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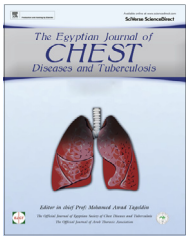

ORIGINAL ARTICLE

Mesothelin and osteopontin as diagnostic and prognostic markers of malignant pleural mesothelioma in Egyptian patients undergoing pleurodesis

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Abstract *Purpose:* In malignant pleural mesothelioma (MPM), early assessment of disease status is important. We evaluated the role of mesothelin and osteopontin biomarkers in distinguishing MPM from benign pleural disease. We also, evaluated whether mesothelin and osteopontin were related to successful pleurodesis or not.

Materials and methods: Mesothelin and osteopontin were assayed in blood and pleural fluid with commercial ELISA kits in a series of 20 patients with malignant mesothelioma and 20 patients with benign pleural effusion (10 patients with tuberculous pleural effusion and 10 patients with benign asbestos pleural effusion). Results were correlated with histological subtypes and pleurodesis outcome.

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Results: Both mesothelin and osteopontin in blood and pleural fluid showed statistically high levels in malignant pleural mesothelioma than benign pleural effusion with a cutoff point of 3.5 nmol/L for pleural mesothelin and 3.3 nmol/L for serum mesothelin and of 280 ng/ml for pleural osteopontin and 260 ng/ml for serum osteopontin. Also, there are statistically significant high levels of mesothelin in epitheliod subtype than sarcomatoid and mixed mesothelioma. Cases of MPM who have a cutoff value of more than (4 nmol/L) for pleural mesothelin and (3.4 nmol/L) for serum mesothelin and (370 ng/ml) for pleural osteopontin and (350 ng/ml) for serum osteopontin had failed pleurodesis but cases that have values less than the cutoff points had successful pleurodesis.

Conclusion: The combined assays of blood and pleural fluid mesothelin and osteopontin biomarkers have a high diagnostic and prognostic yield in malignant pleural mesothelioma patients undergoing pleurodesis.

Introduction

Malignant pleural mesothelioma (MPM) is an aggressive tumor which usually has a poor prognosis. Its incidence is increasing throughout most of the world, and it is predicted that it will rise in the next 10–15 years as a result of widespread exposure to asbestos in the past decades [1]. The management of patients with MPM remains controversial. Due to the usually advanced stage at presentation, the discovery of a marker that would permit an earlier diagnosis could lead to an increase of the proportion of patients diagnosed with early-stage mesothelioma. To date, there is no recognized marker for the early diagnosis of mesothelioma or for screening of at risk asbestos – exposed individuals.

Recent reports have raised the interest of soluble mesothelin-related peptides and osteopontin as possible markers for diagnosing MPM [2,3]. Osteopontin is an extracellular cell adhesion protein involved in non mineral bone matrix formation, but is also a key cytokine in mediating type immune responses [4]. It was implicated in the regulation of metastatic spread of tumor cells [5]. In fact, osteopontin was first described as being secreted by transformed malignant epithelioid cells [6]. Mesothelin is a physiologically expressed membrane bound peptide on the surface of normal mesothelial cells and is also found expressed in various cancers, including malignant mesothelioma [7], pancreatic or ovarian carcinoma, sarcomas and in some gastrointestinal or pulmonary carcinomas [8].

A soluble form, released from the membrane bound mesothelin, can be detected in sera from mesothelioma patients. The mechanism of release of mesothelin from the cell surface into the blood is unknown. Serum mesothelin level is low in healthy subjects exposed to asbestos [9].

Aim of the work

To assess the diagnostic value of both blood and pleural fluid mesothelin and osteopontin biomarkers in the diagnosis of malignant pleural mesothelioma and to evaluate their prognostic value in the success of pleurodesis in those patients.

Patients and methods

The study included 40 patients with pleural effusion suspected of having mesothelioma. Patients were selected from Chest, Internal Medicine and Cardiothoracic Surgery departments, Zagazig University Hospitals from June 2009 to June 2012.

All patients had clinical symptoms suggestive of a diagnosis of MPM (i.e. at least chest pain and dyspnea), associated with pleural thickening, or pleural nodules and pleural effusion on the thoracic computed tomography scan [10].

