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Research article

Evaluation of c-Kit (CD117) expression in patients with squamous cell carcinoma (SCC) and basal cell carcinoma (BCC) of the skin

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Abstract: Squamous cell carcinoma (SCC) and Basal cell carcinoma (BCC) of the skin are the most common malignant tumors in humans. The c-Kit (CD117) is a tyrosine kinase receptor protein that affects the behavior of some tumors and can be a target for new treatments. This study was designed to determine the expression of CD117 in patients with SCC and BCC. In this retrospective study, 69 paraffin blocks of specimens with a diagnosis of SCC and BCC from limbs, head/neck, trunk, and unknown sites were selected. Tumor tissue samples of 40 SCC and 29 BCC cases were then analyzed by immunohistochemistry. The monoclonal CD117 antibody was used. The severity and extent of tumor staining were grouped as follows: negative; weakly positive; moderately positive; and strongly positive. The CD117 was detected positive in 26 (89.7%) BCCs and only in 5 (12.5%) of SCCs. We detected a significant difference between the expression of CD117 in patients with SCC and BCC ($P < 0.05$). No significant relationship was shown between the marker expression and the lesion site in patients with SCC or BCC ($P > 0.05$). On the contrary, a significant relationship between CD117 expression and histopathologic grading was identified in patients with SCC ($P < 0.05$). According to our results, the

expression of CD117 is significantly increased in BCCs than SCCs and this may be of benefit for diagnostic purposes in challenging cases and also for therapeutic purposes including targeted therapy if indicated.

Keywords: squamous cell carcinoma; basal cell carcinoma; immunohistochemistry; c-Kit; CD117

1. Introduction

Keratinocyte carcinomas account for the most common human malignancy and are composed by different entities, mainly represented by the Basal Cell Carcinoma (BCC) and cutaneous Squamous Cell Carcinoma (cSCC) [1]. Regarding frequency, BCC is much more common than SCC, but the latter has higher mortality [1]. Most cases of cSCCs present on the head/neck and upper limbs, while most cases of BCCs present on the head/neck and trunk [2]. The incidence of cSCC may be underestimated [3]. The stem cell/mast cell growth factor receptor (SCFR), also called proto-oncogene c-Kit or CD117 or tyrosine-protein kinase kit, is a tyrosine kinase receptor protein encoded by the KIT gene in humans [4]. This tyrosine kinase is mainly expressed in melanocytes, mastocytes, and germinal cells [5–7], but also in secretory cells of the breast, basal layer of the epidermis, thymic epithelial cells, interstitial cells of Cajal, and spermatogonia [6]. CD117 receptor has a role in cellular differentiation, proliferation, and anti-apoptotic properties. As it may be expressed in some tumors, it has a role in tumor occurrence, development, metastasis, and recurrence [4]. Results of previous studies in the expression of CD117 in SCCs and BCCs are controversial [6,8–12]. The CD117 pathway is a target for many therapeutic agents (so-called targeted therapy; including Imatinib mesylate) [4,6,13]. It is clear that evaluating the expression of CD117 in patients with SCC and BCC can result in diagnostic and therapeutic repercussions. Therefore, we aimed to evaluate the expression of CD117 in patients with SCC and BCC of the skin.

2. Material and method

The study is approved by the Ethical Committee of Kermanshah University of Medical Sciences, Kermanshah, Iran (Code: IR.KUMS.REC.1396.632). The study population consisted of paraffin blocks with SCC and BCC retrospectively identified in two centers, Imam Reza Hospital and Special Clinic Kermanshah University of Medical Sciences. Histological diagnosis of SCC and BCC was performed by a previous biopsy and microscopic examination of the target tissue by a pathologist and reconfirmed by a dermatopathologist. Ten normal skin samples derived from mammoplasty specimens were used and stained as a normal control for comparison. Initially, paraffin blocks were obtained from tumor and control tissue specimens and fragments of 4 μm thickness were prepared from these blocks. Rabbit anti-human CD117 Monoclonal Antibody (Clone EP10) from Master diagnostic, Granada (Spain) as primary monoclonal antibody was used. Incubations of time, temperature, and antibody concentration were performed according to the kit instructions. After washing the slides with saline phosphate buffer, the secondary antibody was poured onto the slides and then streptavidin bound to HRP (Horse Radish Peroxidase) was used. Then, the tissues were stained with hematoxylin for light microscopy. Slides were

