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Original Article

Diagnostic Utility of CD10, EMA and BCL2 Markers in Differentiating Between Squamous Cell Carcinoma and Basal Cell Carcinoma of Skin

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ABSTRACT

Introduction: Non Melanocytic Skin Cell Carcinomas are the commonest malignancies among Caucasians. According to a study conducted 59.5% BCC cases showed CD10 positivity whereas 100% SCC cases showed negativity. Expression of Bcl-2 found positive in 96.5% of BCC cases and 100% negative in SCC. Studies demonstrate EMA to be positive in 100% cases of SCC while showing 100% negativity for BCC. Differentiating BCC from SCC on the basis of IHC is vital as both have diverse managements based on their different prognosis, metastasis and recurrence.

Objectives: To study expression of CD10, BcL2 and EMA in BCC and SCC and correlate this expression in differentiating between BCC and SCC.

Methods: Study had 64 samples taken from 1st September 2018 - 31st August 2019 at department of Histopathology, PIMS, SZABMU, and Islamabad. After H&E staining, IHC was done with CD 10, EMA and Bcl 2 markers and the results evaluated.

Results: CD10 showed positivity in 81.6% of BCC cases and negativity in 84.8% cases of the SCC. Bcl2 immunoreactivity was seen in 84.4% BCC cases whereas SCC showed negativity in 84.9% cases. Considering immune-expression of EMA in SCC 90.6% cases showed positive staining; only 9.4% cases were negative for EMA. 96.9% cases of BCC were negative for EMA.

Conclusion: We suggest that concurrent application of CD10, BCL2 and EMA Immuno markers has high diagnostic value where distinction between BCC and SCC cannot be made on routine microscopy.

Keywords: basal cell carcinoma, squamous cell carcinoma, CD10, EMA and Bcl2

Introduction

Non Melanocytic Skin Cell Carcinomas (NMSC) are the commonest malignancies among Caucasians worldwide with a continuous increase in incidence. World health organization (WHO) has estimated 2-3 million cases annually[1]. Common risk factors for NMSCs include fair skin, exposure to UV radiation, genetic susceptibility, male sex and previous occurrence. The amount of pigment in the skin has an inverse relation to the occurrence of NMSCs[1, 2]. As a result, the incidence of these cancers is relatively low in Asians. Skin cancers are 1-2% of all the diagnosed cancers. However a recent study conducted in Punjab (India) showed a prevalence of 3.18%.

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The design of UV toxicity in skin neoplasia is by DNA damage, which is caused by the photons .These photons deliver energy in a direct or indirect fashion by oxidative action of reactive oxygen species (ROS), which are short-lived and formed from reaction of water with bio macromolecules [3]. Important factors in the etiology of the both BCC and SCC include down regulation of cutaneous inflammatory response and keratinocyte neoplastic transformation

The American Joint Committee on Cancer (AJCC) has done the staging of skin cancers. Variable factors like histological type of tumor, its site, size and penetration (depth) affect treatment of the cancer. Overall health, age and cosmetic outcome also affect the treatment. Mode of treatment for SCC and BCC is quite different. Surgical excision is preferred mode of treatment in SCC. Radiotherapy is an alternative only when surgery can't be performed[4]. In contrast BCC is more radiosensitive. Topical Imiquimod is quite effective in BCC whereas its efficacy has not been established in

SCC [5]. Basal layer of epidermis gives rise to BCC. A significant risk factor is exposure to sunlight. Commonly loss of protein patched homolog1 PTCH1, produces the activation of the Hedgehog pathway which ultimately causes BCC. Fifty percent cases of BCC show point mutations in Tp53 gene and is considered the 2nd most common genetic modification. Deletion of Ptch1 causes hair follicle stem cell to become malignant, whereas the intrafollicular epidermis stem cells do not become tumorigenic [5]. Study conducted over a 10 year period in Larkana (Pakistan) on skin malignancies showed 61% of cases were BCC subtype [6]. Lighter skin, extreme sunburns and appearance of freckles in childhood, and Northern European nationals show a direct relation with the appearance of BCC. Radiotherapy given as ionizing radiation is linked to BCC development [7] .

