Pleural Mesothelioma: Diagnostic Problems and Evaluation of Prognostic Factors

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ABSTRACT

Background: Malignant pleural mesothelioma (MPM) in Egypt is mainly attributed to an environmental origin i.e exposure to asbestos, with a high incidence in women and young adults. Immunohistochemistry and ultrastructural features aid in the diagnosis. The p27Kip1 is a kinase inhibitor protein acting as a cell cycle regulator and a putative tumor suppressor gene playing a critical role in the pathogenesis of several human neoplasms.

Aim: A clinicopathologic, immunohistochemical and ultrastructural study of mesothelioma in Egyptian patients, with identification of different prognostic factors.

Material and Methods: Sixty-one cases of MPM were collected from the department of pathology at the NCI, Cairo. Cases were stained by monoclonal antibodies against CK5/6, calretinin, vimentin, CD15, CEA and p27.

Results: More than half (57.4%) of the patients were residents in endemic areas; 50.8% were of epithelioid type. CK5/6 was positive in 45 (73.8%) cases, 39 (63.9%) cases were positive for vimentin, 49 (80.3%) cases were positive for calretinin. One case showed a focal weak positive reaction to CD15. None of the cases stained for CEA. There was a statistically significant relation between p27 expression and the histopathologic type ($p=0.02$) between overall survival and age ($p=0.01$), histopathologic type ($p=0.02$) and stage ($p=0.036$).

Conclusion: MPM is an increasing disaster in Egypt which is underestimated and neglected. A panel of immunohistochemical markers should be used for proper evaluation. p27 has proven to be a potential biologic prognostic marker for mesothelioma and more studies as regard its significance are recommended on a larger number.

Key Words: Mesothelioma – Asbestos – CK5/6 – Calretinin – p27.

INTRODUCTION

Malignant pleural mesothelioma (MPM) is an uncommon neoplasm, yet is the most frequent neoplasm affecting the pleura and remains a major health threat for many years to come. Although the causation by asbestos is firmly established since more than 50 years, in many world regions the use of this dangerous carcinogen peaked between 1970 and 1990 [1]. MPM has a progressively downhill course with a median survival in the order of 10 months [2].

Cytokeratin (CK) filaments are the intermediate filaments forming the skeleton of epithelial cells, providing support to maintain cell integrity and the structure of epithelial tissues. Human epithelia can present 20 different types of CKs [3]. CK5/6 is one of the positive markers for mesothelioma with high sensitivity (80-100%) and sufficient specificity for practical use in distinguishing it from metastatic adenocarcinomas [4,5].

Calretinin is a 29 KDa protein that is a member of a large family of calcium-binding proteins. Several studies have indicated the importance of calretinin as a positive marker for the diagnosis of mesothelioma [6-8]. Leu-M1 is one of a large group of antibodies against an antigen defined by the cluster designation group 15 (CD15) [9]. Leu-M1 is considered a highly specific marker for separating adenocarcinomas from mesotheliomas; however, its sensitivity is rather low, especially when compared with other negative mesothelioma markers [10].

p27 is a cyclin dependent kinase inhibitor which blocks the cell cycle and prevents its progression from G1 to S phase. Reduced expression of p27 has been correlated with an adverse clinical outcome of a number of neoplasms including MPM [11]. The identification
of prognostic factors is important to properly counsel patients and their families, monitor treatment options and guide the development of novel therapies [2].

The aim of this work was to study the clinicopathologic features and immunoprofile, as well as to identify the prognostic factors, of MPM in Egyptian patients.

MATERIAL AND METHODS

Patients and samples:
Sixty-one cases of MPM were collected from the department of pathology at The National Cancer Institute, Cairo University, from January 2000 to December 2003. Only patients with clear-cut clinicopathologic diagnosis of malignant mesothelioma and adequate surgical and biopsy specimens were included in the study.

The ages of the patients ranged from 20 to 80 years. The patients were asked about their residence to identify areas with endemic asbestos exposure, e.g. Shobra El-Khema, Helwan and El-Hawamdia.

The histopathologic type of MPM was defined according to the morphologic criteria of the WHO classification [1]. Staging was performed according to the staging system provided by the UICC [12].

Survival was taken as the duration between histologic diagnosis of MPM and date of death or last follow-up. Follow-up data were available for 51 cases only.

Methods:

Immunohistochemistry:

Six unstained positively charged slides were prepared from each paraffin block for immunostaining with the following primary antibodies: CK5/6 (Lab Vision, dilution 1:50), calretinin (Dako, dilution 1:50), p27 (Dako, dilution 1:25), vimentin (Dako), CEA (Dako) and CD15 (Dako), the latter 3 antibodies were ready to use.

