigenic immunophenotype of ameloblastomas revealed that all the three proteins involved in control of the cellular cycle and apoptosis (p53, bcl-2, p16) have been extensively expressed in the follicular solid variant, such a phenotype being significant for a less aggressive behavior and a terminal differentiated phenotype of the last two versions of ameloblastomas comparative to the typical follicular ameloblastoma.

• regardless of histology, higher reactivity to p53 and bcl-2 cells from the cubico -cylindrical cells from the perifery of the neoplastic proliferative islands and from stellate reticulum for p16 protein suggests the presence of two celular compartments with different properties , one central differentiated pro-apoptotic and another peripherical anti-apoptotic proliferative.

• the highest proliferative potential had the solid follicular variant of ameloblastoma, peripheral basal cells being the most reactive to Ki-67 marker.

• investigation of the imunoreactivity for MMP-9 and TIMP-2 in ameloblastomas showed that on the one hand at parenchymal level is a higher reactivity in the peripheral cells as compared with the

central ones, and on the other hand, this reactivity was significantly higher in the stroma adjacent to the tumoral invasion front. So ameloblastoma's invasive potential in adjacent tissues is proved byits double parenchymal and stromal capacity to secrete matrix metalloproteinases and their inhibitors.

• the qualitative and semiquantitative assessment of E-cadherin expression showed a higher expression at he level of intercellular junctions of the stellate reticulum and a decreased expression in the peripheral cellular compartment in the follicular variant of ameloblastoma and in the keratinized areas of acanthomatous ameloblastoma and aggregates of granular cells in the corresponding version of ameloblastoma. Such a profile would be suggestive on the one hand for the terminal type differentiation of the cells from the central compartment in acanthomatous and granular cell variations, and on the other hand, the lowering of the reactivity in the perifeic compartment would be in part responsible for promoting the invasion of these tumors.

• investigation of the vimentin imunoreactivity showed maximum reactivity in stromal cells adjacent to the invasion front, such an imunoprofile being suggestive for the local invasive potential of follicular solid variant of ameloblastoma.

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