Prevalence and histopathological types of skin carcinomas in Arges County, Romania

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Abstract

Non-melanoma skin cancers presented a significant incidence increase in the last decades, worldwide. Even though the impact upon mortality is a relatively low one, through the incidence increase, their impact upon the public healthcare systems is a considerable one. In our study, we evaluated 109 cases of skin carcinomas hospitalized during 2012 in the Department of Plastic Surgery of the Emergency Hospital of Pitesti, Romania, for a surgical treatment. The gender distribution showed slight lesion predominance in women, being recorded 56 (51.38%) tumors in women and 53 (48.62%) in men. The highest incidence of skin carcinomas (75.23%) was recorded in the persons aged over 60-year-old. Of 109 cases of skin carcinomas, 80 (73.4%) carcinomas developed on indignant tegument areas, while 29 (26.6%) on premalignant skin lesions (fiberconjunctive papillomas, keratocantomas, keratosic verrucas). The histopathological study highlighted the fact that of 109 skin carcinomas, 87 (79.82%) were basal cell carcinomas and only 22 (20.18%) were squamous cell carcinomas. The immunohistochemical reaction to 34βE12 cytokeratin was highly positive in the cells of the basal cell carcinomas and well-differentiated squamous cell carcinomas (except for the "keratosic pearls") and moderately positive in the moderately differentiated squamous cell carcinoma.

Keywords: non-melanoma skin cancers, basal cell carcinoma, squamous cell carcinoma, solar radiations, 34βE12 cytokeratin.

Introduction

By a high morbidity and obvious severity, cancer represents a real and current issue of public health all over the world. In developed countries, cancer is the second cause of death after cardiovascular diseases [1]. Worldwide, there has been observed an increase of the malignant neoplasias incidence. If in 1991 the global incidence of cancer was of 210.5 in 100 000 inhabitants, in 2007 it went up to 254.5 in 100 000 inhabitants [2]. In the last 15 years, the increase of every cancer type incidence was extremely variable. For example, at a global level, there increased the incidence of colon, thyroid and lung cancer and there decreased the incidence of gastric and cervix cancer.

Within tumoral pathology, skin cancers represent approximately 20% of the new cases of malignant neoplasia, their values being much higher in the areas where external carcinogenic factors (such as solar radiations) manifest with a high intensity (Texas 33%, Australia >50%) [3]. Non-melanoma skin cancers (NMSC) are the most frequent malignant skin tumors diagnosed in the United States of America [4], with approximately 900 000–1 200 000 new cases diagnosed every year [5].

The two major types of non-melanoma skin cancers (NMSC), the basal cell carcinoma (BCC) and the squamous cell carcinoma (SCC), showed dramatic increases of their incidence in the last decades in the USA [6, 7]. For example, in New Hampshire, there were mentioned SCC incidence increases of 235% in men and 350% in women and more than 80% of BCC, both in men and in women aged over 14-year-old [8]. Even though the impact upon mortality is relatively low, through incidence increase, their impact upon the health public systems is a considerable one.

In Romania, at the end of 2005 there were recorded 354 572 patients with malignant tumors, of which 10.8% had skin localization [3]. In Romania, the incidence of skin tumors also varies from one geographical area to another.

In the present study, we proposed an evaluation of the skin cancers hospitalized during 2012 in the Department of Plastic Surgery within the Emergency Hospital of Pitești, Argeș County, Romania.

Materials and Methods

The studied group included 176 patients that were hospitalized in the Department of Plastic Surgery of the Emergency Hospital of Pitești between January 1 and December 31, 2012, for the surgical treatment of some skin tumors. The clinical statistical analysis of the studied group aimed at: the gender and age of patients, localization of skin lesion, existence of some precancerous lesions of
the histopathological type. The clinical and paraclinical data were taken from the observation records, the Hipocrate software system, surgical protocols, laboratory test records and registers of anatomical pathology. For highlighting some important clinical aspects, these data were transposed into charts.

Regarding the patients, after obtaining the informed consent, there was performed a surgical removal of the tumor, in accordance with the oncological safety limits. The resection samples were immediately fixed in 10% neuter formalin and sent to the Anatomical Pathology Laboratory of the Hospital of Pitești and to the Research Center for Microscopic Morphology and Immunology of the University of Medicine and Pharmacy of Craiova, Romania.