Slides were deparaffinized in xylene twice for 10min, rehydrated through graded ethanol to distilled water, incubated for 15min with 3% hydrogen peroxidase to inhibit endogenous peroxidase activity, and then heated in 0.01mol/l citrate buffer (pH6.0) in a microwave oven for 5min at 100ºC for antigen retrieval. After cooling down to room temperature for 30min, the sections were incubated for 15min in a blocking solution containing 10% normal goat serum in PBS. Detection was by Biotin-Streptavidin Amplified (B-SA) system with diaminobenzidine chromogen as in routine protocol. Meyer’s hematoxylin was used for counter staining. The slides were then dehydrated in increasing grades of ethanol, cleared in xylene and mounted. Positive controls were performed by concomitant application of the antibody to tissues known to be positive for each marker.

Criteria for marker evaluation:

Cytokeratin 5/6 positivity was encountered when diffuse cytoplasmic staining was observed with occasional perinuclear enhancement. Cases were considered positive for calretinin if finely granular cytoplasmic and nuclear immunostaining was recognized [6]. Cases were considered vimentin positive when diffuse cytoplasmic staining was encountered [9]. Immunopositivity for CEA was considered with diffuse cytoplasmic staining with occasional membrane enhancement [13]. CD15 immunopositivity was considered when there was diffuse or focal, finely to coarsely granular, predominantly cytoplasmic, immureactivity [9]. The percentage of tumor nuclei expressing p27 was determined by counting 1000 cells per slide. Nuclear staining only was considered positive [2].

Statistical analysis:

SPSS version 12 was used for data analysis. ANOVA (non parametric) was used for comparisons of the means of more than 2 independent groups. Chi-square and Fisher’s exact tested proportion independence. Kaplan Meier’s method estimated overall survival. Log rank test compared survival curves. Cox-regression analysis was used to calculate Hazard ratio (odds ratio) and to show independent variables affecting overall survival. Significance level ≤0.05 was used throughout all statistical tests in the study [14].

RESULTS

The mean age of the studied patients was 47.2 years, ranging from 20 to 80 years. Thirty seven cases were males (60.7%), and 24 (39.3%) cases were females. Thirty five (57.4%) cases were resident in endemic areas (Shobra El-Khema, Helwan, and El-Hawamdia), 10 (16.4%) cases were resident in non endemic areas, and 16 (26.2%) cases could not be traced according
to habitat. Twenty four (39.3%) patients were subjected to open surgical biopsy, 2 (3.3%) patients underwent decortications and 35 (57.4%) patients underwent pleuropneumonectomy. Histopathologic type of MPM was defined according to morphologic criteria of the WHO classification [1]. Thirty one (50.8%) cases were of epithelioid type, 21 (34.4%) cases were of biphasic type, and 9 (14.8%) cases were of sarcomatoid type. Staging was performed according to the staging system provided by UICC [12]. Twenty one (34.4%) cases were in stage IV, 29 (47.6%) were in stage III and 11(18%) cases were in stage II.

Immunohistochemical results:

Forty-five (73.8%) cases were positive for cytokeratin 5/6 (Fig. 1), 25 (25/31) cases were of epithelioid type, constituting 80.6% of epithelioid cases and 55.6% of all cases. Fourteen (14/21) cases of the biphasic type and 6 (6/9) cases of the sarcomatoid type were positive for CK 5/6. Forty nine (73.8%) cases were positive for calretinin, 25 (25/31) cases were of epithelioid type constituting 90.3% of all epithelioid cases and 57.1% of all cases. Seventeen (17/21) cases of the biphasic type and 4 (4/9) cases of the sarcomatoid type were positive for calretinin (Fig. 2). Thirty-nine (63.9%) cases were positive for vimentin, 9 cases were of epithelioid type constituting 29% of all epithelioid cases and 23.1% of all cases. All 21 cases of the biphasic type as well as all the 9 cases of sarcomatoid type were positive for vimentin. All 61 cases were negative for CEA. Only one epithelioid case was focally and weakly positive for CD15 (1.6%).

Regarding p27, the median percentage among all examined cases was 1.6% ranging from 0-70%. As regards epithelioid mesothelioma, the median percentage was 2.2 ranging from 0-34%, while in biphasic mesothelioma (Fig. 3), the median percentage was 0.3 ranging from 0-8.1% and in sarcomatoid mesothelioma, the median percentage was 0 ranging from 0-70%. The relation between median percentage of p27 and histopathologic type recorded a highly statistically significant relationship; \( p=0.004 \). A level of 10% was taken as a convenient cut-off point [15], so that those recording less than or equal to 10% positive cells for p27 were considered low expressors, while those expressing more than 10% positive cells were considered high expressors. Most of the epithelioid cases were high expressors. A significant relation was obtained when comparing p27 score at 10% level with the pathologic type \( (p=0.02) \) (Table 1).

