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BRCA Associated Protein-1 Immuno-Histochemical Stain in Diagnosis of Malignant Mesothelioma

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ABSTRACT

Objective: To differentiate malignant mesothelioma from benign mesothelial proliferations and other histological mimickers such as adenocarcinoma and squamous cell carcinoma, utilizing BRCA Associated protein1 (BAP1) immune-histochemical stain.

Study Design: Retrospective longitudinal study.

Place and Duration of Study: Shaukat Khanum Memorial Cancer Hospital and Research Center, Lahore Pakistan, from 2015 to 2020.

Methodology: We examined BAP1 IHC expression in 82 cases, including 56 malignant mesotheliomas, 12 Adenocarcinomas, and 14 Squamous cell carcinomas. Fifty-six malignant mesotheliomas included 49 epithelioid, 6 Biphasic and one sarcomatoid subtype. Loss of expression was established for cases showing complete loss of nuclear staining.

Results: Of 56 malignant mesotheliomas, 38(67.85%) showed loss of BAP1 expression, whereas 18(32.14%) showed retained expression. Of 12 adenocarcinoma cases, 10(83.33%) showed retained expression. Similarly, out of 14 squamous cell carcinomas, 12(85.71%) showed retained expression.

Conclusion: Loss of nuclear expression of BAP1 IHC serves as a valuable diagnostic ancillary tool in distinguishing malignant mesothelioma from its histological mimickers.

Keywords: BAP1, Immunohistochemistry, Malignant mesothelioma.

How to Cite This Article: *Sarwar S, Mustaq S, Hameed M, Hassan U, Maqbool H. BRCA Associated Protein-1 Immuno-histochemical Stain in Diagnosis of Malignant Mesothelioma. Pak Armed Forces Med J 2023; 73(4): 1045-1049. DOI: https://doi.org/10.51253/pafmj.v73i4.6122*

INTRODUCTION

Malignant mesothelioma is a malignancy of mesothelial origin that arises predominantly from the lining of pleural, pericardial and peritoneal cavities.[1] According to an estimation, 28,000 to 43,000 people worldwide die annually of malignant pleural mesotheliomas.[2] The 2015 World Health Organization (WHO) Classification of Tumors of the Pleura describes three major histologic types of diffuse malignant mesothelioma, including epithelioid, sarcomatoid/ desmoplastic and biphasic types.[3] Patients with sarcomatoid and biphasic tumours tend to behave aggressively with poorer prognoses than epithelioid subtype.[4] Histologically, assessment of these tumours, especially of diffuse type, is often difficult due to bland cytological features and may pose difficulty in separating these from benign mesothelial proliferations, entrapped reactive mesothelial cells within organizing pleuritis and other malignant neoplasms such as metastatic adenocarcinomas and squamous cell carcinomas.[5,6]

Recently, aberrations in the DNA damage repair genes in the pathogenesis of malignant mesothelioma have been studied using next-generation sequencing (NGS) platform and familial-germline molecular aberrations associated with BRCA1 Associated Protein-1 (BAP1), MutS homolog 3(MSH3), breast cancer gene one associated ring domain 1(BARD1), RecQ-like helicase 4(RECQL4), breast cancer gene 2(BRCA2), MRE11 homolog, double-strand break repair nuclease (MRE11A), SHQ1 and H/ACA ribonucleoprotein assembly factor (SHQ1) have been identified.[7,8]

Of these, an association of malignant mesotheliomas with BAP1, which is essentially a tumour suppressor gene located on chromosome 3p21.1, has been found significant. This discovery describing the association of abrogated BAP1 activity with malignant mesothelioma is not just remarkable from a diagnostic point of view. However, it also confers and directs new screening, preventive, and therapeutic approaches such as immunotherapy and platinum-based chemotherapy.[9,10] In this study, we address the diagnostic utility of BAP1 IHC in malignant mesothelioma and compare results with some of the previously mentioned histological differential diagnoses.

METHODOLOGY

The retrospective longitudinal study was conducted at Shaukat Khanum Memorial Cancer Hospital and Research Center, Lahore Pakistan, after approval from Shaukat Khanum Memorial Cancer Hospital and

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Received: 16 Jan 2021; revision received: 29 Mar 2021; accepted: 31 Mar 2021

Research Center Internal Review Board (Ltr no. EX-12-12-19-02), from the pathology archives using institution's database from 2015 to 2020.

