ORIGINAL RESEARCH PAPER
Volume - 12 | Issue - 07 | July - 2023 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

UTILITY OF BEREP4 IN VARIOUS NEOPLASTIC LESIONS

Pathomorphology

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ABSTRACT

Background: The purpose of this study was to assess the immunohistochemical expression of BerEP4 in various neoplastic lesions and for differential diagnosis of various neoplastic lesions. Biopsy samples from 50 individuals with various neoplastic lesions were included, and immunohistochemical staining was performed. BerEP4 showed strong positive expression in all basal cell carcinoma(100%). In conclusion BerEP4 was a useful marker in detecting various neoplastic lesions like multifocal BCC, BCCs with mixed histomorphology, to differentiate lung adenocarcinoma from malignant mesothelioma due to its strong expression in all lesions. Focal positive staining was detected in squamous cell carcinoma and benign sebaceous neoplasms. This finding could be a potential diagnostic pitfall when evaluating sebaceous neoplasms and squamous cell carcinomas.

Objectives:

- To assess efficacy of berep4 as an IHC marker.
- To prove usefulness of BerEP4 immunohistochemistry marker for diagnosis and subtyping of various epithelial lesion.
- To use BerEp4 for differentiating lung adenocarcinoma from malignant mesothelioma.

Materials and Methods:

The present study was carried out on 50 patients. Types of samples include various specimen received in histopathology section of the pathology department. The cases were diagnosed based on light microscopy and confirmed by immunohistochemistry. **Result:** A total of 50 patients were studied. Age distribution varied from 31 to 80 years. The histopathological subtypes were diverse, It includes 29 cases of Basal Cell Carcinoma 04 cases of basaloid squamous cell carcinoma, 01 case of other squamous cell carcinoma, 09 cases of lung adenocarcinoma to differentiate it from malignant mesothelioma and 07 cases of malignant mesothelioma to rule out lung adenocarcinoma. **Conclusion:** BerEP4 proved to be a reliable marker, showing strong expression in all basal cell carcinoma, regardless of their histological subtype or topographic location. Its use is particularly beneficial to differentiate basal cell carcinoma from poorly differentiated squamous cell carcinoma, to differentiate malignant mesothelioma from lung adenocarcinoma where it is strongly positive. BerEP4 can also aid in identifying tumor excision margins, especially in cases where hematoxylin and eosin stained slides are insufficient or unreliable, such as in recurring BCCs.

KEYWORDS

Immunohistochemistry, Basal Cell Carcinoma, Adenocarcinoma.

INTRODUCTION:

BerEP4 is a iimunohistological stain primarily used to assist in the diagnosis of basal cell carcinoma (BCC). It targets the EpCAM (epithelial cell adhesion molecule), which is a glycoprotein found in the cell membrane of healthy epithelial cells and various carcinomas. Clinical trials are being conducted using anti-EpCam antibodies for cancer patients. BerEP4 exhibits high sensitivity and specificity specifically in BCC cells. BCC arises from the basal cells of the epidermis and hair follicle. BCC is the most common skin cancer in human.[1,2] It typically shows negative results in squamous epithelium and mesothelium, but is positive in most epithelial cells throughout the body. It can also aid in distinguishing pulmonary adenocarcinoma (positive for BerEP4) from mesothelioma (generally negative for BerEP4). Accurate differentiation between metastatic adenocarcinoma (MAC), malignant mesothelioma (MM), and reactive mesothelial cells (RM) is crucial for staging and treatment decisions. However, distinguishing malignant cells from reactive mesothelial cells, especially in cases involving malignant mesothelioma versus adenocarcinoma, can be challenging. Ber-EP4 is a monoclonal antibody that identifies 34-kD and 39-kD cell surface glycoproteins present in the membrane of human epithelial cells but not in reactive or malignant mesothelial cells.

Numerous studies have demonstrated its potential as a marker for distinguishing Adenocarcinoma from Malignant mesothelioma. Analyzing these data systematically can help confirm the applicability of Ber-EP4 as a diagnostic marker. Therefore, a meta-analysis was conducted to explore the potential value of Ber-EP4 in the differential diagnosis of basal cell carcinoma from basaloid squamous cell carcinoma and other squamous cell carcinoma, diagnosis of Adenocarcinoma from Malignant mesothelioma and diagnosis of other BerEp4 positive lesions, which, to the best of our knowledge, has not been previously performed.

