SENSITIVITY AND SPECIFICITY OF BEREP4 IMMUNOSTAINING IN BASAL CELL CARCINOMA CASES IN ASIAN SKIN

Asher Ahmed Mashhood, Tariq Sarfraz*, Mohammad Atique*, Mohammad Asif*

Military Hopital Rawalpindi, *Armed Forces Institute of Pathology Rawalpindi

ABSTRACT

Objective: To evaluate the BerEP4 stain positive cases of baral cell carcinoma in our population (BCC).

Design:A non-interventional descriptive study.

Place and Duration of study: Military Hospital and Armed Forces Institute of Pathology (AFIP) Rawalpindi from 1st January 2009 to 31st August 2009.

Patients and Methods: Patients who reported to the skin department of Military Hospital Rawalpindi with clinical impression of BCC were biopsied. Only those cases which were easily diagnosed on Haematoxylin & Eosin (H&E) were included. All such sections were then subjected to BerEP4 immune marker and the intensity of staining was noted. In addition, 11 cases of straight forward squamous cell carcinoma (SCC) were also stained with BerEP4, which served as controls.

Results: The study group (BCC cases) included 17 males (59%) and 12 females (41%), who were histologically diagnosed as BCC. All such cases were subjected to BerEP4 immune staining. All the tumors showed positive staining, the intensity/staining pattern was however variable. Twenty-two out of twenty-nine cases showed diffuse (75.9%), while 7 out of 29 cases showed partial (24.2%) staining; irrespective of the histological subtype and site of tumor. All the SCCs were negative for BerEP4 staining.

Conclusion: BerEP4 was positive in 100% of the cases. Its intensity was however variable. Nevertheless, it must be used with confidence in all difficult to diagnose cases of BCC, especially when it is difficult to differentiate from SCC.

Keywords: Basal cell carcinoma, BerEP4, immunohistochemistry, squamous cell carcinoma,

INTRODUCTION

Basal cell carcinoma (BCC) is the commonest skin malignancy, which is 4-5 times more frequent than SCC. It is relatively more common in fair skin, red hair individuals and those chronically exposed to ultraviolet radiations[1]. The highest percentage of tumors occur over the nose (20.9%) followed by other areas on the face, like periorbital region, cheeks and chin (17.7%)[2]. The male to female ratio is 1.5:1. It is commonly seen in 6th and 7th decades but may manifest earlier. The most common clinical type is nodulo-ulcerative (42.1%)[3].

BCC has very specific histological features and is usually easily picked up by a histopathologist. Occasionally some atypical tumors are encountered which pose some difficulty. It is a common trend to call such tumors as metatypical or basosquamous carcinomas. With the modern use of immunohistochemistry, the true histogenesis of a tumor is more easily determined. No specific marker existed for BCC, until BerEP4 was incidentally discovered to be diffusely positive in almost 100% of BCC[4]. The BerEP4 is derived from the MDF-7 breast cancer cell line. It reacts with glycoproteins 30 and 34 kD present on the surface and the cytoplasm of all epithelial cells except the superficial layers of squamous epithelial, hepatocytes and parietal cells[5]. According to various studies the positivity of this stain is 100% in all BCCs. This is a very significant finding and if we can prove this intensity in our patients too, it could be a very useful diagnostic tool in difficult to diagnose basiloid and metatypical epithelial tumors.

The purpose of this study was to see whether this stain was really 100% positive in BCC in our population, to gain confidence in the use of this immunostain and to encourage

Correspondence: Lt Col Asher Ahmed Mashhood, Asst Prof of Dermatology, MH Rawalpindi
Email: asher.mashhood@yahoo.com
Received: 07 Dec 2010; Accepted: 04 March 2011

the fellow histopathologists to use this stain in difficult cases of epithelial tumors especially in combination with epithelial membrane antigen (EMA). Although this stain is gaining popularity in European countries, it is still very new in Pakistan. Before the start of this study, there was no documented evidence of the use of this stain for the diagnosis of BCC in this region.