The preparation of the biological material was performed through the classical histological technique of paraffin inclusion. The paraffin-included material was sectioned with a microtome (Microm HM350) equipped with a special section transfer system (STS) on a water bath. There were performed histological sections with a thickness of 4 μm that were harvested on special blades covered with a layer of positive amino acid residues, poly-L-Lysine layered blades (Sigma), for the purpose of increasing the section blade confluence. After a rapid dry period of 5–10 minutes on a hot plate, the sections were transferred into an incubator at 60°C and preserved overnight, during which the biological material perfectly adhered to the surface of the slide histological blade. For the histopathology studies, we used the Hematoxylin–Eosin (HE) staining, while for the immunohistochemistry studies we used the following antibodies (Table 1).

Table 1 – Antibodies used in our study

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Target</th>
<th>Host species</th>
<th>Isotype Clone</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK 34βE12</td>
<td>High molecular weight cytokeratin</td>
<td>Mouse</td>
<td>IgG1 kappa</td>
<td>34βE12 1:100</td>
</tr>
<tr>
<td>Ki-67</td>
<td>Proliferation marker</td>
<td>Mouse</td>
<td>IgG1 kappa</td>
<td>MIB-1 1:50</td>
</tr>
<tr>
<td>P53</td>
<td>Wild-type p53 protein</td>
<td>Mouse</td>
<td>IgG2b kappa</td>
<td>DO-7 1:50</td>
</tr>
</tbody>
</table>

Through the immunohistochemistry studies, we wanted to highlight the differences between the two types of skin cancers regarding their proliferative activity, TP53 gene alteration, alteration of some cytokeratins in the neoplastic cell structure.

Results

During 2012, in the Department of Plastic Surgery of the Emergency Hospital of Pitești there were hospitalized and surgically treated 176 patients with skin tumors, of which 109 (61.93%) cases were skin carcinomas, and the rest of 67 (38.07%) cases were represented by melanomas, lipomas, cysts, verrucas, pigment nevi (Figure 1). Of the 109 patients with skin carcinomas, 102 (93.58%) patients needed hospitalization for a continuous period of 5–7 days and only seven (6.42%) patients required a day hospitalization period.

The gender distribution (Figure 2) of skin carcinomas showed slight lesion predominance in women, being recorded 56 (51.38%) tumors in women and 53 (48.62%) in men. At the level of the entire tumoral cases, the difference between men and women was a similar one.

Figure 1 – Number of skin epitheliomas of the tumoral cases.

Figure 2 – Gender and age distribution of skin carcinomas in the studied group.

The age distribution of the cases allowed us to establish that skin carcinomas emerged in the patients aged between 20 and over 80-year-old. The number of individuals with skin carcinomas increased with age (Figure 2): if in the age group between 21 and 30-year-old there was only one case, in the age group between 71 and 80-year-old there were 57 cases. In our study, the most affected age group, both for men and women, was the one between 71 and 80-year-old, followed by the one between 61 and 70-year-old. In these age intervals there were recorded a number of 82 cases, representing 75.23% of the studied group. We may state that the persons aged over 60-year-old are the ones presenting the highest risks of developing a skin carcinoma.

Regarding the localization of skin carcinomas, most of them were found at face level. At the level of the frontal, malar, temporal and nasogenian regions there were recorded 53 (48.62%) cases; at nose level 16 (14.68%) cases, at ear level nine (8.25%) cases, at eyelid, upper and lower limbs five (4.59%) cases each and other localizations four (3.68%) cases (Figure 3). We may easily observe that most of the skin carcinomas manifested at scalp level, with a number of 91 (83.49%) cases, while at trunk and limbs level there were recorded only 18 (16.51%) cases (Figure 3). It should also be noticed the relatively significant percentage of the localization of skin epitheliomas at nose level, the emergence of tumoral lesions at this level being mostly due to the fact that the nose is the most prominent extremity of the human body and, therefore, it is the most exposed to sunlight, known as etiopathogenic factors in skin tumoral pathology.

Knowing the fact that some of the skin tumors locate on the site of a preexistent lesions, we also investigated this aspect in our study and, from the anamnesis and from the analysis of some medical records, as well, we
observed that in 29 (26.6%) patients skin carcinomas developed from other lesions, namely: 15 (13.76%) developed from fibroconjunctive papillomas, nine (8.25%) from keratoacanthomas, while five (4.59%) from keratosic verrucas (Figure 4).

Figure 3 – Distribution of tumoral lesions according to the anatomical region (face, nose, ears, lips, eyelids, trunk, upper and lower limbs, other regions of the body).

Figure 4 – Preexistent lesions from which there developed skin carcinomas.

The analysis of skin carcinoma relapses in the studied group highlighted that of the total of 109 surgically treated cases, there were recorded two local relapses, both being well-differentiated spinocellular carcinomas, one located retro-auricularly in a 74-year-old man, and a second one located at nasal pyramid level in a 70-year-old woman. Both carcinoma cases arise at a period of three and four months, respectively, from the first surgical intervention.

