

IMMUNOHISTOCHEMICAL (IHC) MARKERS AND MALIGNANT MESOTHELIOMA (MM) IN PLEURAL EFFUSIONS PRESENTING TO A TERTIARY CARE HOSPITAL

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ABSTRACT

Objective: To see the importance and frequencies of different IHC markers in the diagnosis of Malignant Mesothelioma (MM) in patients with suspected mesothelioma presented with pleural effusion.

Material and Methods: This was a cross sectional analysis, conducted at Pulmonology unit, Lady Reading Hospital Peshawar, Pakistan from January 2016 to December 2017. Patients with pleural effusion and suspected mesothelioma were included. Pleural biopsies were sent for IHC and SPSS was used for statistical analysis. Those with Granulomatous inflammation, possible TB and chronic non specific inflammation on histopathology were excluded from the study.

Results: A total of 141 cases were identified of which 60 patients were excluded who turned out to be Granulomatous inflammation/chronic non specific inflammation or non diagnostic biopsies report. Remaining 81 patients were included with median age 55.04% years range, 24–75 years \pm 15.688 SD, 46 patients (56.8%) were males and most of the cases (one fourth) were from age group above 50 years. MM was relatively common in males as compared to female (56.8% vs 43.2%). Of those 53 (65%) were diagnosed with MM and 28(34.6%) were Metastatic Adeno-carcinoma on the basis of histopathology and IHC markers. Calretinin was the most frequent marker present in 46 (86.8%) cases of MM followed by WT1 and Cytokeratin in 45 (84.9%) cases each. Similarly the most commonly found marker in cases of Metastatic Adeno-carcinoma (MA) was TTF1 in 22 (78.5%) which was absent in 100% cases of MM. Similarly, Calretinin and HBME was absent in 26 (92.8%) cases of MA. On the other hand Cytokeratin was also present in 42.8% cases of MA. Calretinin was found to have more sensitivity (86.79%), specificity (92.86%), PPV (95.83%) and accuracy (88.89%) in diagnosing MM on histological samples and TTF1 was the most suitable IHC for the identification of MA

Conclusions: Calretinin, Cytokeratin, HBME and WT1 are useful IHC marker in the diagnosis of MM. Calretinin is the most sensitive and specific IHC markers on histopathological samples in MM. While in case of MA, TTF1 was the most suitable marker.

Keywords: Mesothelioma; Pleural effusion; IHC markers.

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INTRODUCTION

Malignant mesothelioma (MM) a types of tumor is found in rare cases, that arises from mesothelial cells lining the serosal membranes of the body, including

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pleural, peritoneal, and pericardial surfaces with an unfavorable prognosis and is still a problem for health authority throughout the world. Frequency of MM is increasing in the entire world and is strongly associated with exposure to asbestos, which remains important global trading goods throughout the world¹. The disease is of great concern as considered still incurable and its genetic profile showed that most of its genes for radio- and chemo resistance are over-expressed.

MM targets either gender and any age, however, traditionally it is more common in males and with

increasing age. Its diagnosis is still challenging and its prognosis is poor². Due to its high latency period i.e. from 20 to 40 years until symptoms of disease point out the presence of disease its early diagnosis is very difficult and in its initial stage it is asymptomatic³. even in the advanced stage of the disease it can present with atypical symptoms. So it's very challenging to diagnose MM early⁴.

For confirmation of diagnosing MM, pathological examination and immune-histochemical analyses are done with radiographic examination such as chest X-rays (CXR) and computed tomography (CT) scans. For this samples can be achieved by two methods i.e. cytological examination or by invasive biopsy. These noninvasive or minimally-invasive methods help us early detection of the disease and hence early treatment and better outcomes and survival^{5,6}. An IHC can be measured that gives an indication of the biological state of the patient. Usually there are three types of markers which are diagnostic, prognostic markers (gives detail about the rate of disease progression) and predictive (which gives detail about a useful outcome of a specific treatment).

The main focus and target of researchers is to find such markers which are easy in use and help in early detection of MM and also help in proper treatment and successful outcome. These markers have the potential not only to help in the detection but also help in distinguishing differentiating Mesothelioma from other malignancies⁷⁻¹². Example of such IHC markers are calretinin, cytokeratin, and thrombomodulin which are positive in MM patients and negative in serous carcinomas¹³.

Moreover it is mandatory to remember that various histological types of MM need to be differentiated from other histologically similar tumor. For example Epithelioid type of mesothelioma from Metastatic Adeno Carcinoma,¹⁴ ovarian serous papillary adenocarcinoma from Peritoneal Mesothelioma,¹⁵ Sarcomatoid carcinoma form sarcomatoid pleural mesothelioma¹⁶ and many more such as, reactive mesothelial hyperplasia must be differentiated from early stage mesothelioma.