Exclusion criteria

- Previous therapy against malignant mesotheliom.
- Other malignant pleural effusion.

Samples taken from patients

1. Blood samples for
   - Mesothelin and osteopontin assays.
2. Pleural fluid samples for
   - Biochemistry: proteins g/L and glucose g/L and LDH.
   - Total and differential cells count of pleural effusion.
   - Cytology for malignant cells.
   - Mesothelin and osteopontin assays.
3. Final diagnosis based on tissue pleural biopsy by thoracoscopic biopsy or through limited thoracotomy in difficult cases for thoracoscopic biopsy or by CT guided pleural biopsy in difficult cases for surgery. Histopathological examination was done for all patients.

So, patients were divided after histopathological diagnosis into (20) cases proved to have MPM and (20) cases who had benign pleural effusion (benign asbestos pleural effusion 10 cases, and 10 patients with tuberculoses pleural effusion).

The malignant mesothelioma cases were subclassified into (14) cases with epithelioid type, (4) cases with sarcomatoid type and (2) cases with mixed type after histopathological examination.

Pleurodesis was done by cardiothoracic surgeons for cases proved to be malignant mesothelioma by talc slurry pleurodesis. Premedication and sedation in the form of benzodiazepine should be appropriate that is, maintenance of verbal communication and cooperation. Sedation employed before pleurodesis should be conducted with continuous monitoring with pulse oximetry and in a setting where resuscitation equipment is available. Lignocaine (3 mg/kg; maximum 250 mg) should be administered intrapleurally just prior to sclerosant administra-

tion [11]. Six grams of sterile talc were instilled, as a slurry, in 200-ml saline solution, through a large syringe. The talc slurry was injected in the chest drain inserted after thoracoscope or primary inserted for pleurodesis. The tube is clamped and the syringe connected to the drain. The slurry is injected and the drain re-clamped. Then the patients were placed in a series of positions by the nursing staff one side first, then the other, with the head up followed by head down – each position for about 10 min. This distributes the talc slurry over the surface of visceral and parietal pleurae. After an hour the drain will be unclamped to allow excess talc and saline to drain out. The drain is usually removed the following day after a check X-ray [12].

Mesothelin and osteopontin assays

Mesothelin levels were measured in both pleural fluid and serum samples of all patients, using the mesomark assay (Fujirebio Diagnostics, Malvern, PA) from serum and pleural fluid stored at −80 °C.

Osteopontin levels in serum and pleural fluid were measured in duplicate using the human osteopontin enzyme – linked immunosorbant assay kits (Immunobiological Laboratories, Minneapolis, MN). The assays were performed according to the manufacturer's instructions where results differed by more than 15%. A triplicate was performed. If at least two results were not within 15% of each other, results were discarded.

Statistical analysis

Data were checked, entered and analyzed by using SPSS (version 19). Data were expressed as mean ± SD for quantitative variables, number and percentage for categorical variables. ANOVA (F test), t test, chi-squared or fisher exact tests were used when appropriate.

$P < 0.05$ was considered statistically significant.

Results

In Table 1 we studied 40 patients suspected of having mesothelioma. They were divided after histopathological examination into 20 cases proved to have mesothelioma and 20 cases with benign pleural effusion (previously asbestoses exposed 10 cases with benign pleural effusion and 10 cases with tuberculous asbestos pleural effusion). Male sex was predominant than female with a mean age of 62.8 ± 9.6 years in the mesothelioma patients. Most of the mesothelioma patients had a history of asbestos exposure (70%).

Table 2 shows that pleural fluid protein, and LDH were higher and pleural fluid glucose level was lower in MPM than in benign pleural effusion and the difference was statistically highly significant. Pleural fluid cytology was positive for mesothelioma in only 40% of MPM patients. It was non conclusive in 60% of them. Thoracoscopic pleural biopsy was conclusive for mesothelioma in 70% of MPM patients. CT guided pleural biopsy was diagnostic in 20% of MPM patients whereas surgical biopsy was the last resort in 10% of patients. The different histological types in the studied MPM patients were epithelioid in 70% of patients, sarcomatoid in 20% and mixed type in 10% of patients.