MATERIAL AND METHODS

This validation study was carried out in Military Hospital (MH) and Armed Forces Institute of Pathology (AFIP) Rawalpindi from 1st January to 31st August 2009.

The study population was the patients who reported in the skin out patient department of Military Hospital Rawalpindi, a tertiary care hospital of Pakistan Army. The patients were mostly army personnel, their relatives and dependent civil population. Those with clinical impression of BCC were biopsied. The clinical details were obtained from laboratory forms.

The inclusion criterion was mainly histological. All the biopsies sent with the clinical impression of BCC were accounted for, but only those in which BCC (any subtype) was diagnosed with confidence on haematoxylin and eosin section by more than one dermatopathologist/ histopathologist, were included in the study (n=29).

All such biopsies were then subjected to BerEP4 immune stain. The degree of staining was graded as 'diffuse' or 'partial'. In order to confirm the validity of the stain, 11 cases of SCC were also included and BerEP4 stain was done on these cases. The photography was done by using Nikon digital camera L5 with 7.2 megapixels and using 4x lens on Olympus microscope model CH-20.

Data was analysed by using SPSS version 15. Descriptive studies were used to describe the data.

RESULTS

All the tumors except one were from head and neck region. Nose was the commonest involved site in 9 (31%), next were lower lid in 4 (13.8%), face in 4 (13.8%), medial canthus in 4 (13.8%) and cheek in 4 (13.8%); forehead in 2 (6.9%), supraorbital region in 2 (6.9%), lateral canthus in 1 (3.4%), chin in 1 (3.4%), and neck in 1 (3.4%) patient. The only odd case was from lower back (3.4%).

The majority were nodular BCC (55.2%). The next frequently encountered tumor was infiltrative BCC (20.7%). Superficial BCC were diagnosed in 3 cases (10.3%), while 2 cases each were of nodulocystic (6.9%) and adenoid BCC (6.9%).

On BerEP4 immunostaining all BCC showed a positive staining. Twenty-two cases showed diffuse (75.9%) while seven cases showed partial (24.1%) staining (Figures 1 & 2). The histological types of BCC and BerEP4 patterns are given in detail in Table 1.

In the 11 cases of SCC, BerEP4 was negative (Figure 3). This group also served as control for our study.

The sensitivity and specificity of BerEP4 was calculated by keeping the histopathological examination as gold standard and plotting the values in 2x2 chart as shown in table 1: -

DISCUSSION

The BerEP4 antibody was obtained from MDF-7 breast cancer cell line. It is attached to two glycoproteins (of 30 and 34 kD)[5], present on the surface and the cytoplasm of all epithelial cells except the squamous cells, hepatocytes and parietal cells. Historically, the stain is used in the differentiation of mesothelioma from adenocarcinoma. It stains only 2% of all mesotheliomas[6], while it stains a majority of pulmonary adenocarcinomas[7]. Systematic reviews of 17 studies have reported sensitivities and specificities of Ber-EP4 in pulmonary adenocarcinoma as 80% and 90% respectively[8].

By 1993 it was realized that there is a strong and diffuse membranous BerEP4 staining in all subtypes of BCC; which were all negative for epithelial membrane antigen (EMA)[9]. Later on several researchers have proven that BerEP4 is a very useful marker to differentiate BCC from[4,10,11]. Similarly, actinic keratosis can be differentiated from BCC by this

immunostain[12]. Its usefulness comes into play especially when dealing with metastatic[13] or metatypical BCCs[14], and in cases where the surrounding inflammatory infiltrate is too intense[15]. It is of limited value in differentiating infiltrative BCC from desmoplastic trichoepithelioma (DTE), although BCC shows diffuse staining and DTE shows partial staining [16]. Trichoblastoma showed positive staining in about 20-40% of the nests. In fibrous papule, perifollicular fibroma, infundibular cyst and proliferating trichilemmal cyst, BerEP4 stains some of the basaloid cells[17]. None of the trichofolliculomas were stained[17]. Similarly trichoepithelomas[18], and cutaneous lymphadenoma shows variable degree of positivity[19]. It is negative in trichilemmomas and sebaceomas[19]. Microcytic adenexal carcinoma is reliably discriminated from BCC by this stain[20].