AIM AND OBJECTIVES :

- To assess efficacy of berep4 as an IHC marker.
- To prove usefulness of BerEP4 immunohistochemistry marker for diagnosis and subtyping of various epithelial lesion.
- To use BerEp4 for differentiating lung adenocarcinoma from malignant mesothelioma.

Type of Study: Prospective & Retrospective study

MATERIALS AND METHODS :

Place of study:
The present study was carried out at the Department of Pathology, Shri M.P. Shah Govt. medical college, Jamnagar in collaboration with the department of TBCD and Dermatology in our hospital.

Design of study: A diagnostic prospective & retrospective study.

Duration of study: 1 year (October 2021 to September 2022)

Sample size: Total 50 cases.

Sample types:
Biopsy specimens with adequate material received in histopathology section of the pathology department. Clinical work up, radiological investigations and detailed history of present complaint were taken from requisition forms in histopathology section.

Biopsy Specimen:
The whole of the specimen was submitted for the histological processing and paraffin embedding. Histological paraffin sections, 3-5um thick were taken. H & E staining was performed and examined by light microscopy. The diagnosis was given according to morphology. IHC Panel IHC using relevant antibodies was done according to histomorphological features. Immunohistochemical studies were carried out with 3-5µ paraffin sections for BerEp4. IHC staining will

be performed using Peroxidase antiperoxidase method (PAP) using paraffin embedded blocks cut into 3-4um thick sections. The sections will be taken on poly L lysine coated glass slides. The secondary antibody kit of Biogenex Ltd will be used for the study. The primary monoclonal ready to use antibodies will be of DAKO, Thermofischer and Biogenex company. Tris-EDTA solution of high pH (8.4) will be used for the antigen retrieval, i.e Heat Induced Epitope Retrieval (HIER), in pressure cooker for up to one whistle. Thereafter the slides will be brought down to room temperature and taken through the steps of the immunostaining protocol using Tris buffered saline (Annexure III) as the wash buffer. The peroxide block will be freshly prepared for each use and protein block will be used as per the company protocol from DAKO, Thermofischer and Biogenex for the use of primary antibodies. The sections will be covered with one to two drops of the primary antibody for incubation for 1 hour in humidity chamber. The slides will be thoroughly washed with tris buffered saline in between each step. After this the polymer HRP provided in the kit will be used. Finally DAB chromogen will be used in the specific concentration as specified by the company.

The slides will be counter stained by haematoxylin and the mounted by DPX. After drying the test slides will be examined under the light microscopy simultaneously with the positive control slides. Negative external control slides will be treated similarly except that the primary antibody was omitted. The positive control section for respective IHC marker will be selected appropriately.

Assessment of IHC markers:
Tumor cells take up brown color.
There could be membranous expression.

- Once the slides will be ready, respective markers will all be viewed under microscope.

Inclusion criteria :

- Patients of any age groups.
- Both sexes.
- Suggestive Clinical and Radiological investigation.
- Any biopsy material received for the histopathological diagnosis of epithelial malignancy and lung carcinoma which is sufficient for IHC.

Exclusion criteria:

- Lesions with extensive necrosis without viable tumor cells.
- Case with inconclusive histopathological diagnosis due to lack of adequate material will be excluded.

RESULTS:

The present study was conducted in the department of pathology with collaboration of the department of TB & Chest Diseases and dermatology in our hospital.