For differentiation between various histological types of mesothelioma and from other histologically similar tumors it is need of the day to use different Immunohistochemical markers. As yet there is no IHC available that is completely specific for mesothelioma. Therefore a combination of markers, both positive and negative is needed to make a confident diagnosis of MM.

For diagnosis of epithelioid type of mesothelioma, calretinin, WT1, Thrombomodulin, Mesothelin

and D2-40 can be applied 17-21 and for diagnosis of adenocarcinoma CEA, T1F1 and Nepsin A are used²². The antibodies in case of sarcomatoid mesothelioma is cytokeratin which exhibits a high specificity and is very useful²³.

Pakistan is among the countries where the use of asbestos is still common. In Khyber Pakhtunkhwa province of Pakistan this disease is ranked high because of asbestos mining and its use in various industries. Various studies have been conducted on the prevalence of MM in this region. However, to the best of our knowledge, studies have never been conducted to identify predictive IHC in suspected cases of MM. This is the first study of its kind in this region.

The aim of study was to predict the probability of diagnosis of MM, based upon the presence of certain IHC in the pleural tissue samples obtained for histopathology. These IHC play important role in the early detection of MM.

MATERIAL AND METHODS

This study was conducted at Pulmonology Unit, LRH Peshawar, Pakistan, from January 2016 to December 2017. The hospital receives patients from all over the KP, and Afghanistan. Data were collected from patients, recruited through the outpatient department (OPD) and admitted patient from different wards of LRH, who attended this hospital for medical care with chest symptoms, pleural effusion and clinical suspicion of MM. Detailed history with socio-demographic information, a detailed occupational history, exposure to asbestos, smoking habits, and other medical history were taken. Initially pleural tap under aseptic technique after informed consent and ultrasound markings. After injecting lignocain and small incision locally in the marked intervertebral space Abram needle is used to take 4-6 biopsies and were sent to standard laboratory in normal saline for histopathology The diagnosis was based on clinical examination, radiographic examination (X-ray and chest CT), biopsy, and IHC. Those who has chronic inflammation and granulomatous inflammation on biopsies were excluded from this study Group. IHC applied to the biopsy specimen consisted of calretinin, cytokeratins (CK5/6), Wilms tumor protein (WT-1), carcinoembryonic antigen (CEA), CK7, CK20, HBME, HBME1, GATA3, PAX8, D240 and thyroid transcription factor 1 (TTF-1). The diagnosis of MM was confirmed by standard histological and immunohistochemical criteria. Data were analyzed using the software, SPSS version 20. Qualitative data (gender, radiological findings, presence of MM/MA) were presented as frequency and percentage; while quantitative data (age) were presented as means and standard deviation. The

research protocol was approved by Ethical committee, Postgraduate Medical Institute, Lady Reading hospital, Peshawar, Pakistan.

RESULTS

141 cases were identified of which 60 patients were excluded whose biopsies shows Granulomatous inflammation/chronic non specific inflammation or non diagnostic report. The remaining 81 patients have median age 55.04 years (range, 24–75 years) standard deviation SD ±15.688 and 46 patients (56.8%) were males (Table 1), one fourth of the MM cases belong to the age group more than 50 years of age (Table 2). Results showed that 65.4% of the study cases were Malignant Mesothelioma and 34.6% were Metastatic Adenocarcinoma (Table 3). For prediction of differentiation between the two types of MM both positive and negative IHC were used. Similarly, both positive and negative IHC were used for prediction of MA. Among MM cases, the

most frequent marker found were Calretinin 44 (86.8%) cases followed by WT1 and Cytokeratin (84.9%) each. Similarly, TTF1 were absent in all MM cases and were found in 78.5% cases of MA cases. Similarly, among MA cases, Calretinin were negative among 92.8% of the MA cases and HBME were negative among 92.8% of MA cases. The Cytokeratin was found in 84.9% and 42.8% in MM and MA respectively shows that it is not very specific for MM (Table 3). Diagnostic accuracy by different IHC was analyzed and it was found that sensitivity for calretinin, cytokeratin, HBME and WT1 is found to be 86.79%, 84.91%, 83.02% and 84.91% respectively. Further it was noted that with sensitivity (86.79%), specificity (92.86%), PPV (95.83%) and accuracy (88.89%), calretinin was the most useful IHC for the purpose of finding of MM and TTF1 was the most suitable IHC for the identification of MA (Table 4).

Table 1: Gender distribution of the study cases (N=81).

Gender	Malignant Mesothelioma Cases		Metastatic Adenocarcinoma Cases	
	N	n%	N	n%
Male	32	60.4	14	50.0
Female	21	39.6	14	50.0

Table 2: Distribution of study cases on basis of age).

Age Group	Malignant Mesothelioma Cases		Metastatic Adenocarcinoma Cases	
	N	n%	N	n%
21 to 40	8	9.9	9	11.1
41 to 60	26	32.1	9	11.1
More than 60	19	23.5	10	12.3

Table 3: Comparison between MM and MA on the basis of Presence of Markers (N=81).

