

**THE UNIVERSITY OF MEDICINE AND PHARMACY
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DOCTORAL THESIS

**CLINICAL AND MORPHOLOGICAL FEATURES
OF THE UPPER LIP BASAL CELL CARCINOMA**

SUMMARY

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1. BASAL CELL CARCINOMA EPIDEMIOLOGY

Oral cancer is a serious and growing problem in various parts of the globe. Together with pharyngeal cancer, it is ranked sixth among the most common cancers in the world. Annually, the incidence of these cancers is 275,000 for the oral and 130,000 for pharyngeal cancers, excluding the nasopharynx [1]. Approximately two-thirds of these cases are found in developing countries. The areas with the highest incidence are: South and Southeast Asia, certain areas of Western and Eastern Europe, certain areas of Latin America and the Pacific Ocean [2].

In 2004, 67,000 new cases of the European Union (EU) were recorded in these countries. Overall, oral and pharyngeal cancers occupy position 7 in the EU [3]. Within the EU, the countries with the highest rate of male incidence are France and Hungary, and the lowest rates are recorded in Greece and Cyprus. In a study, the male oral cancer rate in France was nearly seven times higher than for men in Greece [4]. The lifetime risk for the development of oral and pharyngeal cancer in Europeans is estimated at 1.85% for men and 0.37% for women. Incidence rates are higher in Western European countries than in northern Europe. However, the highest mortality rates are reported in Eastern Europe.

As far as the distribution of lip cancer is concerned, the scientific literature shows various statistics. In Madrid, according to gender, lip cancer occurred predominantly in males (749 cases, 85%, $X^2: 432.1, P < 0.001$). Women accounted for 15% of the total (132 cases). The male-female ratio was 5.6:1.

NMSC care costs are ranked fifth after prostate, lung, colon and breast cancers. Costs for NMSC care depend on 2 factors: care types and treatment options. Treatment options include surgical excision, microscopically controlled surgical excision (Mohs surgery), radiotherapy, curettage, cryosurgery, photodynamic ablation and topical chemotherapy [5].

Regarding the relative survival estimates for patients with oral cancers, the overall estimate for five years as far as lip cancer is concerned (ICD-10: C00) was 86.5%. Survival decreased from 85.7% in patients aged 15-64 years to 79.9% in patients aged 65-74 years and eventually again increased to 93.8% for patients with age 75 or older. Relative survival was higher in males (88.1%) than in females (81.2%). Tumors and grade have contributed to the survival rate. It ranges between 86.7% (local stage) and 62.0% (regional stage), and between 93.0% (grade 1) and

70.9% (grades 3 and 4). The overall survival estimate at five years for patients with tongue cancer (ICD-10: C01-02) was 48.1% [34].

2. ETIOLOGICAL FACTORS. RISK FACTORS.

Basal cell carcinoma is the most common form of skin cancer, which accounts for about 75% of skin malignant tumors [6]. Chronic exposure to sunlight is considered to be the main cause of these lesions [7]. CBC is more common in people over 40 with a history of chronic sun exposure, the lesions being often on the face [6-8].

Risk factors for lip cancer have been reviewed by many authors [9-12]. These factors include smoking (especially pipe), actinic radiation, trauma, fishing, inflammatory processes, viral infections and endogenous factors [13]. On the other hand, there is supposed to be an etiological similarity between lip cancer and skin cancer: actinic radiation has been suggested as a risk factor for both cancers [14-17].

3. BASAL CELL CARCINOMA PROGNOSTIC FACTORS

The treatment of head and neck cancer requires an exact stratification of risk to determine the type and extent of the therapy and the expected clinical outcome.

Physical examination, diagnostic imaging studies and pathological examination allow the clinician to determine the size and magnitude of the primary tumor, the state of cervical lymph nodes and the probability of removing a metastatic disease, thus generating a precise TNM staging, depending on the primary tumor, the lymph nodes and the metastasis for each patient. In addition to TNM staging, other clinical and pathological factors that are not systematically incorporated into the staging system have been shown to influence the response to therapy and the probable outcome.

These factors can be classified as follows:

1. prognostic factors related to the primary tumor;
2. prognostic factors related to cervical lymph nodes;
3. prognostic factors related to the patient's demographics;
4. prognostic factors related to the patient's medical condition [18].

Welsch et al. highlighted the correlation of the different histopathological subtypes with the invasion depth in 100 biopsied CBC specimens and found that the micronodular tumors had the highest average depth, followed by infiltrative, nodular, and superficial subtypes. In another study, correlating the depth of the invasion with

the histopathological subtypes of CBC, the Clark level was comparable between micronodular and infiltrative CBC, while nodular CBC showed a more superficial level compared to both groups [19].