The 2nd most common NMSC is SCC. Keratinocytes belonging to epidermis or adnexal structures give rise to SCC. Over 700 000 cases of cSCC are seen annually in the United States. Study conducted in Larkana (Pakistan) showed 32% of cases of skin cancers were SCC [6]. Child-hood and youth, cumulative sun exposure is an important causative factor for SCC. Recently, immune suppression especially with organ transplantation is considered an important factor in skin carcinogenesis. Head and neck and the extensor surfaces of the arms and hands, are common anatomic locations for SCC because of their frequent sun exposure. Cluster of differentiation (CD10) or common acute lymphoblastic leukemia antigen (CALLA) is a metallopeptidase which is dependent on Zinc and is a beneficial marker in the classification and diagnosis of leukemia/ lymphoma. CD10 has been reported in both epithelial carcinomas and mesenchymal neoplasms. It is found positive in BCC whereas SCC shows negativity to it[8]. According to a study conducted, 59.5% BCC cases showed CD10 positivity whereas 100% SCC cases showed negativity[9]. In another study 75.8% of BCC cases showed reactivity for CD10 while 100% SCC cases showed negativity to the same stain[10].

Antiapoptotic protein B Cell Lymphoma 2 (Bcl-2), exists on the outer or external mitochondrial membrane. Uninterrupted self-replication of the stem cells and cell survival are maintained by Bcl2. Expression of Bcl2 is demonstrated in BCC. According to a study this immunomarker was found positive in 96.5% of BCC cases and was negative in all cases of SCC[10] . A number of glandular epithelial cells and the neoplasms arising from these epithelial cells express Epithelial membrane antigen (EMA) on their surface which is basically a glycosylated protein. According to a study EMA is found to be positive in 100% cases of SCC while showing 100% negativity for BCC and [10].

The rationale of this study is that differentiating BCC from SCC is vital for the reason that both, are managed differently depending on their variable prognosis, metastasis and recurrence. The rate of recurrence of SCC is roughly double that of the BCC. Furthermore 2% of SCC cases show metastasis while in BCC it is infrequent[8] . NMSC is associated with high morbidity and the subsequent cost associated with its treatment is quite high hence making an exact diagnosis absolutely necessary[11]. The guidelines and course of action for BCC and SCC management are also different[12]. In some cases it becomes very difficult to differentiate between them only on clinical evaluation and histopathologic staining. For this reason immunohistochemistry has a fundamental role to play in differentiating these tumors. In this era of targeted therapy exact diagnosis using specific markers is very helpful. Globally various markers have been used for this purpose, however very limited studies have been done in Pakistan in which immunologic markers are used for differential diagnosis of BCC and SCC. This study focuses on the use of three immune markers i.e. CD10, Bcl2 and EMA for differentiating between BCC and SCC.

It is a cross-sectional study conducted at department of Histopathology, PIMS, SZABMU, Islamabad from 1st September 2018 to 30th August 2019. Sixty four samples, 32 each of BCC and SCC were collected by consecutive non-probability sampling. Following approval from hospital ethics committee, informed consent was taken from the patients. All skin biopsies and surgical resection specimens (in 10% buffered formalin) brought to the Department of Pathology; Pakistan Institute of Medical Sciences (SZABMU, PIMS) Islamabad with clinical impression of NMSCs were collected. Tissue processing was carried out in automated tissue processor LEICA TP-1020. Sections of 3 - 4 microns thickness were cut from paraffin embedded skin biopsy specimens. These sections were mounted on slides and then stained with H&E in a tissue stainer Shandon Varistan 24-4.Microscopy of the cases was carried out on (H&E) slides. All cases of skin biopsies with SCC and BCC were histologically analyzed.

