EXPRESSION OF P16 MUTANT PROTEIN AND KI67 IN CUTANEOUS AND NON-CUTANEOUS MELANOMA

Farhan Akhtar, Mohammad Hassan Mohammad Ali, Rabia Ahmed, Muhammad Asif, Ghazala Sadaf, Muhammad Tahir Khadim

Armed Forces Institute of Pathology/National University of Medical Sciences (NUMS) Rawalpindi Pakistan

ABSTRACT

Objective: To determine expression of p16 mutant protein and ki67 in cutaneous and non-cutaneous melanoma.
Study Design: Cross sectional study.
Place and Duration of Study: Armed Forces Institute of Pathology, Rawalpindi, from July 2017 to July 2018.
Methodology: All cases of melanoma diagnosed by histopathological examination were included. P16 and ki67 antibodies for immunohistochemical studies were applied in all cases. Patient's gender, age, tumor site and results of immunohistochemical (IHC) stains were noted.
Results: A total number of 40 cases of melanoma were studied. It was more common in males 28 (70%) than females 12(30%). Mean age of presentation was 51.1 ± 1.17 years. Most of the patients i.e., 25 (62.5%), were in age group above 50 years, Cutaneous melanoma was the commonest type being 22 (55%), followed by ocular melanoma being 14 (35%) and mucosal melanoma 4 (10%). The immunohistochemical staining for p16 was positive in all cases (100%). Ki67 proliferative index was high in most cases 34 (85%). It was low in the rest of cases being 6 (15%).
Conclusion: Melanoma was found more common in males in the age group above 50 years. Cutaneous melanoma was the most common type. There was strong correlation between p16 and ki67 expression. P16 was seen in all cases. High ki67 was seen in 85% of cases.

Keywords: Cutaneous, Ki67, Non-cutaneous melanoma, P16 protein.

INTRODUCTION

Melanoma is an aggressive malignant tumor. The estimated global incidence rate of this tumor is 2.8-3.1/100000 with more frequent cases in western countries[1,2,3]. The highest incidence rates have been reported in New Zealand, Australia and USA. It is relatively rare in Asia and Indian subcontinent[1,2]. This tumor arises from melanocytes, which are derived from the neural crest cells and they migrate to various organs. They are first recognizable in the skin during the 3rd month of embryonic life[4]. Due to the wide distribution and early appearance of melanocytes, melanoma and naevi can be seen at many sites and in differentage groups. The diagnosis of neoplastic melanocytic lesions, in addition to routine stains may require immunohistochemical stains such as S100, HMB45 and Melan A for confirmation. Cutaneous melanoma is the most common type of melanoma, followed by ocular melanoma and mucosal melanoma[5]. Histologically, subtypes of melanoma include: superficial spreading melanoma, nodular melanoma, lentigo malignamelanoma and acral lentiginous melanoma. In addition to these types there are less than 5% of rare variants such as desmoplastic, amelanotic and polypoid melanomas[6].

The pathogenesis of melanoma is multifactorial. It is a rising from interaction of environmental and host factors. Ultra violet (UV) rays are considered the most important environmental factor. Number and size of nevi are important host risk factors. More than 100 nevi and giant nevi (>20 cm) have high risk potential for melanoma. Male patients have a poor outcome compared to females[7]. The prognosis of melanoma depends on Breslow's depth or vertical tumor thickness which measured from the epidermal

Correspondence: Dr Farhan Akhtar, Consultant Histopathology, Army Medical College, Rawalpindi Pakistan
Email: fakhtar690@yahoo.com
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granular layer to the deepest point of invasion, histologically recognized ulcer which identified by full-thickness epidermal defect with host response and thinning, effacement or reactive hyperplasia of the surrounding epidermis. Mitosis also has an impact on prognosis[5,8].

It has been found that melanoma is associated with mutations in CDKN2A and BRAF genes. These mutations result in loss of function of p16 protein, either by the absence of protein or production of abnormal mutated protein. This protein, which can be detected by immunohistochemistry, has an important role in the development of cancer. This is achieved by blocking Rb protein activity[9,10]. Other mutations are also identified such as NRAS mutation seen in about 15% of patients, NF1 mutations which is identified in about 10% of cases of cutaneous melanomas, and c-KIT mutations which have been identified in patients with acral and mucosal melanomas[5].