Survival analysis:

The minimum follow-up period was 2 years, while the maximum was 5 years. Overall survival of the cases ranged from one month to 35.33 months (median: 5 months). A median survival of 4.13 months was observed in the epithelioid type, 9.63 months in the biphasic and 3 months in the sarcomatoid type. A statistically significant relation was detected on comparing overall survival and histopathologic type, recording a \( p \)-value of 0.025, (Fig. 4). Pair wise comparison showed that the difference between survival was the greatest between biphasic and sarcomatoid types, recording a highly significant statistical relation \( (p=0.004) \). A statistically significant relation was recorded between age and survival (Fig. 5); patients less than or equal 45 years showed a median survival of 8 months, while those older than 45 years had a median survival of 2.47 months \( (p=0.01) \). Although median survival in males (3.6 months) was much lower than females (8.43 months), the difference did not reach statistical significance \( (p=0.21) \). A highly statistically significant relation was obtained on comparing survival of patients in stage II and III (median survival of 8 months) to that of patients in stage IV (median survival of 2.47 months), recording a \( p \) value of 0.006 (Fig. 6). As regards p27, despite the obvious trend for longer survival (8.47 months) in the high expressor group than low expressor group (4.40 months), this difference was statistically insignificant \( (p=0.2) \). Multivariate analysis of different clinicopathologic parameters and p27 immunostaining revealed that both age and stage were the only independent prognostic factors affecting survival in patients with MPM (Table 2).

<table>
<thead>
<tr>
<th>Histologic type</th>
<th>No. of cases</th>
<th>p27%* Median (range)</th>
<th>Overexpression (&gt;10%)**</th>
<th>p27 No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelioid</td>
<td>31</td>
<td>2.2 (0-34)</td>
<td>8</td>
<td>25.8</td>
<td></td>
</tr>
<tr>
<td>Biphasic</td>
<td>21</td>
<td>0.3 (0-8.1)</td>
<td>–</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Sarcomatoid</td>
<td>9</td>
<td>0.0 (0-70)</td>
<td>1</td>
<td>11.1</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>61</td>
<td>1.6 (0-70)</td>
<td>9</td>
<td>14.8</td>
<td></td>
</tr>
</tbody>
</table>

\* \( p=0.004 \), \** \( p=0.02 \).
Table (2): Cox regression analysis for overall survival in relation to age and stage.

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>SE</th>
<th>OR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &gt;45</td>
<td>0.87</td>
<td>0.3</td>
<td>2.4 (1.3-4.3)</td>
<td>0.004</td>
</tr>
<tr>
<td>Stage IV</td>
<td>0.3</td>
<td>0.3</td>
<td>2.7 (1.4-5.1)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

B = Regression coefficient. OR = Odds ratio. SE = Standard error of B. CI = Confidence interval.

Fig. (1): Epithelioid mesothelioma positive for CK5/6 with perinuclear enhancement (x400).

Fig. (2): Biphasic mesothelioma, both components are positive for calretinin (x100).

Fig. (3): Biphasic mesothelioma with p27 positive epithelioid & sarcomatoid components (x400).

Fig. (4): Median survival among various types of malignant mesothelioma.

Fig. (5): Survival in relation to age.

Fig. (6): Survival in relation to stage.
DISCUSSION

Malignant mesothelioma remains a highly lethal cancer. Recent advances in both surgical and medical therapy have improved survival, but the treatments remain toxic and the selection of appropriate patients for these therapies is difficult [16]. Mesothelioma in Egypt is mainly attributed to an environmental origin i.e exposure to asbestos, with a high incidence in women and young adults. The incidence of MPM is rising in Egypt [17].

In our study, the mean age of patients was 47.2 years and the median was 45 years, ranging from 20 to 80 years with a male to female ratio 1.5:1. These results lie in accordance with those obtained by El-Shafiey [18] who recorded a mean age of 47.4 years ranging from 17-85 years and M:F ratio 1.7:1, as well as the results obtained by Abou Elkasem [19] who recorded a median age of 46 years and M:F1.4:1.