blinded by two individuals in 10 fields and studied in the same conditions. Light microscopy with a magnification of 400 times was used to count the number of cells and estimate the percentage of positive cells. Based on the intensity and percentage of staining of the cells, the slides were classified into negative, weak, medium, and strong positive degrees. Melanocytes and mast cells were used for internal control and color intensity comparison. Cases stained like these internal controls considered as 2+. The cells with less or more staining intensity than controls considered as 1+ and 3+, respectively. The percentage of total cells in the tumor tissue was estimated. Sweat ducts, melanocytes, and mast cells were stained as internal controls. According to the work of Went PT et al. [6], the severity and extent of tumor staining were grouped as follows:

Negative: No staining;

Weakly positive: 1+ staining intensity in less than 60% of cells and 2+ intensity in less than 30% of cells;

Moderately positive: 1+ staining intensity in ≥60% of cells and 2+ intensity in 30–79% of cells and 3+ intensity in less than 30% of cells;

Strongly positive: 2+ intensity in ≥80% of cells or 3+ intensity in ≥30% of cells.

Only membrane or cytoplasmic staining was considered for analysis, as cytoplasmic staining alone was proven to be false positive (Figure 1).

Finally, ten mammoplasty skin samples were stained as normal control for the basal layer and sweat glands (Figure 2). Positive controls were selected during the work of confirmed strong positive melanoma specimens. Then all demographic data along with the results of the tests were entered into SPSS software and the relevant analyzes were performed with the help of a biostatistician.

Statistical analysis

After entering data in SPSS 20 software, CD117 expression in SCC and BCC samples was determined. A comparison of CD117 expression between SCC and BCC was performed by the Chi-square test. The mean and standard deviation were used to describe quantitative data. The Kolmogorov-Smirnov test was used to investigate the normality of the distribution of continuous quantitative variables. A comparison of quantitative data between groups was performed using the t-test and Mann–Whitney test if not normal distribution.