The histopathological and immunohistochemical study highlighted that of the 109 skin carcinomas, 87 (79.82%) were basal cell carcinomas and only 22 (20.18%) were squamous cell carcinomas ones (Figure 5). Of the 22 squamous cell carcinomas, 17 were well differentiated and only five were moderately differentiated.

Basal cell carcinomas appeared as basophil cellular islands or cords, similar to the cells in the basal strata of the epidermis, poorly differentiated, with big, round or oivalary nuclei and with little cytoplasm (Figure 6). The island or cellular cords were well demarcated by a structure with basal membrane function that separated them from the surrounding conjunctive tissue. The arrangement of tumoral cells was a particular one, in that the cells at the tumoral island margins had a relatively ordered arrangement, “in a palisade”, with parallel nuclei among them and perpendicular on the membrane structure, while the cells inside the islands had a disordered arrangement.

The squamous cell carcinomas appeared made of polyhedral cells, similar to those from the Malpighi spinous layer of the epidermis, arranged as islands or cellular cords, poorly demarcated. The differentiation degree of these tumors may be a variable one. In our study, most of the squamous cell carcinomas were well differentiated, generating “keratosic pearls” or patterns of “keratosic pearls” (Figures 7 and 8).

The histological analysis highlighted, both in the basal cell carcinomas and in the squamous cell carcinoma ones, the presence of numerous atypias and nuclear monstrosities, more frequent in the moderately differentiated forms.

The tumoral stroma was different from the normal structure of the dermis. In basal cell carcinomas, the stroma was rich in fibroblasts and in collagen fibers with a tendency of organizing in fascicles, placed around the islands of neoplastic cells; in the squamous cell carcinomas the stroma had a character of lax conjunctive tissue, strongly infiltrated by round mononuclear cells of the lymphocyte and macrophage type. The stroma vascularization of the tumor appeared more intense in the squamous cell carcinomas, especially where the inflammatory infiltrate was more abundant and less developed in the basal cell carcinomas.

The evaluation of the reaction to 34\(\beta\)E12 cytokeratin showed that all the cells of the basal cell carcinoma were positive, the intensity of the reaction being directly proportional with the cytoplasm quantity in every cell (Figure 9). In squamous cell carcinomas, the reaction to 34\(\beta\)E12 cytokeratin was quite intense in the basal and parabasal strata, but it was absent at “keratosic pearls” level (Figure 10), while in moderately differentiated squamous cell carcinomas the reaction was present in all the cells, but it was less intense proving that in this type of carcinomas cytokeratins 1, 5 10 and 14 are more poorly represented in the cytoplasm of tumoral cells (Figure 11).

Using the Ki67 cellular proliferation antibody, allowed us to observe that all the basal cell skin carcinomas were intensely positive to this antibody (Figure 12), while the well-differentiated squamous cell carcinomas were moderately positive (Figure 13); moderately differentiated carcinomas were also intensely positive to Ki67 (Figure 14).

The immunohistochemical study of p53 protein showed a weak reaction in the case of basal cell carcinomas (Figure 15), while in the squamous cell carcinomas ones, the reaction was an intense one (Figure 16).
Figure 6 – Islands of basal cell carcinomas placed in a stroma rich in fibroblasts and collagen fibers. HE staining, ×100.

Figure 7 – Well-differentiated squamous cell carcinoma, made of tumoral islands generating “keratotic pearls” included in a well-vascularized tumoral stroma, made of lax conjunctive tissue strongly infiltrated with inflammatory cells. HE staining, ×200.

Figure 8 – Microscopic image of a moderately differentiated squamous cell carcinoma, made of fusiform, ovalary, polyhedral cells of various sizes, with a tendency of organizing in cellular cordons or islands. HE staining, ×200.

Figure 9 – Basal cell carcinoma with an intense reaction to 34βE12 cytokeratin. Immunomarking with 34βE12 anti-cytokeratin antibody, ×200.

Figure 10 – Spinocellular carcinoma with an intense reaction to 34βE12 cytokeratin. Immunomarking with 34βE12 anti-cytokeratin antibody, ×200.

Figure 11 – Microscopic image of moderately differentiated squamous cell carcinoma with a moderately positive reaction to 34βE12 cytokeratin. Immunomarking with 34βE12 anti-cytokeratin antibody, ×200.
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Figure 12 – Basal cell carcinoma with an intense reaction to Ki67. Immunomarking with anti-Ki67 antibody, ×200.