Table 3 illustrated that both serum and pleural Mesothelin are significantly higher in cases of mesothelioma pleural effusion than in benign pleural effusion. Also, there is a cutoff value of (3.5 nmol/L) for pleural mesothelin and (3.3 nmol/L) for serum mesothelin to differentiate between benign and malignant pleural effusion secondary to mesothelioma. As regards to pleural fluid and serum osteopontin, their levels were significantly higher in MPM than in benign pleural effusion. There is a cutoff value of (280 ng/ml) for pleural osteopontin and (260 ng/ml) for serum osteopontin to differentiate between benign and malignant pleural effusion secondary to MPM.

Table 4 shows that both serum and pleural mesothelin and osteopontin biomarkers are statistically highly significant in epithelioid type than sarcomatoid and mixed types.

Table 5 shows cases of MPM that had cutoff values of (4 nmol/L) for pleural mesothelin and (3.4 nmol/L) for serum mesothelin. As regards osteopontin, cases had cutoff values of pleural. Osteopontin (370 ng/ml) and of serum osteopontin (350 ng/ml) had failed pleurodesis but cases below these cutoff points had successful pleurodesis.

Table 6 illustrates that pleural mesothelin shows higher sensitivity than specificity (95.0% compared to 90.0%). On the opposite side, pleural osteopontin shows higher specificity than sensitivity (95.0% compared to 90.0%) in the diagnosis of malignant pleural mesothelioma.

Table 1 Demographic data of the studied patients.

Demographic data	Mesothelioma No = 20 patients	Benign pleural effusion No = 20 patients	*P*-value
Age in years			
mean ± SD	62.8 ± 9.6	60.3 ± 7.5	> 0.05
Sex [*n* (%)]			
Male	13 (65%)	12 (60%)	> 0.05
Female	7 (35%)	8 (40%)	> 0.05
Asbestos exposure			
Yes	14 (70%)	10 (50%)	> 0.05
No	2 (0%)	8 (40%)	< 0.05
Likely	4 (20%)	2 (10%)	> 0.05

Table 2 Diagnostic methods used in the studied patients.

Method of diagnosis	Mesothelioma (20 patients)	Benign pleural effusion (20 patients)	*P* value
Pleural fluid biochemistry			
– Total protein g/L	54.1 (50–58.2)	48 (43–53.1)	< 0.001
– Glucose g/L	0.93 (0.58–1.17)	1.48 (1.12–1.14)	< 0.001
– LDH (IU/L)	1562 (304–1820)	554 (373–735)	< 0.001
Pleural fluid cytology			
– Positive for mesothelioma	8 (40%)	0.0 (0%)	< 0.05
– Negative for mesothelioma	0 (0%)	14 (70%)	< 0.05
– Non conclusive	12 (60%)	6 (30%)	> 0.05
Tissue biopsy			
– Thoracoscopy	14 (70%)	12 (60%)	> 0.05
– Surgery	2 (10%)	2 (10%)	> 0.05
– CT guided pleural biopsy	4 (20%)	6 (30%)	> 0.05
Histopathology			
– Epithelioid	14 (70%)	–	
– Sarcomatoid	4 (20%)	–	
– Mixed	2 (10%)	–	

Table 3 Pleural fluid and serum cutoff values of mesothelin and osteopontin in malignant pleural mesothelioma and benign pleural effusion patients.

Tumor biomarkers	Malig. pl. mesothelioma (median and range)	Benign pl. eff (median and range)	*P*-value
Pleural mesothelin cutoff (3.5 nmol/L)	3.8 (3.3–4.3)	3.1 (2.9–3.6)	< 0.001
Serum mesothelin cutoff (3.3 nmol/L)	3.6 (3.2–4.1)	2.8 (2.2–3.4)	< 0.001
Pleural osteopontin cutoff (280 ng/ml)	390 (270–460)	210 (205–285)	< 0.001
Serum osteopontin cutoff (260 ng/ml)	370 (250–440)	220 (210–270)	< 0.001

Table 4 Pleural fluid and serum values of mesothelin and osteopontin in the different histological types of mesothelioma.