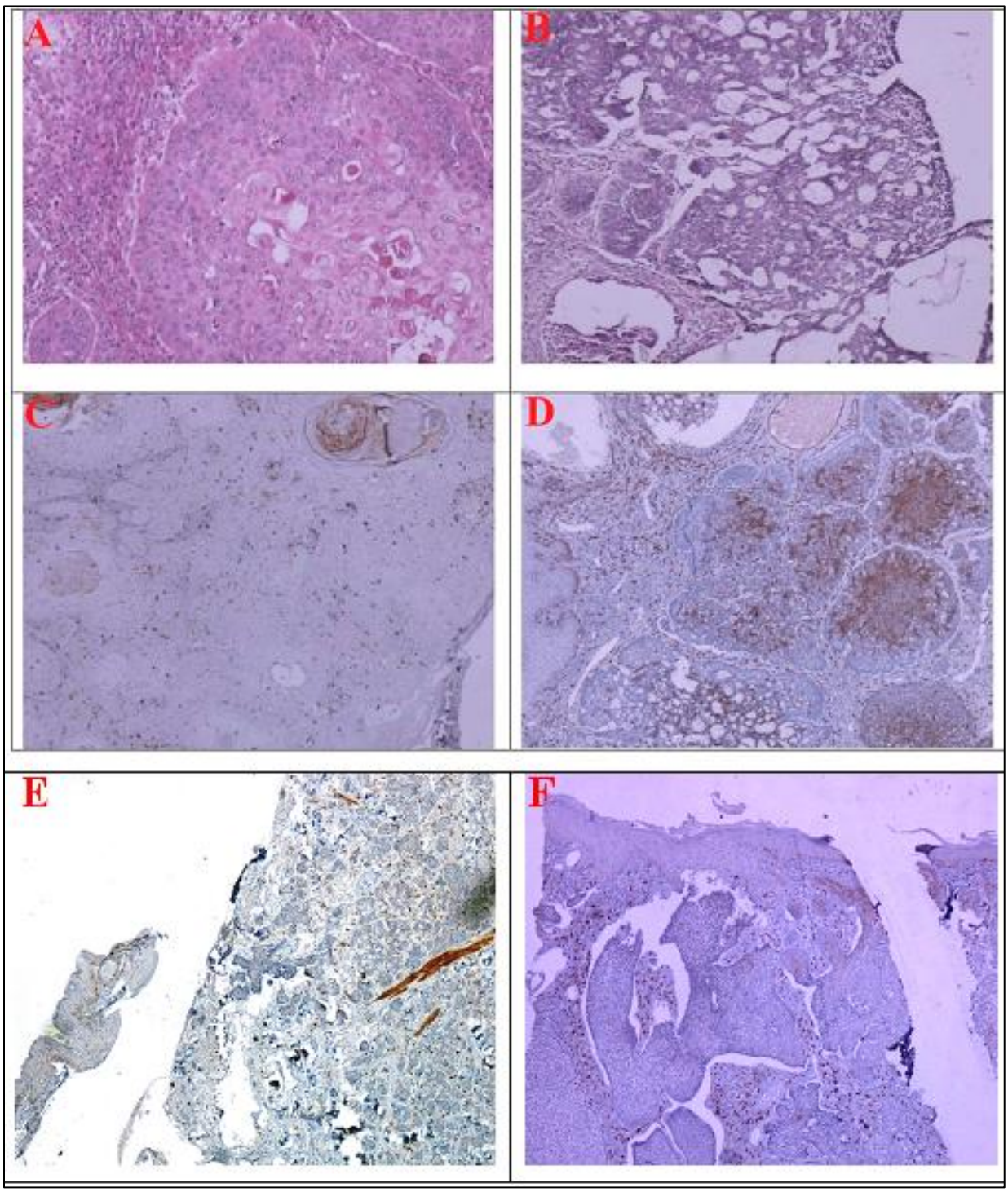

Figure 1. Hematoxylin-Eosin staining and Immunohistochemistry for CD117. Hematoxylin-Eosin staining (X100 magnification): (A) SCC and (B) BCC, Immunohistochemistry (X40 magnification), (C) Weak positivity in SCC, (D) Moderate positivity in BCC, (E) Negativity in BCC, and (F) Weak positivity in BCC.

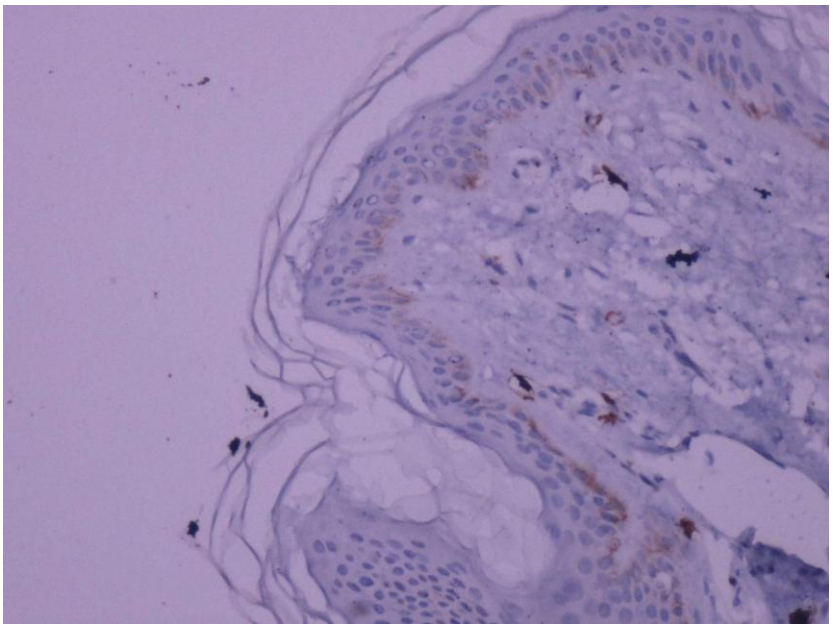

Figure 2. Immunohistochemistry for CD117. Mammoplasty control sample (X200 magnification).