Markers	MM (53)				Metastatic Adenocarcinoma (28)			
	Positive		Negative		Positive		Negative	
	n	n%	n	n%	N	n%	N	n%
Calretinin	46	86.8	7	13.2	2	7.1	26	92.8
Cytokeratin	45	84.9	8	15.0	12	42.8	16	57.1
TTF1	0	0.0	53	100.0	22	78.5	6	21.4
HBME	44	83.0	9	16.9	2	7.1	26	92.8
WT1	45	84.9	8	15.0	3	10.7	25	89.2

Table 4: Sensitivity and Specificity of different IHC for the presence of MM.

Characteristics	Calretinin	Cytokeratin	HBME	WT1	TTF1
Sensitivity	86.79%	84.91%	83.02%	84.91%	0.0%
Specificity	92.86%	57.14 %	92.86 %	89.29 %	70.67%
Positive Likelihood Ratio	12.15	1.98	11.62	7.92	0.00
Negative Likelihood Ratio	0.14	0.26	0.18	0.17	1.42
PPV	95.83%	78.95%	95.65%	93.75%	0.0
NPV	78.79	66.67 %	74.29 %	75.76 %	89.83
Accuracy	88.89%	75.31%	86.42%	86.42%	65.43%

DISCUSSION

MM is an aggressive cancer which pose challenge in its diagnosis in its early stages. It is very important to find out new possible way for the detection of MM as early as possible. A newly introduced method is using a IHC markers. A Immunohistochemical marker is a characteristic that can be measured and may be used for the identification any biological change in the patient biological state. By using reliable and validated IHC the diagnosis process of MM in early stages may be speed up^{9,24,25}. IHC have to be sufficiently robust for their application in clinical practice. But this advance technology in some countries especially in¹⁰ under-developed countries like Pakistan, the resources for histopathological diagnosis of suspected cases are limited. In Pakistan especially in Khyber Pakhtunkhwa, the higher rate of MM remains a major health problem in the few areas where use of asbestos is still used in different ways²⁶.

It was very important to find out new ways for speedy findings of the disease at early stages as soon as possible and hence this was the first ever study conducted in this region to find out predictors IHC for the detection of MM on histopathological diagnostic workup of tissue samples for which Biopsy specimens were obtained via closed pleural biopsies from all suspected cases attending in the hospital. Our study suggest that MM can be diagnosed with IHC alone for which further studies need to be done^{27,28}.

This study also shows that male are more affected with MM than female. The possible explanation for this could be that male were more exposed to different factors and most of them belonged to poor families like workers/farmers as discussed in a study from the same area by Jehan N and Ahmad I 2007²⁹⁻³¹. Here in this project 39.6% female declared MM and most of them were housewives so it seems that either something is present in the environment or is genetically targeting these females²⁶.

Another important finding of the present study is about the age of the study cases. Usually this illness develop in late ages and most as of the cases from this study were from age group above 50 years and fourth

part of the MM cases also belong to this age group and which is also similar in other studies^{26,29}. It is due to exposure to risk factors for long time and its latent period is of long duration.

In the present study, we also presented the presence of IHC among the study cases. We conclude from our study that Carletinin, WT1 and Cytokeratin help in diagnosing MM with negative TTF1. On the hand in MA cases shows that TTF1 is positive in most cases and carletinin and HBME has low sensitivity for MA.

Finding of the present study about Carletinin, a best IHC for the diagnosis of MM is also described by few other studies³²⁻³⁴. Along with Carletinin another important biomarker present here among MM cases of this project was WT-1 which was present among 84.9% cases. HMGB1 is a proteinous diagnostic biomarker among potential cases 35 which is released by necrotic death cell. A study conducted by Napolitano A et al., for determining the sensitivity and specify of HMGB1 showed importance of this biomarker in comparison of MM between two groups, one asbestos-exposed and other healthy controls. But for further validation and diagnostic accuracy of this biomarker more high and larger prospective studies are required³⁶.

CONCLUSIONS

In conclusion cytokeratin, calretinin, HBME and WT1 were the IHC of interest . Calretinin with more sensitivity and specificity help identify it the most useful IHC in diagnosing MM and TTF1 was the most suitable IHC for the identification of MA. All the studied IHC were found to be useful in diagnostic work up in both MM and MA in patients presented with pleural disease.

Recommendations

More large scale and robust studies are needed to give guideline

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AUTHOR'S CONTRIBUTION

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

- Iqbal Z:** Conceived of the presented idea, Acquisition of data, Drafting of manuscript
- Khan MY:** conceived of the presented idea, Critical revision
- Khan MA:** Acquisition of data, Analysis and interpretation of data, Drafting of manuscript
- Alam J:** Analysis and interpretation of data, Critical revision.
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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.