Inclusion Criteria: Incisional/needle core and excisional biopsies exhibiting unequivocal histological features of malignant mesothelioma and its differential diagnoses, including squamous cell carcinomas and adenocarcinomas centred clinically and radiologically around pleura and distant sites, were included.

Exclusion Criteria: Specimens with poor fixation, processing artefacts, extensive necrosis and scanty leftover tissue, excluded.

Hematoxylin and eosin stained slides of 4-5 micron thick sections were prepared using Leica Peloris for processing, Thermo Histostar for Embedding, Leica RM 2245 for microtomy, Leica ST 5020 for staining and Leica CV 5030 for coverslips. IHC analysis was performed in all the cases. 4-5 micron-thick serial paraffin sections from representative paraffin blocks were processed using a primary antibody against BAP1 (clone BSB-109, mouse monoclonal [Bio SB, Santa Barbara, CA, USA). Antibody retrieval time was 40 minutes, and incubation time was 15 minutes. Non-neoplastic cells acted as internal positive controls, such as vascular endothelium, stromal, or inflammatory cells. BAP1 expression was considered positive/ retained when a weak, moderate or strong nuclear positivity was shown. Some cases showed cytoplasmic localization of expression varying from weak to strong staining. However, for this study, loss of expression was referred to as complete loss of nuclear expression. An Olympus 75 microscope was used to assess the morphology of tumours and IHC evaluation. Each case was evaluated by at least two pathologists at our institute.

Statistical Package for Social Sciences (SPSS) version 22.0 was used for the data analysis. Quantitative variables were expressed as Mean±SD and qualitative variables were expressed as frequency and percentages

RESULTS

Eighty-two cases were analyzed in the study. Diagnosis-wise cases were malignant mesotheliomas (n=56), Adenocarcinoma (n=12), and Squamous cell carcinoma (n=14). Malignant mesotheliomas comprised 49(87.5%) epithelioid, 6(10.71%) Biphasic and 1 (1.78%) sarcomatoid subtypes. Of 56 malignant mesotheliomas, 38(67.85%) showed loss of BAP1 expression, whereas 18(32.14%) showed retained expression. Retained expression was seen in the Biphasic subtype (2 of 6 cases, 33.33%) and sarcomatoid type (1 of 1 case, 100%). 15 of 49(30.6%) epithelioid subtypes also showed retained expression (Figure-1). Site distribution and prevalence of malignant mesotheliomas incorporated in this study are elaborated in Figure-2. Demographics of the cases selected for malignant mesothelioma show a male predominance of 38(67.85%) over 18 females (32.14%) and the mean age of 56±14 years.

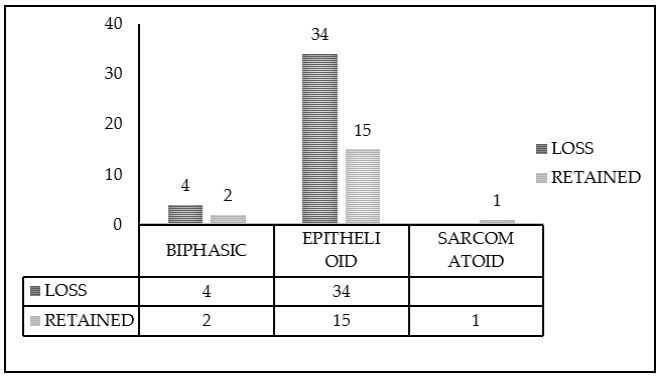

Figure-1: Majority of malignant mesotheliomas, especially Epithelioid subtype shows significant loss of BAP1 IHC expression. Note that Sarcomatoid subtype shows retained expression

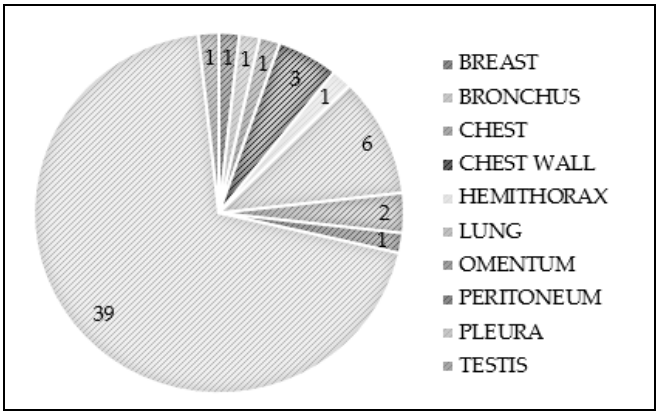

Figure-2: Majority of the malignant Mesotheliomas incorporated in this study show pleural localization. However, a wider spectrum of sites involved by disease is noted