By IHC staining with immunomarkers CD10, BCl2 and EMA, antigens in individual cells of a tissue section were detected by using monoclonal or

polyclonal antibodies targeted against those specific antigens. IHC staining was done after initial selection of H&E stained slide. The expression was evaluated according to the criteria discussed below. CD 10 immunoreactivity was considered negative if < 10% cells showed expression of CD10. Positivity was only considered if expression of CD10 is seen in more than 10% of the cells. The percentage of positivity in entire section was scored as 1+ 10-15%,2+>50%.[13]

Brown cytoplasmic and /or nuclear membrane staining is considered a positive expression for Bcl2. With regards to Bcl2 staining < 5% staining was considered as negative and > 5% was regarded as positive. The percentage of positively stained tumor cells in entire section was scored as 1+ 5-24%,2+ 25-50% and 3+>50%[10]

Coloring the membrane and infrequently cytoplasm brown without staining the background tissue is an indication for positive EMA staining. EMA reactivity was considered negative if < 5% of cells stain. If staining was seen in >5% cells, it was considered as positive staining. The percentage of positively stained tumor cells in entire section was scored as 1+ >5% weak staining,2+ >5%moderate staining,3+>5%strong staining[14].

Statistical Analysis

Data was analysed using SPSS version 21. Mean and standard deviation was calculated for measureable variables like age and immunohistochemical scores for all three markers. Frequency and percentages were presented for qualitative variables like histopathological subtypes, site of the lesion and age group of the patients. Chi-square test was applied to compare histopathological subtypes on the basis of IHC markers (CD10, Bcl2 and EMA). P value <0.05 was taken as statistically significant.

Results

Out of these 64 cases of NMSC, collected over a year, 42 (65.6%) belonged to the male gender while 22 (34.4%) to the female gender. This gives male to female ratio of 1.9:1 The age ranged from 41 years to 110 years with a mean age of 62 ± 15 years and median age of 63.50. The peak incidence of NMSC was in 60-79 years of age group having a total of 29/64 (45.3%) patients. Followed by age group of 40-59 years with 27/64 (42.2%) patients. Our study had 32 (50%) cases of SCC and 32 (50%) cases of BCC. As far as differentiation/grade of SCC was concerned, well differentiated SCC was the commonest one comprising of 16 (50%) cases out of 32 cases. The next common subtype was moderately differentiated i.e. 12/32 (37.5%). Poorly differentiated SCC was the least common type with 4/32 (12.5%) cases. Cases of BCC were not sub classified.

We found that in the SCC cases majority of the lesions were seen in nose and temple / forehead region i.e 7/32 (21.9%) cases each, followed by scalp 6/32 (18.8%) cases and ear 4/32 (12.5%) cases. With regards to BCC, nose was the commonest site with 10/32 cases (31.3%) followed by temple/forehead, chin and eyelid each having 5/32 (15.6%) cases. Scalp lesions were seen in 3/32 (9.4%) cases.

Table 1 Site of Lesion and Histopathological Diagnosis

site of lesion	Histopathological diagnosis		Total
	Squamous cell Carcinoma	Basal cell Carcinoma	
Ear	4 (12.5%)	2 (6.3%)	6 (9.4%)
Nose	7 (21.9%)	10 (31.3%)	17 (26.6%)
Temple and forehead	7 (21.9%)	5 (15.6%)	12 (18.8%
Scalp	6 (18.8%)	3 (9.4%)	9 (14.1%)
Cheek skin	2 (6.3%)	5 (15.6%)	7 (10.9%
Leg and foot	2 (6.3%)	0 (0%)	2 (3.1%)
Arm	2 (6.3%)	0 (0%)	2 (3.1%)
Buttock skin	1 (3.1%)	0 (0%)	1 (9.4%)
Eye lid and eye brow	1 (3.1%)	5 (15.6%)	6 (1.6%)
Neck	0 (0%)	1 (3.1%)	1 (1.6%)
Chin	0 (0%)	1 (3.1%)	1 (1.6%)
Total	32 (100%)	32 (100%)	64 (100%)