In Western studies, the recorded median age was so much higher ranging from 60 to 69 years and M:F ratio higher than 10:1 [2,20,21]. The lower figures obtained by our study could be explained by the fact that most of our patients live in endemic areas, so the chance of exposure among both sexes is nearly equal with a slight increase in males due to their outdoor activities most of the time in the vicinity of the asbestos plants and due to occupational hazards.

In our study, 77.8% of patients with known residence live in endemic areas (Shobra El-Khema, Helwan, and El-Hawamdia), reflecting to some extent also the percentage of asbestos exposure. Asbestos exposure in mesothelioma of 67%, 75.5% and 80 were reported in other studies [19,22,23]. This observation was properly analyzed by Testa and colleagues [22], who reported that approximately 80% of mesotheliomas were associated with asbestos exposure and that finding established an indisputable link between asbestos and mesothelioma.

According to the histopathologic type, most of our cases were of the epithelioid type (50.8%), followed by the mixed type (34.4%) and the sarcomatoid type (14.8%). These results lie in accordance with other studies reporting highest frequency for epithelioid type and lowest for sarcomatoid type [2,19,23].

In our study, the most recent TNM classification system was adopted [12] and supported by proper accurate histopathologic evaluation, 18% of our cases were of stage II, 47.6% were of stage III, and 34.4% were of stage IV. None of our studied cases were of stage I, a finding which may highlight the aggression of the disease and the late presentation among Egyptian patients. Abou Elkasem [19] staged MPM cases according to the International Mesothelioma Interest Group (IMIG) staging system [24], with differences between preoperative and postoperative staging. Preoperative staging included 8 cases of stage I, 13 cases of stage II, and 1 case of stage III, while post operative staging included 3 cases of stage I, 5 cases of stage II, and 14 cases of stage III. This difference underlines the importance of the histopathology in identifying different categories of the TNM staging system. When the Butchart classification system [25] was adopted, stage I was reported in 35.1%, 52.5% and 66.9% of cases in different studies [18,21,23]. The high percentage of stage I was probably an overestimation because of the inadequacy of the classification system, a debate which was highlighted by Boutin and co-workers [23] who reported median survival for Butchart Stages I and II as 13.4 and 9.4 months, respectively. This slight difference underlined the limitations of this classification.

In our study, 73.8% cases were positive for CK5/6, including 80.6% of epithelioid mesothelioma, 66.7% of biphasic mesothelioma, in which 10 cases showed positivity in both components, while 4 cases showed positivity in the epithelioid component only. In sarcomatoid mesothelioma 66.7% of the cases were positive. In other studies, CK5/6 was reported in 87-100% of cases of mesothelioma [4,10,13], indicating that CK5/6 is one of the most sensitive and specific available markers of malignant mesothelioma. It has been concluded that CK5/6 was diagnostically useful in distinguishing sarcomatoid mesotheliomas from most sarcomas and that positive staining with any antimesothelial antibody in conjugation with cytokeratin would favor the diagnosis of mesothelioma over spindle cell carcinoma [26].

Calretinin was positive in 80.3% of our cases, including 90.3% epithelioid mesothelioma, 44.4% sarcomatoid mesothelioma, and 81% mixed mesothelioma. The reaction was both
nuclear and cytoplasmic. Kayser et al. [27] reported reactivity for calretinin in 82.2% of malignant mesotheliomas, including 84.8% epithelioid mesotheliomas, 75% mixed mesotheliomas, and 57% sarcomatoid mesotheliomas. They recorded a sensitivity of 81% and a specificity of 86% in distinguishing mesothelioma from metastatic carcinoma in the pleura. Comin et al. [7] concluded that calretinin seemed to be the most sensitive (100%) and specific (91.3%) mesothelioma marker. Moreover, the staining pattern for calretinin seemed to be highly specific (100%) in differentiating mesothelioma from lung adenocarcinoma because the former always showed nuclear and cytoplasmic staining, whereas the latter was either negative or showed only cytoplasmic staining. Ordonez [8] recorded reactivity for calretinin in 100% of epithelioid mesothelioma and only in 8% of lung adenocarcinoma. He concluded that calretinin is one of the best positive markers for differentiating epithelioid malignant mesothelioma from pulmonary adenocarcinoma. In accordance with our results on sarcomatoid mesothelioma, both nuclear and cytoplasmic expression for calretinin was obtained in 39% of sarcomatoid mesotheliomas with no expression in sarcomas or sarcomatoid carcinomas examined [26]. From these results, it is worth mentioning that although calretinin was not particularly sensitive for sarcomatoid mesothelioma, its specificity was 100%. Others concluded that although calretinin appeared one of the most useful markers for differentiating sarcomatoid mesothelioma from sarcoma, it was of no value in differentiating sarcomatoid mesothelioma from sarcomatoid carcinoma [28].