This study was planned at Armed Forces Institute of Pathology Rawalpindi, with the intention to check the specificity and sensitivity of BerEP4 staining in diagnosed cases of BCC in our setting. While including the cases we were very particular in choosing only straight forward cases, having all or most of the diagnostic histopathological features of BCC. Basosquamous or other difficult to diagnose cases were deliberately excluded. In our laboratory the immune staining was done manually although we followed strict protocols for quality control.

Based on the results it was proved that BerEP4 is a very useful marker for BCC with sensitivity and specificity of 100%. The pattern of positivity was however variable. It was diffuse staining in only 75.9% of the cases. These results were in contrast to many previous studies which showed diffuse positive staining in all the BCC[4,16-19]. This variation in staining pattern could be due to different skin type in our population. However, this hypothesis requires further studies in this region to clearly demonstrate that BCC in Asian population shows less diffuse staining. Another possibility of unusual staining pattern was due to manual staining method in our study. Had it been done by an automated immunostainer the results could have been different.

To our knowledge no such study was previously conducted in Asia, and all the work published is from Europe and USA. This study will not only introduce the use of this immune marker in the above indication but also build the confidence of practicing dermatopathologists in this region for its use to resolve the issue in difficult to diagnose epithelial skin tumors.

CONCLUSION

It may be concluded in the light of the results of this study that BerEP4 is a specific and sensitive immune marker for BCC. It is positive in 100% of the cases, whether it is partial or diffuse, Hence BerEP4 can be used with confidence in the diagnosis of all the difficult cases of BCC.

REFERENCES

1. Crowson AN. Basal cell carcinoma: Biology, morphology and clinical implications. Mod Pathol 2006: 19(Suppl 2); S127.
2. Kennedy C, Bajdik CD. Descriptive epidemiology of skin cancer in Aruba 1980-1995. Int J Dermatol 2001; 40: 169.
3. Weshah S, Smadi R, Helalat M. Basal Cell Carcinoma: A retrospective analysis of 76 patients. Pak J Med Sci 2007: 23; 556-560.
4. Beer TW, Shepherd P, Theaker J M. BerEP4 and epithelial membrane antigen aid distinction of basal cell, squamous cell and basosquamous carcinomas of the skin. Histopathology 2000; 37:218-223.
5. Ordonez NG. Desmoplastic small round cell tumor: an ultrastructural and immunohistochemical study with emphasis on new immunohistochemical markers. Am J Surg Pathol 1998; 22: 1314-27.
6. Carella R et al. Immunohistochemical panels for differentiating epithelial malignant mesothelioma from lung adenocarcinoma. Am J Surg Pathol 2001; 25: 43-50.S
7. Comin CE, Novelli L, Boddi V, Paglierani M, Dini S. Calretinin, thrombomodulin, CEA, and CD15: a useful combination of immunohistochemical markers for differentiating pleural epithelial mesothelioma from peripheral pulmonary adenocarcinoma. Hum Pathol 2001; 32: 529-536.
8. King JE, Thatcher N, Pickering CA. Sensitivity and specificity of immunohistochemical markers used in the diagnosis of epithelioid mesothelioma: a detailed systematic analysis using published data. Histopathology 2006; 48:223-32
9. Tellechea O, Reis JP, Domingues JC, Baptista AP. Monoclonal antibody BerEP4 distinguishes basal-cell carcinoma from squamous-cell carcinoma of the skin. Am J Dermatol 1993; 15: 452–5.
10. Ryan W, Shelly N, Mark FE, Winifred T, Peter K, Abdelmonem E. Ber-EP4, CK1, CK7 and CK14 are Useful Markers for Basaloid Squamous Carcinoma: A Study of 45 Cases. Head Neck Pathol 2008; 2: 265-71.
11. Jones MS, Helm KF, Maloney ME. The immunohistochemical characteristics of the basosquamous cell carcinoma. Dermatol Surg 1997; 23: 181–4.
12. Whitney DT, Mehran NR, David A, Kist BA. Ber-EP4-Positive Phenotype Differentiates Actinic Keratosis from Superficial Basal Cell Carcinoma. Derm Surg 2000; 26: 415-18.