Tumor biomarkers	Epithelioid sub type No = 14	Sarcomatioid No = 4	Mixed No = 2	*P*-value
Pleural mesothelin (nmol/L)	4.2 (4.0–4.4)	3.7 (3.5–3.9)	3.9 (3.8–4.0)	< 0.001
Serum mesothelin (nmol/L)	4.1 (3.9–4.3)	3.6 (3.5–3.7)	3.8 (3.6–4.0)	< 0.001
Pleural osteopontin (ng/ml)	430 (440–420)	380 (390–370)	390 (410–370)	< 0.001
Serum osteopontin (ng/ml)	410 (430–390)	370 (380–360)	380 (400–360)	< 0.001

Table 5 The impact of pleural fluid and serum values of both mesothelin and osteopontin on the success of pleurodesis.

Tumor biomarkers	Failed pleurodesis (7 patients) Median (range)	Successful pleurodesis (13 patients) Median (range)	*P*
Pleural mesothelin (nmol/L)	4 (3.9–4.3)	3.2 (3.1–3.3)	0.016*
Serum mesothelin (nmol/L)	3.8 (3.5–4.1)	2.8 (2.2–2.4)	0.026*
Pleural osteopontin (ng/ml)	420 (380–480)	300 (240–390)	< 0.001*
Serum osteopointin (ng/ml)	360 (365–440)	295 (250–360)	< 0.001*

Discussion

Mesothelioma is a highly aggressive cancer, which is often diagnosed up to 30–40 years after asbestos exposure. Because tumor growth is generally insidious and the usual clinical signs (dyspnea, cough and chest pain) are non-specific mesothelioma diagnosis is generally obtained too late [13]. Thus disease markers have been searched in an attempt to help early diagnosis. Recently, osteopontin and mesothelin have been proposed as early markers for MPM diagnosis [3].

Some studies showed that osteopontin is a high sensitivity marker but with poor specificity and mesothelin had a good specificity but with a lower sensitivity for MPM, so, we evaluated the diagnostic and prognostic value of the combination of the two markers in the assessment of MPM patients.

Table 6 Validity of both mesothelin and osteopontin biomarkers in the diagnosis of malignant pleural mesothelioma.

	Sensitivity	Specificity	PV		Accuracy
			+ve	–ve	
Pleural mesothelin	95.0	90.0	90.5	94.7	92.5
Serum mesothelin	95.0	95.0	95.0	95.0	95.0
Pleural osteopontin	90.0	95.0	94.7	90.0	92.5
Serum osteopontin	95.0	85.0	86.4	94.4	90.0

In the present study, 40 patients suspected of having mesothelioma were enrolled. They were divided according to histopathological examination into 20 cases with benign pleural effusion and 20 proved mesothelioma (MPM) patients. Male sex was predominant than female with a mean age of (62.8 ± 9.6) years. Male predominance is related to their occupational exposure to asbestos dust more than females. This agrees with Steven et al. [10].

Also (70%) of MPM cases had a history of asbestos exposure. This agrees with Daniel and Steven [14] who reported that asbestosis is the principal carcinogen associated with malignant pleural mesothelioma, indeed, malignant pleural mesothelioma was rare before the widespread use of asbestos, also the lifetime risk of developing MPM among asbestos workers is 8–13%. They also reported that, there is typically a long latency period of approximately 30–40 years from the time of asbestos exposure to the development of mesothelioma. This also explains the predominance of old age among the studied MPM patients with an average of 62.8 y.

Table 2 showed that biochemical variables of pleural fluid are good indicators of (MPM) in which glucose is markedly decreased while LDH and protein is markedly increased.

The results mentioned previously by Silvia Bielsa et al. [15] that pH < 7.2, glucose < 60 mg/dl and LDH > 600 μ/L with karnosfsky index <70 and massive effusion defined as the one that exceeds the hilar region can be used as predictive factors of decreased survival in malignant mesothelioma.

Also Table 2 showed that thoracoscopic biopsy gave the highest diagnostic yield than other biopsies (70% in MPM and 60% in benign pleural effusion). This agrees with Murray and Nadel's [16] who reported that, the preferred technique for surgical biopsy is via pleuroscopy. Not only does pleuroscopy have the advantage of obtaining large samples, but, also, it permits the drainage of effusions and freeing up of a trapped lung. In addition, if the lung is not trapped, talc can be insufflated at the end of the procedure to achieve pleurodesis. Also Carbone et al. [17] mentioned that video assisted thoracoscopy is a useful technique among patients of mesothelioma.