Total 50 cases were studied, which include CT- guided biopsy specimen and various skin biopsy. The age range was 31 to 80 years and maximum cases (32%) were seen in the 40-60 years of age group. There was slightly male preponderance (60%) and male to female ratio was 3:2. (Table no. 1)

Table 1: Age and Gender distribution of cases (n=50)

Age group	Male	Female	Total
31-40	02	01	03
41-50	12	08	20
51-60	14	10	24
61-70	01	01	02
71-80	01	00	01
	30	20	50

Table 2: Distribution of cases according to lesion and positivity (n=50)

Lesion	Strong positive	Weak positive	Negative	Total
Basal cell carcinoma	27	02	00	29
Basaloid squamous cell carcinoma	00	00	04	04
Other squamous cell carcinoma	00	00	01	01
Adenocarcinoma of lung	08	01	00	09
Mesothelioma	00	01	06	07

Table no. 2 shows, among 50 cases, basal cell carcinoma shows strong positivity in 90% cases, while squamous cell carcinoma and other squamous cell carcinoma shows negative expression for BerEp4.Lung Adenocarcinoma shows positivity for BerEp4 while Malignant mesothelioma shows negative expression for BerEp4.

DISCUSSION:

The initial evaluation of BerEP4 in detecting cases of basal cell carcinoma (BCC) was conducted by Beer et al. in 2000[3]. They reported successful detection of all 39 BCC samples included in their study. Similarly, Ishida et al.[4] in 2008 studied 20 BCC samples and obtained the same positive results. Krahl & Sellheyer[5] in 2007 conducted tests on 28 sclerosing and infiltrative BCCs using BerEP4. In all 28 BCC cases, varying degrees of positive results were observed, with 27 showing moderate to strong positivity and one showing weak positivity. Sellheyer et al.[6] in 2013 conducted further immunohistochemistry tests to assess the utility of BerEP4 in diagnosing morpheaform BCCs. Among the 17 specimens tested, 16 exhibited an immunological reaction of more than 75%, while one specimen showed a reaction of over 25%. Ansai et al.[7] in 2012 reported a 97% sensitivity of BerEP4 in detecting BCC cells based on their study of 31 BCC cases, including 18 nodular type, 9 superficial type, and 4 morpheaform type.

BerEP4 also plays a significant role in diagnosing basosquamous carcinomas (BSC), which are challenging to identify and definitively diagnose. Accurate diagnosis of BCC and BSC is crucial due to differences in prognosis and treatment approaches.

Linskey et al. (2013)[8] reported that BerEP4 alone was unable to differentiate bSCC and BSC as both were stained by BerEP4, although the mean percentage of cells stained was significantly higher in BSC group compared to bSCC. In detecting bSCC, BerEP4 was reported to have a sensitivity of 60%, specificity of 44%, positive predictive value (PPV) of 65%, negative predictive value (NPV) of 39%, and false positive rate of 25% [10]. While in our study all basaloid Squamous cell carcinoma are negative for BerEp4 immunohisytochemical staining.

Karahan et al. in 2006[9] demonstrated that BerEP4 staining can facilitate the identification of BSC. Since BSC is a tumor with squamous differentiation resembling BCC, it exhibits a similar immunohistochemical profile. However, the difference lies in the extent of staining observed in the specimen. In BSC, patchy stains covering only part of the tumor are typically observed.

R Carella et al. in his study included 46 Specimens of mesotheliomas and 20 lung adenocarcinomas were examined with BerEp4. Of the mesotheliomas , four of 46 (9%) are positive with Ber-EP4. Of the lung adenocarcinomas, 20 of 20 (100%) are positive with Ber-EP4.

The specificity and accuracy of BerEP4 antibody for cutaneous basal cell carcinoma and lung adenocarcinoma shown in this study serves as a fundamental proof of concept for the clinical application of this antibody both as a potential in-vivo diagnostic probe and potentially as a therapeutic agent.

CONCLUSION:

Immunohistochemistry testing has become an important technique in differential diagnosis, particularly for exploring parameters that cannot be detected, characterised, or analysed using traditional staining methods. BerEP4, a monoclonal antibody, is used to identify EpCAM antigen in basal cell carcinoma (BCC) cells. It has proven to be capable of detecting all forms of BCC, including rare occurrences in intraoral, axillary, metatypical, and granular sites.

BerEP4 has been shown to be useful in distinguishing BCC from other cutaneous disorders such as basaloid squamous cell carcinoma, squamous cell carcinoma, collision tumours, sebaceoma, microcystic adnexal carcinoma, and others which are BerEp4 Negative.

Furthermore, BerEp4 is very useful marker for differentiating lung adenocarcinoma from malignanat mesothelioma which shows overlkapping morphology in histopathology sometimes.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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