4. CLINICAL AND STATISTICAL STUDY OF THE LIP TUMOURS AND OF THE UPPER LIP BASAL CELL CARCINOMA

A retrospective study on patients admitted to the Oro-Maxillofacial Surgery Department of the County Emergency Clinical Hospital of Craiova was carried out during the period 2012-2016, who presented the lip tumor diagnosis and for whom the surgical procedure was performed.

The main clinical subtypes of CBC are: nodular, superficial and sclerodermiform. From time to time, combinations of these two latter types can occur with nodular form. Variable quantities of melanin may be present in these tumors, which are often referred to as pigmented CBC [20].

There are several options for the treatment of upper lip basal cell carcinomas :

- surgical:
 - Standard excision;
 - Mohs micrographic excision.
- destructive:
 - electrodesiccation;
 - curettage;
 - cryosurgery;
- non-surgical:
 - topical therapy: imiquimod;
 - 5-fluorouracil;
 - photodynamic therapy;
 - radiotherapy.

THE CONCLUSIONS OF THE CLINICAL-EPIDEMIOLOGICAL STUDY:

- Nodular basal cell carcinoma is the most commonly met clinical subtype, accounting for 50-79% of the total CBC;
- Standard excision with a predetermined margin is useful for CBC, this being also the method of treatment applied in our clinic;

- 62% of the subjects diagnosed with tumorous pathology were men and 38% were women;
- The age ranged between 6 and 92 years old, with an average age of 61;
- The distribution of the participants to the study, according to their home residence, showed that the majority of the subjects came from rural areas;
- As far as the etiology of lip cancer is concerned, the most frequently mentioned factors are solar radiation, smoking and viruses;
- In this study, 64% of the subjects were farm workers, so the prevalence of lip cancer was higher in subjects exposed to solar radiation;
- Lip cancer is commonly associated with smoking, as shown in this study;
- With regard to the main epidemiological characteristics, in subjects diagnosed with basal cell carcinoma of the upper lip, we found that 37% of the subjects in this study were under 60 years of age, while 63% were aged over 60;
- The gender distribution study showed that upper lip CBC was encountered in 59.26% of cases for females and in 40.74% for male cases;
- From the point of view of the topography of the lesion, the results revealed that the lesion has only spread to the tegument for 90.74% of the cases and was also found at the vermilion level for 9.26% of the cases.

5. HISTOPATHOLOGICAL STUDY OF THE UPPER LIP BASAL CELL CARCINOMA

The investigated material was human material, coming from the County Clinical Emergency Hospital (SCJU) in Craiova, consisting of surgical exeresis parts, obtained in the Oro-Maxillofacial Surgery, ENT Surgery, Plastic Surgery and Dermatology departments. This material was subsequently sent to the Pathological Anatomy Laboratory of the same hospital for histopathological diagnosis, during 2012-2016. In the histopathological study of investigated casuistry we were interested in the macroscopic and microscopic parameters, namely macroscopic variables such as: dimensions, appearance, color, consistency, aspect on the sectional area, the existence of necrosis zones, hemorrhagic areas and the presence of adjacent structures invasion and microscopic variables respectively: histopathological type, histopathological subtype, tumor growth pattern, cytological

differences of studied tumors, the aspect of tumor stroma, the presence/absence of lympho-vascular invasion, the presence/absence of perineural invasion, the presence/absence of the invasion of surgical resection limits.

THE CONCLUSIONS OF THE HISTOPATHOLOGICAL STUDY OF THE INVESTIGATED CASUISTRY:

- In the time span 2012-2016, basal cell carcinoma of the upper lip accounted for about 49% of the total clinical lesions developed with this localization;
- From a histopathological point of view, we recorded the presence of 7 subtypes of basal cell carcinoma: nodular, adenoid-cystic, micronodular, keratotic, mixed, superficial and sclerodermiform carcinoma;
- The most common histopathological subtype that we diagnosed in the upper lip area was the nodular type of basal cell carcinoma (39%), followed at a certain distance by the adenoid-cystic subtype (17%), and the last places were superficial and sclerodermiform types, each with 7.4%;
- The histopathological aspects common to the various subtypes of basal cell carcinoma consisted of: 1) basal cell-like neoplastic cells, small, cubic, with a round-oval, trachychromatic nucleus, surrounded by a small amount of amphophilic cytoplasm; 2) the peripheral palisade in the shape of a “small fence” of the neoplastic cells; 3) artifacts retractions of the peritumoral stroma; 4) in many cases, the origin of the tumor was highlighted in the surface epithelium;
- Except for the nodular and superficial types, the other varieties, given the particular histological aspects they have presented, have often imposed a differential diagnosis with a number of other neoplastic or non-neoplastic lesions that may develop in the area;
- In a fifth of the investigated casuistry, the invasive local character was remarked, most of them invading the deep dermis and hypodermis and 45% of these cases the underlying striated muscle was also invaded;
- From a histopathological point of view, the most invasive forms were sclerodermiform (100% of the cases), followed by the micronodular type (2/3 of the cases) and the mixed form (60% of the cases);