Table 3 showed that both serum and pleural mesothelin are significantly higher in cases of (MPM) than in benign pleural effusion. Also, serum and pleural osteopontin are higher in cases of mesothelioma than in benign pleural effusion. This result is concomitant with Bogdan-Dragos et al. [18] who detected, that the serum osteopontin level was higher in MPM patients compared with healthy asbestos exposed subjects and a good capability of osteopontin to distinguish between these two populations. They also revealed that serum mesothelin had a good ability for diagnosing MPM but was unable to identify patients with non epithelioid mesothelioma subtypes. Also Pass et al. [3] reported that osteopontin has been proposed as an early marker for MPM diagnosis.

Creaney et al. [19] illustrated that most (>95%) patients with mesothelioma have pleural effusion, it would be logical to assess the usefulness of soluble mesothelin in the pleural fluid as a diagnostic test for mesothelioma. They also reported that soluble mesothelin is detectable in pleural effusions and is significantly higher in pleural mesothelioma than in effusions of benign pleuritis.

Harvey et al. [20] illustrated that osteopontin mediates cell – matrix interactions and cell signaling through binding with integrin and is regulated by proteins in cell – signaling pathways that are associated with asbestos – induced carcinogenesis. Moreover, high levels of osteopontin correlate with tumor invasion, progression and metastases. So, osteopontin is a useful biomarker in pleural mesothelioma.

Table 4 showed that both serum and pleural mesothelin are higher in epitheliod type than in mixed or sarcomatoid type.

This result is concomitant with Daniel and Steven [14] who reported that, soluble mesothelin is a useful marker for epitheliod mesothelioma but not for other histological variants of mesothelioma. The result is concomitant with Bogdan-Dragos et al. [18] who found that mesothelin had a good ability for diagnosing MPM but was unable to identify patients with non epithelioid mesothelioma subtypes.

This result can be explained by the fact that mesothelin is a glycoprotein found predominantly in normal mesothelial cells in pleura so, when MPM is formed the tumor burden increases by an increase in the size of tumor and its secretions. Patients with epithelial MPM and larger tumor had higher concentrations of soluble mesothelin related peptides (SMRP) than those with sarcomatoid histotype and smaller tumor size. Also, a trend toward increasing SMRP concentrations with disease progression was observed while SMRP levels fell after debulking surgery [21].

Table 5 showed that cases of mesothelioma with cutoff values of 4 nmol/L for pleural mesothelin and 3.4 nmol/L for serum mesothelin, 370 ng/ml for pleural osteopontin and 350 ng/ml for serum osteopontin or more had failed pleurodesis and those with cutoff values less than these values had successful pleurodesis.

This result can be explained by the fact that high levels of these biomarkers indicate increased tumor burden that impairs pleurodesis success. Siliva et al. [15] explained that cases with low glucose and pH in the pleural fluid traditionally have increased tumor burden and decreased pleurodesis success.

Table 6 illustrates that pleural mesothelin shows higher sensitivity than specificity (95.0% compared to 90.0%). On the opposite side, pleural osteopontin shows higher specificity than sensitivity (95.0% compared to 90.0%).

This can be explained by the fact that mesothelin is a glycoprotein attached to the cell surface; it is not a cancer-specific antigen but a differentiation antigen thought to have a role in cell adhesion and in cell-to-cell recognition and signaling. It is over-expressed in several tumors such as mesothelioma, pancreatic cancer and ovarian cancer [8], so it is a highly sensitive but less specific marker. Pass et al. [3] investigated the role of osteopontin in MPM, they found significantly higher specificity 85.5% than sensitivity 77.6%. This higher specificity correlates with its role in tumor invasion, progression and metastasis.

Conclusion

The combined assays of blood and pleural fluid mesothelin and osteopontin biomarkers have a high diagnostic and prognostic yield in malignant pleural mesothelioma patients undergoing pleurodesis.

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