In our study, we reported vimentin positivity in 63.9% of the cases, including all biphasic and sarcomatoid cases and 29% of the epithelioid type. It is noteworthy to mention that co-expression of vimentin and CK5/6 was detected in 44.3%, thus confirming the value of co-expression of vimentin and CK5/6. Wick et al. [29] detected vimentin in 41% of epithelioid mesotheliomas in comparison to 17% of pulmonary adenocarcinomas Mayall and colleagues [30] identified vimentin in 54% and 74% of epithelioid and mixed mesotheliomas, respectively, and in 87% of sarcomatoid mesotheliomas. The variability in immunoreactivity for vimentin can be attributed to; first: Differences in tissue fixation [31] and second: The antibodies employed [8]. Kayser et al. [27] reported positivity for vimentin in 83% of malignant mesotheliomas, including 80% of epithelioid mesothelioma as well as 100% of sarcomatoid and biphasic mesothelioma, recording sensitivity of 82% and specificity of 52% in distinguishing mesothelioma from metastatic carcinoma in the pleura.

None of our cases stained for CEA, while only one case of the epithelioid type showed a focal and weak positive reaction to CD15. It has been recommended that anti-CEA and CD15 should always be included in the panel of antibodies used for immunohistochemical diagnosis of mesothelioma, since CD15 and CEA were the most specific for separating mesothelioma from adenocarcinoma [7]. As regards CEA, conflicting results have been reported from different series, recording CEA positivity in 15% to 45% of mesotheliomas and 100% to as few as 25% in adenocarcinomas. These discrepancies are believed to be attributable to different sensitivities and specificities of the different antibodies used. At present, this problem is not so much encountered as there are several anti-CEA monoclonal antibodies that do not stain mesotheliomas but do react with most adenocarcinomas [10].

A median survival ranging from 4 to 14.3 months in either untreated or treated patients was reported in MPM [19,22]. In our study, the median survival was as low as 5 months and ranged from one month to 35.33 months, pointing strongly to the aggression of the disease among our studied cases of Egyptian patients. We found a statistically significant correlation between overall survival and age (p=0.01). Moreover, by Cox regression analysis, age was proved to be an independent prognostic factor (p=0.004). It has been reported that the best survival is associated with young age [33,34]; yet, others did not find any correlation [19,23]. In our study, there was an obvious trend for female patients to have a better survival than males, however, the difference did not reach the point of statistical significance. Edwards et al. [35] succeeded in detecting a statistically significant correlation confirming the association between poor prognosis and male gender. There was a statistically significant relation between the histopathologic type and survival (p=0.025) observed in our cases. It is noteworthy to men-
tion that pair wise comparison has identified that the great difference between survivals among histopathologic types was estimated when comparing patients with mixed mesothelioma to those with sarcomatoid mesothelioma ($p=0.004$). A highly significant statistical relationship between histopathologic type and survival ($p=0.001$), with the best survival obtained in patients with the epithelioid and mixed types, was recorded [21]. Patients with the epithelioid type were categorized as long-term survival group [36].

In our study, there was a highly statistically significant relationship between survival and stage of malignant mesothelioma ($p=0.006$). Moreover, stage was proved to be an independent prognostic factor when subjected to Cox regression analysis ($p=0.002$). In accordance with our results, Abou ElKasem [19] reported a statistically significant relationship between survival and stage ($p=0.02$).

In our study, the median survival of low expressors ($\leq 10\%$) of p27 was 4.40 months, while high expressors ($>10\%$) had a median survival of 8.47 months. Inspite of this obvious trend for the high expressor group to live more, this relationship did not reach the level of statistical significance, most probably due to small sample number.

There was a highly statistically significant relation between p27 expression and histopathologic type ($p=0.004$). The median percentage of p27 immunoreactivity was higher in the epithelioid type in comparison to the biphasic and sarcomatoid types. This statistically significant relation was also obtained when comparison between p27 and histopathologic type was done at the level of 10% as a cut-off point ($p=0.02$). It has been reported that low p27 expression was associated with a statistically significant decrease in survival, however, this observation showed border-line significance in the multivariate analysis [15]. Others also reported that alterations in cell cycle control proteins such as p16, p21, and p27 also offer information on prognosis and represent potential targets for therapy [16].

**Conclusion:**

MPM is an increasing disaster in Egypt which is underestimated and neglected. A panel of immunohistochemical markers should be used for proper evaluation. p27 has proven to be a potential biologic prognostic marker for mesothelioma and more studies as regards its significance are recommended on a larger number of cases.

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