CD 10 expression was seen in 31 (48.5%) cases out of 64. IHC scoring of 1+ was found in 13 (40.6%) cases and 2+ in 13 (40.6%) cases of BCC .With respect to SCC 1+ staining was seen in 4/32 (12.5%) cases while 2+ intensity was found in 1/32 (3.1%) SCC cases. Thus, CD 10 negative cases were mostly observed in SCC while a reverse trend of CD 10 positivity was seen in BCC as enumerated in the table below:

Table 2 CD10 Immunoexpression in NMSC

CD 10 Intensity and Tumor Type							
		Tumor Type				Total	P value 0.0001
		Well Differentiated SCC	Moderately Differentiated SCC	Poorly Differentiated SCC	Basal Cell Carcinoma		
Cd 10 intensity	Negative	13	11	3	6	33	
Positive	1+	3	1	0	13	17	
	2+	0	0	1	13	14	
Total		**16**	**12**	**4**	**32**	**64**	

Of the total 64 cases Bcl2 immunostaining showed positive results in 31 (41.4%) cases. Out of 31 positive cases of Bcl2, 27 (84.4%) belonged to BCC with IHC score of ,1+ in 2 (6.3%) cases , 2+ in 4 (12.5%) cases and 3+ in 21 (65.6%) cases. Only 4 (12.6%) cases of SCC showed positive staining with Bcl2. However when the negative immunoexpression was considered , 28 (84.8%) cases were SCC while 5 (15.2%) cases were BCC, so showing an obvious positive staining pattern in BCC cases as shown in the table below:

Table 3: Bcl2 Immunoexpression in NMSC

Bcl2 Intensity and Tumor Type							
		Tumor Type				Total	P value 0.0001
		Well Differentiated SCC	Moderately Differentiated SCC	Poorly Differentiated SCC	Basal Cell Carcinoma		
Bcl 2 intensity	Negative	15	10	3	5	33	
Positive	1+	1	1	0	2	4	
	2+	0	0	0	4	4	
	3+	0	1	1	21	23	
Total		16	12	4	32	64	

Thirty out of 64 cases showed positive staining with EMA 29 of which belonged to SCC. Intensity of EMA expression in SCC was 2+ in 4 (12%) cases and 3+ in 25 (78.1%) cases).

Table 4 EMA Immunoexpression in NMSC

		Tumor Type				Total	P value 0.0001
		Well Differentiated SCC	Moderately Differentiated SCC	Poorly Differentiated SCC	Basal Cell Carcinoma		
EMA intensity	Negative	2	0	1	31	34	
Positive	1+	0	0	0	1	1	
	2+	2	1	1	0	4	
	3+	12	11	2	0	25	
Total		**16**	**12**	**4**	**32**	**64**	

Discussion

Epithelial tumors of the skin are some of the most commonly encountered tumors by the histopathologists. Histopathologically, most cases of NMSC are easily diagnosable. SCC and BCC are quite distinguishable from each other in routine H&E stained slides. However, diagnostic challenges are occasionally faced by the histopathologists in case of tiny and non-representative biopsies that sample only portion of the lesion. Some cases are challenging when overlapping morphology is seen in other tumors, particularly BCC. Differentiating basaloid SCC from keratinizing BCC and superficial SCC from BCC poses difficulties for pathologists because of similarities in histapathology[15]. Accurate diagnosis of these malignancies is of utmost importance because SCC tends to have more hostile clinical behavior and greater ability to metastasize .Delayed diagnosis of SCC is associated with high morbidity and can cause local tissue destruction, metastasis and eventually death. SCC also requires radical treatment and closer follow-up. BCC is localy invasive and rarely metastasizes [12]. In the current study, male to female ratio was 1.9:1. The ratio showed clear male predominance for NMSC. Similar to the study by M Ramezani et al in which male to female ratio was found to be 1.6:1[10]. Comparable results were established by Soomro FR et al in his study conducted in Larkana district Pakistan, where he found male to female ratio of 1.85:1[6]. Slight preponderance of NMSC in males was also found in study conducted by Ahmed et al[16]. Male predominance in appearance of NMSC is associated with outdoor jobs in males