Ki67 protein is a non-histone protein with two isoforms of different molecular weights; 345 and 395. It is expressed during the cell cycle (G1, S, G2 and M) phases. It is highly expressed in malignant tumors[10]. Melanoma shows high ki67 level. Ocular and mucosal melanomas are comparatively rare but they carry a poor prognosis if compared to the cutaneous melanomas.

Melanoma is extensively studied in western countries. Most of the epidemiological and clinical data was obtained from these countries. The objective of this study was to determine the expression of p16 and ki67 in melanoma in Northern Pakistan.

METHODOLOGY

This descriptive cross-sectional study was carried out at the department of histopathology, Armed Forces Institute of Pathology (AFIP), Rawalpindi from July 2017 to July 2018 after taking approval from the Institutional Review Board. A total of 40 specimens diagnosed as melanoma, on routine histopathology and immunohistochemical stains, were included in this study. The samples were selected regardless of gender; age or site of tumor by non-probability consecutive sampling technique. The sample size was calculated by WHO sample size calculator with confidence level at 95% and margin of error at 5%. Non-melanocytic malignant tumors, benign melanocytic lesions such as naevi, poorly fixed tissues and specimen with scanty tissue were all excluded from this study. Immunohistochemical staining for Ki67 and p16 was performed on formalin-fixed, paraffin embedded tissue. Tissue slides of 3 µm thickness were prepared by microtome from the selected blocks, deparaffinized in xylene and rehydrated with decreasing concentration of ethanol. The epitopes were retrieved by heat method in Tris/EDTA buffer at pH9.0. Ki67 and p16 antibodies from Dako Company were applied. Age, gender, anatomical sites and immunohistochemical staining patterns of ki67 and p16 were noted and analyzed by using SPSS-20. Mean ± SD were calculated for continuous variables. Categorical variables were presented by frequency and percentages.

For Ki67 the hottest area was studied by using power 40X. No nuclear staining was considered as negative and any nuclear staining was considered positive. Weak, less than 10% was taken as low proliferative index and more than 10% as high proliferative index.

For p16 no nuclear staining was interpreted as negative and any nuclear staining was interpreted as positive.

RESULTS

In this study a total of 40 cases of melanoma were analyzed. There were 28 (70%) males. The mean age of patients was 51.2 ± 1.17 years with the range of 4 to 70 years. Most of the patients were in age group above 50 years being 25 (62.5%), followed by patients in the age group 30-50 years being 13 (32.5%). Cutaneous melanoma was the commonest type being 22 (55%), followed by ocular melanoma being 14 (35%) and mucosal melanoma being 4 (10%).

DISSCUSSION

Melanoma is an aggressive skin cancer with great histological and genetic diversity. At the

molecular level, it has been confirmed that p16 protein has a fundamental role in the pathogenesis of melanoma. It's expression or absence may predict some morphological or biological features of melanoma[11,12]. This protein is usually expressed in early stages of melanoma, superficial spreading melanoma and vertically growing melanoma with spindle cells morphology[13,14]. With deep extension expression of p16 becomes weaker and it is totally lost with metastasis[15].

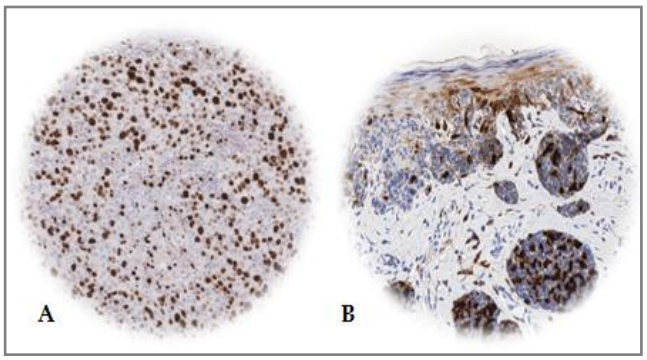

Figure-1: Immunohistochemistry stains A: High level of Ki67 nuclear stains and B: p16 positive nuclear stains.

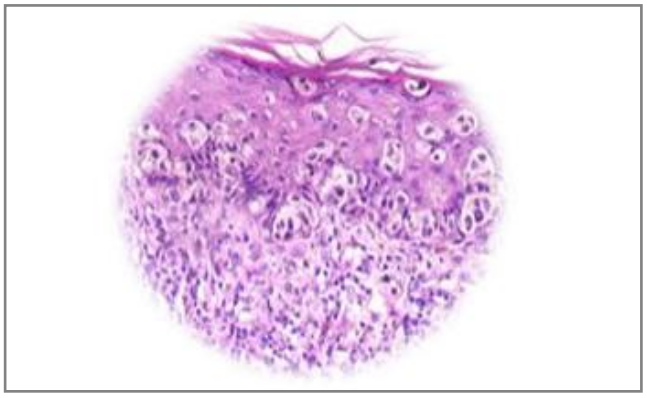

Figure-2: Cutaneous melanoma (H&E stain).