Of 12 adenocarcinoma cases, 10(83.33%) showed retained expression. The 2 cases with loss of expression showed acinar and poorly differentiated morphology (Figure-3). Of 14 cases of squamous cell carcinoma, 12(85.71%) showed retained expression. The 2 cases with loss of expression showed poorly differentiated morphology (Figure-4). Microscopic findings on H&E stained slide along with BAP1 IHCs are demonstrated in Figure-5A (top) and Figure-5B (bottom).

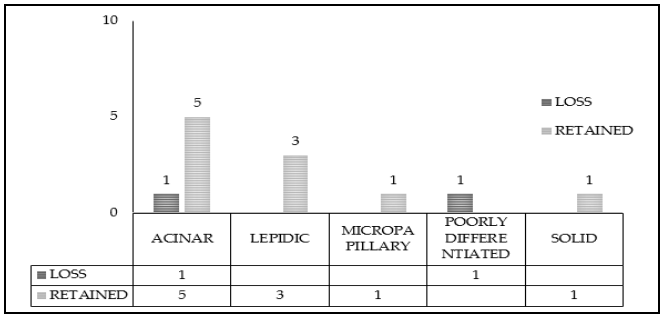

Figure-3: Significant number of Adenocarcinomas show retained BAP1 IHC expression in contrast to malignant Mesotheliomas where loss of expression is predominantly noted

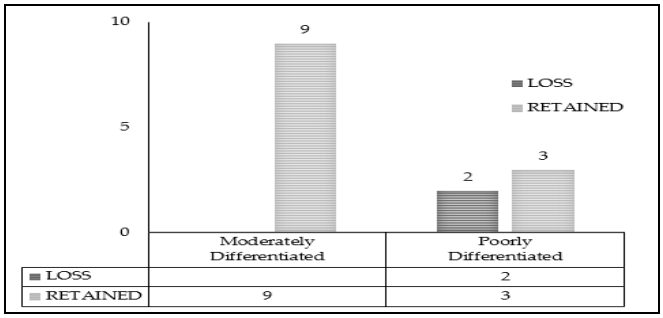

Figure-4: Retained expression of BAP1 IHC is noted in majority of Squamous Cell Carcinomas

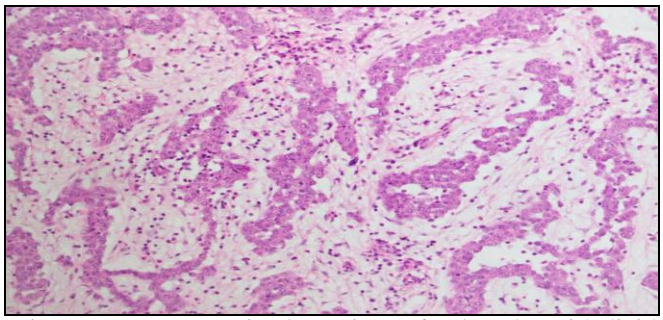

Figure-5A: H&E stained section of pleural Epithelioid Malignant Mesothelioma. Note branching gland-like lumina lined by relatively bland cuboidal cells showing round nuclei, moderate amounts of Eosinophilic cytoplasm and Conspicuous nucleoli

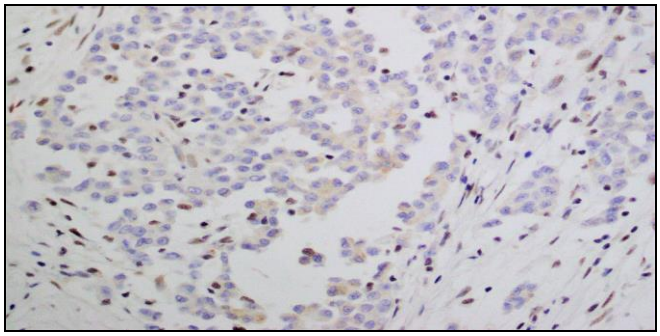

Figure-5B: Loss of nuclear BAP1 IHC expression can be seen. Background Stromal Tissue and Inflammatory cells show nuclear Expression (Positive Internal Control)