3. Results

In the present study, 40 cSCC paraffin blocks and 29 BCCs paraffin blocks were included. Descriptive and comparative characteristics of age and gender of the patients, lesion site, and CD117 expression in SCCs and BCCs are summarized in Table 1. According to the results of Table 1, no significant difference was detected between patients' age and gender in SCC and BCC (P-value of 0.851 and 0.382 for age and sex respectively). A Chi-square test was used to compare the site of lesion in patients with SCC and BCC. According to the results, a significant difference between the lesion site of patients with SCC and BCC was shown ($P = 0.035$), unless both the neoplasms were mainly located in the head and neck region (BCC: 72.4%; SCC: 67.5%). Furthermore, a Chi-square test was used to compare the expression of CD117, with the above mentioned quantitative classification, in patients with SCC and BCC. A significant difference between CD117 expression in patients with SCC and BCC was obtained ($P < 0.001$). BCC patients showed a significantly higher positivity for CD117 in comparison with those affected by SCC. On the contrary, strong positivity for CD117 was seen in none of the samples from patients.

Histopathology grading of patients with SCC consists of 3 groups with well-, moderate-, and poorly-differentiated. Since in 4 small specimens the grading was not possible, a group of neoplasms with unknown differentiation was added. Thirty (75%), 4 (10%), and 2 (5%) of SCC patients showed well-, moderate-, and poorly-differentiated, respectively. Unknown differentiation was reported in 4 cases (10%).

A Chi-square test was used to investigate the association between CD117 expression and histopathological grading in patients with SCC, and results are summarized in Table 2. A significant relationship between the expression of CD117 and histopathological grading of patients with SCC ($P = 0.002$) was detected. CD117 was more intensely expressed in poorly differentiated tumors.

Table 1. Characteristics of the patients.

Variable	SCC (n = 40)	BCC (n = 29)	*P*-value
Age (year)			
Mean	65.15 ± 19.64	66.03 ± 18.74	0.851
Sex			0.382
Male	33 (82.5%)	21 (72.4%)	
Female	7 (17.5%)	8 (27.6%)	
Site			0.035
Limbs	10 (25%)	1 (3.4%)	
Head/Neck	27 (67.5%)	21 (72.4%)	
Trunk	1 (2.5%)	1 (3.4%)	
Unknown	2 (5%)	6 (20.7%)	
CD117 expression			< 0.001
Negative	35 (87.5%)	3 (10.3%)	
Weakly Positive	4 (10%)	15 (51.7%)	
Moderately Positive	1 (2.5%)	11 (37.9%)	

Note: Abbreviations: SCC; Squamous cell carcinoma, BCC; Basal cell carcinoma.

Table 2. Relationship between histopathological grading of squamous cell carcinoma and CD117 expression.

Histopathology grading	Negative	Weak Positive	Moderate Positive	*P*-value
Well	27 (77.1%)	3 (75%)	0 (0%)	0.002
Moderate	3 (8.6%)	1 (25%)	0 (0%)	
Poor	1 (2.9%)	0 (0%)	1 (100%)	
Unknown	4 (11.4%)	0 (0%)	0 (0%)	
Total	35	4	1	40

4. Discussion

In this retrospective and analytical study, we evaluated the expression of CD117 marker by immunohistochemistry method in cSCC and BCC paraffin blocks. It is well known that this marker is expressed in different tumors [4]. Indeed, Kriegsmann and colleagues evaluated the expression of CD117 in lung SCC, and they found 34 positive and 548 negative cases. Regarding the immunohistochemical results, diffuse membranous and cytoplasmic positivity in at least 1% of tumor cells was considered positive [14]. In another study, Pelosi and colleagues selected a 5% cut-off for membranous or cytoplasmic staining. Thirteen percent (15/113) membrane positivity and 8% (7/113) cytoplasmic positivity was found in lung SCC cases [13]. Moreover, Nakagawa et al. [15] found 20% positivity for lung SCC. The choice of different cut-offs can justify the differences in the results of different studies.