Diagnosis of classical melanoma is usually straight-forward. Sometimes, in early stages, the diagnosis may be difficult. This in part may be due to histological diversity or great mimicers of some naevi. There are some evidences supporting the diagnostic utility of p16 in these conditions, but this value of p16 as a confirmatory or differentiating marker is limited by its expression by some types of naevi. In these circumstances assessment of the proliferative capacity of the lesion can aid in the diagnosis[16].

The proliferative capacity of a neoplasm can be assessed by many proteins such as Ki67 and PHH3. Ki67 is the most studied proliferative marker. The association of high Ki67 with p16 is strong enough to diagnose melanoma in most cases[17]. Moreover in some melanomas, such as oral mucosal melanoma Ki67 is considered as independent prognostic factor. In addition to its diagnostic and prognostic roles, there is increasing evidence that Ki67 is a good target in cancer therapy[10].

Table: Immunohistochemical expression of 16 and Ki 67 in melanoma cases (n=40).

Parameter	No. of cases	Percentage
p16 Expression		
Positive cases	40	100
Negative cases	-	-
Ki67 Expression)		
High ki67 index	34	85
Low ki67 index	6	15

The understanding of the complex relationship of genes and related proteins with melanoma, has also led to a breakthrough in the treatment of melanoma by introducing new treatment modalities of targeted therapy. In this era the anti BRAF kinase inhibitors such as vemurafenib and dabrafenib are good examples[17].

Detailed analysis of this study showed that cutaneous melanoma was the dominant type 22 (55%). This finding followed the same patterns that were noted in the literature. In this context, studies conducted by Botti *et al*, in 2016 and Kuk, in Turkey, reported that cutaneous melanoma was the dominant type with percentages of (75% & 78.2%) respectively[18,19]. This difference in the percentages coincided with the global epidemiological studies of the rarity of this tumor in the Indian subcontinent. The difference might also be due to small sample size in our study compared with the other studies n=3454 and n=78 vs n=40 cases in our study. Concerning the gender our study showed that males are commonly affected being 28 (70%), this finding was similarly

demonstrated by Botti *et al*, Kuk *et al*, and Sula *et al*, where they reported that males were commonly affected[18-20].

Characteristically our study showed that the mean age was ten years less (51.2 ± 1.17 years) than that reported by Kuk *et al*, and Sula *et al*, studies in which the mean age group was around 61 years in both[19,20].

Results of immunohistochemistry analysis showed strong correlation between p16 and high Ki67 proliferative index. In this study p16 was expressed by all cases (100%), this finding was uniquely higher than all comparable studies such as Blokhin *et al*, study in 2013 and Uguen *et al*, study in Poland in which they found less number of positive cases for p16 (45.5% and 41%), respectively[18,21]. In France Mackiewicz-Wysocka *et al*, they reported a bit higher percentage (60%) in their studies for p16 expression[22]. The analysis of ki67 in this study showed that high ki67 index was seen in (n=34, 85%) of cases. This finding was in concordance with Anghel *et al*, study in Romaniain which high ki67 was seen in 75% of cases[23]. Our study showed higher value of ki67 when compared with Tu *et al*, study in USA in which high ki67 was reported in 50% of cases[24].

Advanced cases of melanoma have very poor prognosis. In high risk European countries early detection of melanoma through cytogenetic screening program has led to reduction of the mortality related to this condition. Due to the high cost effects and shortage of cytogenetic screening services in many centers in Pakistan and based on the high correlation of p16 and Ki67 in this study we suggest the usage of these markers in any suspicious lesion in patients with family history of melanoma. However, due to small sample size in this study, we recommend extended study with inclusion of naevi.

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CONCLUSION

This study has proven the strong association of mutant p16 with melanoma. Due to the relationship of p16 with BRAF gene and it's therapeutic effects we recommend detection of this molecule through immunohistochemistry in such patients. In this study there are a significant number of cases expressing high ki67 proliferative index, so these patient may get benefits from anti–ki67 therapy.

CONFLICT OF INTEREST

We, all the authors, declare that there is no conflict of interest in the designing, data collection and publication of this original research.

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