DISCUSSION

BAP1 is the most frequently mutated gene in sporadic cases as well.[11] BAP1 gene aberration can be demonstrated by Immunohistochemistry. Loss of its function translates into nuclear negativity for BAP1 expression by immunohistochemical (IHC) staining with high concordance between the two events.[12] Loss of nuclear BAP1 IHC expression has been recently studied, and its utility in differentiating malignant mesothelioma from its histological mimickers such as metastatic adenocarcinoma, squamous cell carcinoma, sarcomatoid carcinoma and reactive mesothelial proliferations is evaluated.[13]

Histological evaluation of pleural biopsies to rule out malignancies is a two-step challenge, i.e. first, to identify the mesothelial origin of the tumour and then differentiate it from benign/reactive mesothelial proliferations. BAP1 IHC stain can help both these challenges as an adjunct to morphological features of the tumour.

Extensive literature is present that putatively allows the differentiation of mesothelial-derived cancers from other histological mimickers.[14] One study listed several lineage-specific IHC stains for mesothelial tumours, of which, Calretinin was the most sensitive (100%), followed by WT1 (94%), CK5/6 (89%) and D2-40 (80%).[15]

Of 56 cases of malignant mesotheliomas included in this study, 49 were epithelioid subtypes, six biphasic and one sarcomatoid subtype. 34 of 49 epithelioid mesotheliomas (69.4%) showed loss of nuclear BAP1 IHC. 2 of 4 biphasic subtypes (50%) also showed loss of expression. One sarcomatoid mesothelioma included in the study showed retained expression. In contrast to the loss of expression in malignant mesotheliomas, BAP1 showed retained expression in other malignant tumours. 10 of 12 adenocarcinomas (83.33%) and 12 of 14 squamous cell carcinomas (85.71%) expressed retained nuclear staining.

A previous study concluded that seventeen (53.1%) of 32 peritoneal aspirates in patients with malignant mesothelioma showed loss of nuclear BAP1 expression.16 Another study, performed a BAP1 IHC stain in 30 cell blocks from effusion cytology specimens and reported loss of expression in 20 of 30 cases (66.66%).[17] Another study reported the loss of BAP1 expression in 31 of 51 malignant mesothelioma cases (60.8%). This study compared the expression of BAP1 IHC in 51 cases of malignant mesothelioma and 25 cases of reactive mesothelial hyperplasia.[18] 100% intact

expression was noted in the latter. In our study, in addition to reporting the loss of BAP1 expression in 67.85% of malignant mesotheliomas, the comparison was made with 12 cases of adenocarcinoma and 14 cases of squamous cell carcinoma. BAP1 had intact expression in 22 of 26 cases (84.61%) and loss in just 4 cases.

A recent study showed the more significant number of cases and reported that 119 of 211(56.4%) cases of malignant mesothelioma showed loss of BAP1 IHC expression having more predilection for epithelioid and biphasic subtypes.[19] These findings align with our results (67.85% with more predilection towards epithelioid and biphasic subtypes). A recent large-scale study evaluates the prognostic role of cytoplasmic expression of non-epithelioid malignant mesotheliomas with loss of nuclear expression.[20]

LIMITATIONS OF STUDY

First, using BAP1 IHC only applies to proliferations that have been established mesothelial. They are analyzed against a limited number of differential diagnoses, i.e. squamous cell carcinoma and adenocarcinoma. Second, the number of cases included in this study for rare subtypes such as sarcomatoid and biphasic types are limited, and a more extensive study with a substantial number of cases is required to establish a more accurate relation with BAP1 IHC expression. Lastly, the results of this study are based on the complete loss of nuclear staining. However, the variable intensity of staining, especially the cytoplasmic expression pattern, might be of diagnostic and prognostic significance in classifying various subtypes of malignant mesothelioma in a more extensive study.

CONCLUSION

In conclusion, this study of 82 cases demonstrates that loss of nuclear expression of BAP1 IHC is a valuable diagnostic ancillary tool in distinguishing malignant mesothelioma from its histological mimickers. Thus, therapies to restore BAP1 activity are potentially relevant to many cancer patients with this disease.

Conflict of Interest: None.

Authors Contribution:

Following authors have made substantial contributions to the manuscript as under:

SS & SM: Data acquisition, data analysis, drafting the manuscript, approval of the final version to be published.

MH & UH: Concept, drafting the manuscript, data interpretation, critical review, approval of the final version to be published.

HM: Critical review, study design, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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