Figure 13 – Well-differentiated squamous cell carcinoma with a moderately positive reaction to Ki67 antibody. Immunomarking with anti-Ki67 antibody, ×200.

Figure 14 – Moderately differentiated squamous cell carcinoma with an intense reaction to Ki67 antibody. Immunomarking with anti-Ki67 antibody, ×200.

Figure 15 – Basal cell carcinoma with a weak reaction to p53. Immunomarking with the anti-p53 antibody, ×200.

Discussion

Skin carcinomas, although emerge in easily to observe regions, have lots of social and economical implications, due to the fact that they are diagnosed quite late, a fact that imposes a complex treatment and an oncological monitoring for several years. Also, they have an aggravating potential upon the individual’s health and life quality.

The incidence of skin carcinomas presents a great variability from one geographical area to another. Most of the cases appear in the white population, in areas with the highest values of solar UV radiations [7]. More clinico-statistical studies showed that this neoplasia incidence is ever growing in various geographical areas: USA [8–11], France [13] and even in North-European countries [14, 15].

In our study, we observed that, of all skin carcinomas, the non-melanoma carcinomas were the most frequent ones, representing 61.95% of all hospitalized tumor cases. Of the two types of skin carcinomas, the basal cell carcinoma was the most frequently met type of skin tumor (79.82%), while squamous cell carcinomas represented only 20.18%. The great majority of the studies emphasized the fact that the basal cell carcinoma is the most frequently met type of skin cancer in white individuals, representing up to 80% of the non-melanoma skin cancers.
[16], and this tumor incidence is in a continuous growth [16–18].

Regarding the gender distribution of skin carcinomas, in our study, we observed slight lesion predominance in women (51.38%). Other studies [1] showed that, in certain geographical areas, skin carcinomas affect more the men. The increase of skin carcinoma incidence in women may be due to the exposure to the same life, work and environment factors of both sexes.

Our study showed that the incidence of skin carcinomas increases with age, most of the cases (82, representing 75.23% of the whole group) being recorded in persons over 60-year-old. These data lead us to the idea that the etiopathogenic factors of skin carcinomas have a cumulative effect and have a clinical expression in old persons due to the decrease of the defense and recovery ability of the organism.

In our study, most carcinomas were detected at head and neck level (83.48%), in the area exposed to solar radiations, which confirms the hypothesis that solar radiations represent the most important etiopathogenic factors.

Numerous studies showed that exposure to UV radiations of the sun or generated by artificial sources determined the increase of skin carcinoma incidence [3, 5, 19, 20]. Chronic exposure of the skin to ultraviolet radiations determines DNA lesions and mutations at the level of tumoral suppressor genes, which leads to a malignant transformation of keratocytes. Ultraviolet radiations act through some free oxygen radicals [21, 22] while B ultraviolet radiations directly act upon the cellular DNA, triggering ruptures of the genetic material or the formation of mutagenic biochemical compounds [23]. Moreover, ultraviolet radiations may promote tumorigenesis by other mechanisms, as well, such as immune-suppression and inhibiting the macrophage migration [24, 25].

The emergence of basal cell carcinomas may intervene on healthy teguments or on preexistent lesions (chronic radiodermites), or may be preceded by benign tumors with a basal cell structure with a transformation potential into invasive basal cell epithelioma (basocellular nevus, premalignant fiberepithelioma). In our study, premalignant lesions were present in 29 (26.6%) patients and were represented by fibroconjunctive papillomas, keratoacanthomas and keratoacanthomas.

Although skin carcinomas may be completely cured, we consider that the disease has a lethal potential, especially the squamous cell carcinoma that may lead to metastases. That is why it is required an early diagnosis of the lesion, the treatment and monitoring of skin premalignant lesions and the intensification of medical education activities regarding the harmful effects of prolonged exposure to ultraviolet radiations.

Conclusions

Of a total number of 176 skin tumors hospitalized and surgically treated during 2012 in the Department of Plastic Surgery of the Emergency Hospital of Pitești, Romania, 109 (61.93%) tumors were represented by skin carcinomas, while the rest of 67 (38.07%) cases were represented by melanomas, lipomas, cysts, verrucas, pigmenitary nevi. The highest incidence was observed in the age group between 71 and 80-year-old (52.29%). Of 109 skin carcinomas, 87 (79.82%) were represented by basal cell carcinomas and only 22 (20.18%) were squamous cell carcinomas. Eighty (73.4%) carcinomas developed on indigent tegument areas, while 29 (26.6%) on premalignant skin lesions (fiber conjunctive papillomas, keratoacanthomas, keratotic verrucas).

References

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