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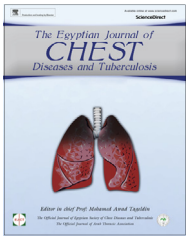

ORIGINAL ARTICLE

Role of fibulin-3 in the diagnosis of malignant mesothelioma

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KEYWORDS

Malignant mesothelioma;
Fibulin-3;
ELISA

Abstract *Background:* Early detection of malignant pleural mesothelioma (MPM) is critical to survival, the use of pleural or blood fibuin-3 might allow this early detection.

Aim: Studying the validity of measuring serum and pleural fibulin-3 in the diagnosis of MPM.

Subjects & Methods: Fibulin-3 levels were measured in serum and pleural fluid by enzyme-linked immunosorbant assay (ELISA) in 45 patients with exudative pleural effusion. Patients with non-conclusive cytology or microbiological examination had undergone medical thoracosope for histopathological examination.

Results: Twenty five was diagnosed as MPM, 11 cases as pleural metastasis of carcinoma (Mets) and nine cases with benign pleural effusions. Patients with MPM had significantly higher pleural effusion and serum fibulin-3 levels than those with metastatic effusion of carcinoma or benign pleural effusion (p-value < 0.001). Using a cut-off point of pleural fluid fibulin-3 (150 ng/ml) with AUC of 0.878 (sensitivity 72.3%, specificity 80) and at a cut-off point of serum fibulin-3 (66.5 ng/ml), with AUC of 0.776 (sensitivity 88%, specificity 81.8%), discrimination between MPM and Mets occurred. Also, using a cut-off point of pleural fluid fibulin-3 (127.5 ng/ml) with AUC of 0.909 (sensitivity 88%, specificity 77.8%), and at a cut-off point of serum fibulin-3 (18 ng/ml), with AUC of 0.931 (sensitivity 100%, specificity 77.8%), discrimination between MPM and benign pleural effusion could occur.

Conclusions: Fibulin-3 in the serum and pleural fluid is a good biomarker in the diagnosis of MPM and in differentiation between MPM from malignant pleural metastasis other than mesothelioma and also from benign pleural effusions.

Introduction

Malignant pleural effusion is a condition in which cancer causes an abnormal amount of fluid to collect in the pleural cavity. Lung cancer and breast cancer account for about 50–65% of malignant pleural effusions. Other common causes include pleural mesothelioma and lymphoma. Malignant pleural mesothelioma (MPM) has a very bad prognosis of about a

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year after diagnosis. Earlier detection of this lethal pleural cancer could conceivably result in earlier treatment and some improvement in life [1]. Early detection is limited by the long latency period, an inability of imaging to detect the disease at an early stage even when it is used as a screening strategy, and the lack of sensitive and specific blood-based markers [2]. Analysis of pleural fluid yields a confirmed diagnosis in a relatively small percentage of MPM patients, and needle biopsy offers only slightly better results. Medical thoracoscopy is recommended in the investigation of patients with MPM, which has a diagnostic yield of >95% [3]. Early detection is critical to survival with mesothelioma, the use of pleural or blood-based biomarkers might allow detection of MPM at an early stage. Tumor markers offer an attractive means of diagnosis, being less expensive and less invasive [4]. Soluble mesothelin related protein (SMRP), the most extensively studied blood based mesothelioma biomarker, is limited by an overall sensitivity of 47% at 96% specificity [5]. Serum biomarkers such as SMRp, osteopontin, CA125 and megakaryocyte potentiating factor (MPF) have been investigated as tools to aid in the diagnosis of malignant mesothelioma, or for screening of 'at risk' group [6]. A positive blood test for mesothelin at a high specificity threshold is a strong incentive for further diagnostic steps, provided there is no renal failure [7]. However, the poor sensitivity of mesothelin at diagnosis (35–50%) limits its value. In screening studies, mesothelin levels are elevated before diagnosis in fewer than 15% of mesothelioma patients in a high risk group, so it is not recommended as a screening tool [8]. Also osteopontin and CA125 lack specificity as diagnostic markers, serum mesothelin and CA125 may have value in monitoring response to treatment [8]. New biomarkers are needed to detect pleural mesothelioma at an earlier stage. Fibulin-3 is an extracellular glycoprotein in the fibulin family; these proteins are frequently associated with vascular and elastic tissues, and become overexpressed in people with pleural mesothelioma [9]. Fibulin-3 is a highly conserved member of the extracellular glycoprotein fibulin family encoded by the gene epidermal growth factor – containing fibulin-like extracellular matrix protein 1 (EFEMP1) on chromosome 2p16 [10]. Gene expression is low in normal tissues, with the highest expression in the thyroid [11]. Fibulin-3 is expressed in condensing mesenchyme, giving rise to bony and cartilaginous structures. It mediates cell-to-cell and cell-to-matrix communication, is inversely related to cell growth, and has variable angiogenic effects. Inactivation of EFEMP1 due to DNA hypermethylation has been reported in lung, prostate, colorectal, breast, nasopharyngeal, and hepatocellular carcinomas [12].