exposing them to sun damage which is considered a risk factor for NMSC[6]. Women on the other hand mostly stay indoors so are less exposed to sun and eventually have lower incidence of NMSC. According to Qureshi MA et al incidence of NMSC was considerably high both in males and females in Karachi mostly associated with ozone depletion[17].

When age distribution in NMSC is considered, our study showed mean age of 62 ± 15 years having range of 41-110 years with 29/64 (45.3%) cases in age group 60-79 years and 27/64 (42.2%) cases in 40-59 years. Age results of study conducted by Ramezani et al. in Iran were in concordance with our study showing mean age of 61 years with 17.5 standard deviation[10]. Another study carried out by Sabeti et al also showed mean age of 64 years, with 40-88 years age range[15]. A study from Lahore in 1999 by Mansoor et al. showed that maximum age of appearance for NMSC was 90 years with mean age of 47 years[18]. Ahmed et al showed mean age of 58 ± 15 and 59 ± 15 for SCC and BCC respectively[19]. Our study showed SCC occurring equally in age range of 40-59 and 60-79, a total of 13/32 (40.6%) cases each. Whereas BCC occurred most commonly in age group 60-79, a total of 16/32 (50%) cases. According to Anindarayan et al the highest prevalence was among the age group 60-80 years (80%) for BCC and 40-60 years (50%) for SCC (20). So the results of our study were quite similar to this study.

Age distribution with respect to gender depicted maximum number of females i.e. 13/22 (59.1%) in 40-59 years of age group while only 6 (6/22) female were seen in age group 60-79 years. Maximum number of male patients 23/42 (54.8%) were in age group 60-89 years and 14/42 (33.3%) patients in age group 40-59 years .The findings of Edwards L et al showed median age at diagnosis for BCC and SCC to be 67 and 73, 69 and 71 for men and women respectively[21]. Well differentiated SCC was the commonest histological grade in our study i.e. 16/32 (50%) cases, followed by moderately differentiated SCC 12/32 (37.5%) cases and poorly differentiated SCC 4/32 (12.5%) cases. Histological analysis of the patients in Ramezani et al specified that well differentiated SCC were 14 (48%) cases, moderately differentiated SCC made up 9 (31%) cases and 6 (21%) cases were poorly differentiated [10]. In the present study BCC was not subcategorized histologically.