- As differences in the aggressiveness between the cases of mixed basal cell carcinoma have been highlighted, the solid-sclerodermiform associations proved to be much more invasive locally than the solid-micronodular associations respectively; it is thus necessary in such cases to establish with certainty the form associated with the most aggressive potential;
- In 10% of the cases investigated, we recorded the existence of at least one resection limit invaded by the tumor. These cases corresponded to the most aggressive forms of basal cell carcinoma, namely: micronodular, sclerodermiform and mixed forms.

6. IMMUNOHISTOCHEMICAL STUDY OF THE LOCAL INVASIVENESS OF THE UPPER LIP BASAL CELL CARCINOMA

Immunohistochemical investigations were performed for 24 cases which were selected from the 54 upper lip basal cell carcinomas, which were the subject of the histopathological study. The choice of cases was made in such a way as to be representative for the studied casuistry.

In this study, the paraffin blocks used in the previous histopathological study were also used and serial sections of 4 µm thickness were performed out of them, which in turn were applied to electrostatically charged glass blades. After being dried at laboratory temperature, the sections were dewaxed and rehydrated.

THE CONCLUSIONS OF THE HISTOPATOLOGICAL STUDY ON THE INVESTIGATED CASUISTRY:

- Tumor immunomarking was more evident in the markers Twist1, COX-2 and WASL, immunoreactivity scores reaching the maximum value, IRS = 12;
- At the opposite end, there was the tumor reactivity for markers MT1-MMP, CD56 and maspin, the scores obtained not exceeding the IRS = 4 value;
- A somewhat intermediate reactivity was obtained for CXCR4 and alpha - SMA markers, the maximum recorded scores not exceeding IRS = 8-9 values;
- Investigating the intrinsic potential for local invasion of the upper lip basal cell carcinomas indicated an immunoprofile of the type: CXCR4 + / MT1-MMP + / CD56 + / Cox-2 + / Maspin, - which was present in cases of net invasion of the hypodermis and of the subcutaneous striated muscle;

- Such an immunoprofile was more evident at the tumor invasion front and at the periphery of the neoplastic proliferation;
- Investigating immunohistochemical analysis with the help of the N-cadherin marker of the acquisition of a mesenchymal phenotype by tumor cells showed the existence of such a process, especially in the invasion front of invasive basal cell carcinoma, in the cells at the periphery of tumor proliferation and obviously in the sclerodermiform forms and micronodulation;
- The tumor immunoreactivity for Twist-1 has highlighted the existence of an active epithelial-mesenchymal transition process in the invasive forms of basal cell carcinoma, especially in sclerodermiform, micronodular and mixed variants;
- The investigation of the intrinsic motility of tumor cells with the help of WASL markers and alpha-SMA has highlighted the existence of such a WASL + / alpha-SMA + profile, especially in the sclerodermiform, micronodular and mixed forms of basal cell carcinoma and especially to the invasion front;
- Positive immunomarking for CXCR-4, MT1-MMP, Twist-1, COX-2, alpha-SMA and WASL markers in tumor stromal micro medium reveals certain parenchymal tumor-stromal interactions to facilitate local invasiveness of upper lip basal cell carcinomas;
- This latter aspect was more evident in the invasion front of the sclerodermiform, micronodular and mixed basal cell carcinoma;
- Following immunohistochemical investigation, we identified a group of basal cell carcinomas with high local aggressiveness that belonged to sclerodermiform, micronodular and mixed histopathological types. Thus, a CXCR4 + / MT1-MMP + / CD56 + / Cox-2 + / Twist-1 + / N-cadherin + / alpha-SMA + / WASL + / Maspin immunoprofile has a prognostic value which identifies cases with the greatest local invasive potential;
- At the same time, some of these markers may be the target of potential therapies that would greatly reduce the rate of mortality, recurrences and complications that may occur in the case of such patients.

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