13. Rao NS, Fiza SBS , Huachen W , Mark GL, Robert GP. Use of Ber-EP4 protein in recurrent metastatic basal cell carcinoma: a case report and review of the literature. Int J Dermatol 2004;43: 600-3.

14. Eric ES, Deba. PS, Mingkui C, Bo W. Metatypical Basal Cell Carcinoma of the Nose. The Inernet J Derm. 2007; 5.

15. Kist D, Perkins W, Christ S, Zachary C. Anti-Human Epithelial Antigen (Ber-EP4) Helps Define Basal Cell Carcinoma Masked by Inflammation. Derm Surg 2008; 23: 1067-1070.

16. Swanson PE, Fitzpatrick MM, Ritter JH, Glusac EJ, Wick MR. Immunohistologic differential diagnosis of basal cell carcinoma, squamous cell carcinoma, and trichoepithelioma in small cutaneous biopsy specimens. J Cutan Pathol 1998; 25: 153–9.

17. Seiji M. Immunohistochemical demonstration of Ber-EP4 expression in neoplasms with follicular differentiation. Jap J Dermatol 2005; 115:2218-22.

18. Swanson PE, Fitzpatrick MM, Ritter JH, Glusac EJ, Wick MR. Immunohistologic differential diagnosis of basal cell carcinoma, squamous cell carcinoma, and trichoepithelioma in small cutaneous biopsy specimens. J Cutan Pathol 1998; 25: 153–9.

19. R.A. Carr, D.S.A. Sanders Basaloid skin tumours: Mimics of basal cell carcinoma. Curr Diag Pathol 2007; 13, 273–300.

20. Krahl D, Sellheyer K. Monoclonal antibody BerEP4 reliably discriminates between microcystic adenexal carcinoma and basal cell carcinoma. J Cut Pathol 2007; 34: 782-7.

.....

Table-1: **Sensitivity and Specificity of BerEP4 immunostainig**

	Histopathology positive	**Histopathology negative**
BerEP4 positive	29 (a)	0 (b)
BerEP4 negative	0 (c)	11(d)
Total	**29**	**11**

Sensitivity = a / a+c x 100=100 %
Specificity = d / d+b x100= 100%
Positive predictive value = a / a+b x100=100%
Negative predictive value = d / c+d x100=100%

Table 2: Histological type and staining pattern of BerEP4 in basal cell carcinoma (n-29)

S/No	Histological types	No of cases	Percentages	Staining Pattern (Diffuse)	Staining Pattern (Partial)
1	Nodular	16	52.2%	13	3
2	Infiltrative	6	20.7%	3	3
3	Superficial	3	10.3%	2	1
4	Nodulocystic	2	6.9%	2	0
5	Adenoid	2	6.9%	2	0

Figure 1: Diffuse positivity of BerEP4 immunostaining in BCC

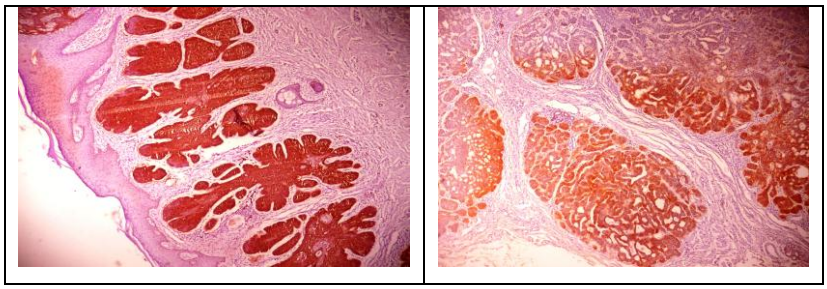

Figure 2: Partial positivity of BerEP4 immunostainig in BCC