Aim

The aim of this study was to assess the validity of measuring serum and pleural fibulin-3 in the diagnosis of malignant pleural mesothelioma and its ability to differentiate between MPM, and both other pleural malignancies or benign pleural effusions.

Subjects and methods

Patients with pleural effusion were admitted to the Chest Department in Menoufiya University Hospitals, Egypt, during the period from January 2013 to August 2013. All patients had undergone history taking including occupational and environmental hazards, general and local examinations, routine laboratory investigations (Serum lactate dehydrogenase (LDH), total protein, albumin, liver and kidney functions, ESR and CBC), Radiological assessment by plain CXR, and CT. Following the previous step, and after the diagnosis of pleural effusion had been confirmed, thoracentesis was done. The routine study of the pleural fluid included the following: pH, biochemical testing of pleura/serum (LDH, glucose, albumin and Adenosine deaminase (ADA), cytology and microbiological testing (Z–N, L–J culture) and differential cell count). Using Light's original criteria (ratio of pleural fluid/serum protein >0.5, ratio of pleural/serum LDH >0.6 or pleural fluid LDH more than two-thirds of the upper limit of normal serum value), 20 patients with transudative pleural effusions were excluded from the study. The remaining 45 diagnosed to have exudative pleural effusion was enrolled in the study. Patients with non-conclusive cytology and microbiological examination had undergone medical thoracosope by which multiple pleural biopsies were taken and sent for histopathological examination. Tuberculous pleural effusion was confirmed either by positive Z–N or L–J culture or by the presence of tuberculous granuloma in the histopathological examination. Pleural effusion was categorized as malignant if pleural fluid cytology or pleural biopsy findings were positive for malignancy. A parapneumonic effusion was the one that developed in a patient with fever, pulmonary infiltrates and complete response to antibiotic treatment. All other exudative effusions were included. An idiopathic pleural effusion was identified as one for which a cause was not determined despite an initial workup that included repeated thoracenteses and thoracoscopic pleural biopsies. Patients with transudative pleural effusion, serious uncontrolled diseases (including renal, hepatic, cardiac diseases, and coagulopathy), and hemodynamically unstable were excluded.

Collection of blood samples and pleural effusion fluid

Serum: using a serum separator tube and 10 ml of whole blood samples were allowed to clot for 2 h at room temperature or overnight at 4 °C before centrifugation for 20 min at approximately 1000*g*. Assay freshly prepared serum immediately or store samples in aliquot at 20 °C or −80 °C for later use. *Pleural fluids:* 10 ml of centrifugated samples for 20 min at 1000*g* with removal of particulates and assay immediately or samples were stored in aliquot at −20 °C or −80 °C for later use. *Measurement of fibulin-3:* the fibulin-3 concentrations in pleural fluid and serum were determined using ELISA. The test required 2–3 h. The assay used two monoclonal antibodies. During incubation, both antibodies reacted with fibulin-3 in a sandwich-like manner. After several washing procedures, the tracer remaining in the test tube was measured using a luminometer; the intensity of the luminescent signal was directly proportional to the fibulin-3 concentration of the serum or pleural fluid sample [13]. All the previous steps were done after a written consent from all patients.

Statistical methodology [14]

The data collected were tabulated and analyzed by SPSS (statistical package for the social science software) statistical