Recently, one study successfully used CD117 as a marker for differentiation between porocarcinoma and SCC; they found 100% positivity in porocarcinoma *versus* 19.4% in SCCs [16]. The same author has previously found CD117 positivity in only 2/55 of keratinocyte tumors [17]. CD117 positivity has also proven to be associated with higher proliferation index and aggressiveness (including grade, stage, size, and microvessel density) in lung SCC [13].

Some researchers used the CD117 immunohistochemistry marker in skin tumors. Membranous and focally cytoplasmic staining for CD117 was found in 93% (61/66) of BCC cases by Terada [8]. Leon et al. [9] found CD117 positive mast cells close to basal cell carcinoma islands, while tumor cells were negative. The intensity of staining may be compared with the normal mast cells in the tissue [13]. One research used CD117 for differentiation between adenoid cystic carcinoma and adenoid BCC and they found 100% positivity in adenoid cystic carcinoma but only 20%, albeit with weak to moderate intensity, in the later [10]. In the study by Yang et al. [11], none of the 19 BCC cases stained with CD117. However, in another interesting research, Castillo and colleagues [12] selected advanced BCC cases and found 43.5% positivity for CD117. The difference between staining results may be also related to advanced and resistant cases that were not evaluated separately from ordinary cases.

CD117 is also used in salivary gland tumors for differentiation between adenoid cystic carcinoma and polymorphous low-grade adenocarcinoma. The first one showed significantly higher expression [5,18,19], although, another study did not confirm such a hypothesis [20]. One of the most comprehensive studies is done by Went and the colleagues [6] on 3000 tumors in 120 different categories for CD117 immunohistochemistry expression by antibody A4502. They selected this antibody after a pilot study on 7 commercially available antibodies for low background staining and high expression in gastrointestinal stromal tumors (GISTs). Positivity was obtained in 2/41 (4%) of BCCs and 14/39 (36%) of melanomas. As Went's study [6] was comprehensive, in the present research we used their method to evaluate the severity and percentage of staining, unless we decided to use another Company's different antibodies due to availability. Both analyses considered only membranous and membranous or cytoplasmic staining as true positive, while cytoplasmic staining alone was considered as false positive. Others [21] showed cytoplasmic staining with more intensity in BCCs than SCCs, but found different patterns and intensities in the tumors of each group. The use of different antibodies, the determination of different cut-offs, and different staining criteria may justify part of the different, and sometimes inconsistent, results in different studies. When considering SCC, the researchers found interesting results: staining was positive in only 2% of SCCs in vulva and lung but negative results in cutaneous and other mucosal (oral mucosa, larynx, penis, vagina, and anus) samples. We found a significantly higher expression of CD117 in BCCs than in SCCs, so such a marker may be considered as a diagnostic marker in difficult cases. Moreover, since the CD117 pathway can be the target for treatment with imatinib mesylate or other related drugs; this may be considered as an alternative therapy in selected cases by clinicians. However recent researches with proposing intratumor genetic heterogeneity and resistant mutations made a challenge for simple targeted therapy [22].

5. Limitations

First, given the small number of similar studies, it was difficult to compare our study with others. Therefore, further studies including meta-analyses are recommended in the future. Second, some studies have evaluated non-cutaneous sites, such as the lung for squamous cell carcinoma, which may show

differences in marker expression with skin tumors. Third, different studies have chosen different cutoffs, criteria of positivity, and markers of different manufacturers. This makes the comparison of the results more difficult. Fourth, genetic analysis was not done in this study. Every mutation is not targetable. Mutations in BCC/cSCC may be the same or different. Finally, analysis of the expression of downstream products of the c-Kit pathway was not done in this study.

6. Conclusions

According to the results of this study, it seems that the expression of CD117 in patients with BCC could be higher than in patients with SCC and therefore CD117 inhibitors may have benefit in the treatment of BCC in some selected cases.

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Conflict of interest

The authors declare that they have no competing interests.

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