In the current study, 59/64 (92.4%) lesions appeared in the head and neck sites with nose being the commonest one with 17/64 (26.6%) cases, followed by temple / forehead 12/64 (18.8 %) cases and scalp 9/64 (14.1%) cases. Nose, temple / forehead were the most common site for the occurrence of SCC with 7/32 (31.3%) cases, followed by temple / forehead, cheek skin and eyelid, each having 5/32 (15.6%) cases. The results were similar to Ramezani et al in which 96.5% lesions occurred in the head and neck region for BCC whereas for SCC 89% lesions were in the same area as well[10]. Sabeti et al also found majority of the lesions in the head and neck area[15]. According to Freitas et al the commonest location on the face was the nose (48.01%), followed by the eyelid (17.64%) ear (14.7%), cheek (7%), and lip[22]. Regarding immuno-expression of CD10 in our study 31/64 (48.4%) cases showed CD10 positivity. SCC showed negativity in 27/32 (84.8%) cases. With regard to BCC 13/32 (40.6%) cases showed 1 + positivity and 13/32(40.6%) cases showed 2+ positivity. Only 6/32 (18.8) cases showed negativity to CD10. Thus, overall CD10 showed positivity in 81.6% of BCC cases and negativity in majority (84.8%) of the SCC cases. These results were comparable to Ramezani et al which demonstrated 100% negativity for CD10 in SCC whereas 1+ positive cases were 50% and 2+ positive cases were 20% in BCC [10]. Sabeti et al showed positivity in 74% cases of BCC and negativity in 88% cases of SCC[15]. Shafaei et al found 59.5% of BCC cases, to be positive for CD10 in tumoral cells while in nearly all SCC cases (90%), no reactivity was seen [9]. Wagoner et al. observed CD10 expression in 14 BCC cases and 13 SCC cases. Immunoexpression of CD10 was intense and strong in BCCs showing 100% positivity .CD10 negativity was seen in all the SCCs[23]. The results of our study were fairly like the other studies showing high percentage of positivity in BCC (81.8%) and high percentage of negativity (84.8%) in SCC. The P value for CD 10 was 0.001. No significant statistical association of CD10 positivity with age, sex and grade was demonstrated.

Immune-expression of Bcl2 in the current study, showed positive expression in 31/64 (48.4%) cases. In BCC , 27/32 (84.4%) cases showing positivity. Two out of 32 (6.3%) cases showed 1+, 4/32 (12.5%) cases showed 2 + and 21/32 (65.6%) cases showed 3+ intensity of staining with Bcl2 marker. Twenty eight out of thirty two (84.9%) SCC cases showed negative Bcl2 staining. Gaballah et al established that Bcl2 was significantly expressed in skin BCC (80%) compared to skin SCC in which 100% negativity was seen[24]. Study conducted by Bartos et al showed 42 (93.4%) BCCs with positive staining for Bcl-2 protein, 10 (23.8%) of which displayed low and remaining 32 (76.2%) cases exhibited high expression[25]. So overall the results of our study were considerably similar to these two

studies. Hence our study demonstrated that Bcl2 shows positivity in majority of the BCC cases with a P value of 0.001 proving to be a useful marker as it was found negative in majority of SCC cases. Age, sex and grade of SCC were found to have no statistical significance with Bcl2 expression. With regards to EMA immune -expression, the present study demonstrated positive expression in 31/64 (46.9%) cases whereas 33/64 (53.1%) cases showed complete negativity to EMA. Considering particularly SCC , 29/32 (90.6%) cases showed 2+ and 3+ intensity staining in 4/32 (12.5%) and 25/32 (78.1%) cases respectively. Thirty one out of 32 (96.9%) cases of BCC were negative for EMA, giving a significant P value of 0.001. According to Villada et al EMA was found to be positive in all SCCs and negative in all BCCs[26]. Ramezani et al showed all BCC patients (100%) were EMA-negative and 24 out of 29 (82.7%) SCC patients were EMA-positive. No statistical implication of age sex and grade of SCC was seen with EMA expression.

This study is new and unique because till date no study in Pakistan has used these immune markers and collected data regarding their immune-expression in NMSC. The above data strongly supports that CD10, Bcl2 and EMA are potential biomarkers that can be effectively used in differentiating between SCC and BCC where conclusive diagnosis based on just histomorphology cannot be achieved. Thus in this age of targeted therapy our study can help the clinicians for effective treatment thus limiting the financial burden and morbidity associated with NMSC.

Conclusion

Our study suggest that concurrent application of CD10, BCL2AND EMA Immuno markers has high diagnostic value in cases where distinction between BCC and SCC cannot be made on routine microscopy as both tumors have different managements plans depending on their variable prognosis , metastasis and recurrence.

Conflict of interest: There is no conflict of interest declared by any author

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B. Active Participation in Active Methodology
C. Interpretation/ Analysis and Discussion