RESEARCH ARTICLE

Fibulin-3 as a Diagnostic Biomarker in Patients with Malignant Mesothelioma

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Abstract

Background: New tumour biomarkers are being intensely investigated for malignant mesothelioma (MM). Fibulin-3 is produced in MM but its role remains uncertain. The aim of this study was to evaluate the validity of measuring serum fibulin-3 in the diagnosis and prognosis of MM. Materials and Methods: This prospective study was performed on 43 patients and 40 healthy controls who were admitted to our hospital between January 2012 and January 2014. Data from MM patients, including demographic and clinical features, routine laboratory data, levels of serum fibulin-3, and treatment outcomes were defined as potential prognostic factors. The receiver operating characteristic (ROC) curve for fibulin-3 was used to detect the cut-off value with highest sensitivity and specificity. Univariate survival analysis was performed using the Kaplan-Meier method in patients with MM. Afterwards, the possible factors identified with univariate analyses were entered into the cox regression analysis. Results: Our results revealed that patients with MM had significantly higher serum levels of fibulin-3 than controls. The results showed that the best cut-off point was 36.6 ng/ml with an AUC (area under the curve)=0.976, sensitivity=93.0% and specificity=90.0. In our study, the initial significant poor prognostic factors were advanced stage, high white blood cell count, high platelet count, high C-reactive protein (p<0.05 for each variable). Later, according to multivariate analysis the results showed only advanced stage as significant parameter (p=0.040). Conclusions: We determined that real use for serum fibulin-3 was not for prognosis but for diagnosis in MM. Also advanced stage was associated with poor MM prognosis.

Keywords: Malignant mesothelioma - serum fibulin-3 - diagnosis - prognosis

Introduction

Malignant mesothelioma (MM) is a lethal tumor that typically has a poor prognosis. The greatest risk factor for the development of MM is asbestos exposure, either occupational or environmental. Asbestos exposure is identified in 50-90% of the patients with MM. Contrary to developed countries, the vast majority of mesothelioma in developing countries is secondary to environmental asbestos exposure (Senyigit et al., 2000; Metintas et al., 2002; Berk et al., 2013).

Early detection is limited because there is a long delay between the onset of symptoms and the diagnosis of MM, an inability of imaging to detect the disease at an early stage, and lack of sensitive and specific blood-based markers. The difficulties in establishing the diagnosis of mesothelioma were explained in a large scale study (Boutin et al., 1993). Therefore, most patients present in an advanced stage of this disease because it is an insidious and relatively nonspecific clinical presentation.

Histopathological examination is required for the definitive diagnosis of the MM. The differential diagnosis of MM includes both benign and malignant processes, especially the differential diagnosis between mesothelioma and adenocarcinoma is difficult. Therefore establishing a tissue diagnosis can be difficult (Ordonez 2003). Mesothelioma patients who are treated with supportive care have a median survival of 4 to 13 months (Anthman 1993). Even those treated with the best available chemotherapy survive 13-25 months (Vogelzang et al., 2003).

Recently, research has focused on evaluating several less invasive biomarkers for MM screening, diagnosis, prognostication and monitoring. It has been well documented that soluble mesothelin-related protein, the most extensively studied blood-based mesothelioma biomarker, is limited by a high specificity but poor sensitivity for early diagnosis of MM (Creaney and Robinson, 2009; Hollevoet et al., 2012). Unfortunately, an ideal biomarker for MM has not been yet identified.

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A new biomarker, fibulin-3, has recently been reported as a potential biomarker to facilitate the identification of patients with mesothelioma. Fibulin-3 is an extracellular glycoprotein that is encoded by the epidermal growth factor-containing fibulin-like extracellular matrix protein 1 (EFEMP1) gene and its expression is low in normal tissues. Fibulin-3 is expressed in condensing mesenchyme, giving rise to bony and cartilaginous structures (Zhang and Marmorstein, 2010). In previous studies, different types of cancer have been associated with fibulin-3, such as brain, hepatocellular, prostate, colorectal, and lung (Hu et al., 2009; Nomoto et al., 2010; Kim et al., 2011; Tong et al., 2011; Kim et al, 2012) however, there are few studies pertaining to the association with MM (Pass et al., 2012; Agha et al., 2014; Creaney et al., 2014). In these studies, levels of fibulin-3 investigated in patients with occupational asbestos exposure. But yet, the precise role of fibulin-3 in MM is still under investigation.

The aim of this prospective study was to determine the diagnostic and prognostic value of fibulin-3 and as well as known clinical prognostic factors in MM. This is the first study, to our knowledge, focusing on patients with a history of environmental asbestos exposure.

Materials and Methods

This was a prospective study on 43 patients with biopsy-proven MM who were admitted to our hospital between January 2012 and January 2014. Additionally, 40 healthy controls were included; they had normal chest X-rays and no history of environmental asbestos exposure. Informed consent was obtained from each participant. The study was approved by the local Ethics Committee. Data from MM patients, including demographic and clinical features routine laboratory investigations, levels of fibulin-3, and treatment outcomes were defined as potential prognostic factors and were recorded at the time of diagnosis.

Clinical Features of Mesothelioma Patients

The following pre-treatment patient characteristics were analyzed for their effect on survival age at the time of diagnosis (≤60/>60 years), gender, body mass index (BMI) according to weight and height information, environmental asbestos exposure (yes or no), duration of exposure (≤20/>20 years), smoking history and pack-years of smoking (≤20/>20), symptoms at the time of diagnosis [presence of dyspnea, chest pain, abdominal pain, weight loss (>5% in the last 3 months)], the interval between the onset of symptoms until the diagnosis (≤6 or >6 months), Karnofsky Performance Status (≤60/>60%), primary site of pleural tumor (right/left), presence of pleural fluid and cytology, diagnostic procedures [operative (video-assisted thoracoscopic surgery, minithoracotomy or non-operative (percutaneous pleural/peritoneal needle biopsy)], histopathological types [epithelioid or others (biphasic sarcomatous and unspecified)], staging system according to International Union Against Cancer Classification [stage I-II (earlier) or stage III-IV (advanced)], treatment approach (three groups: best supportive care, systemic chemotherapy, or multimodal therapy), follow-up time

(the interval between the diagnosis and death or the last visit) and patient status (alive or dead).

Laboratory results

The upper limit of the laboratory reference range of different tests is as follows: hemoglobin concentration (Hb, <12.3 or ≥12.3 g/dl), white blood cell count (WBC, ≤8.3 or >8.3/μl) platelet count (≤400.0 or >400.0/μl), levels of serum albumin (≤3.0 or >3.0 g/dl), lactate dehydrogenase (LDH, ≤500 or >500 U/l), C-reactive protein (CRP, ≤50 or >50 mg/l), and erythrocyte sedimentation rate (ESR, ≤50 or >50 mm/h).

Collection of blood samples for fibulin-3 measurement

Ten milliliters of venous blood samples were obtained from all participants. They were allowed to clot for 30 minutes at room temperature before they were centrifuged at 4000 rpm and +4°C for 10 minutes. Following this, the obtained sera were transferred into microcentrifuge tubes and later serum samples were stored -80 °C until analyzed. Fibulin-3 levels of patient and control groups were determined using commercially available the enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturers' protocols.

Statistical analysis

The data collected were tabulated and analyzed by using the SPSS® for Windows version 15.0. Characteristics were reported using frequency and descriptive analysis. The Student t-test (parametric) or Mann-Whitney U test (nonparametric) was used to compare two independent subgroups, as appropriate. The receiver operating characteristic (ROC) curve for fibulin-3 was used to detect the cutoff value with highest sensitivity and specificity Sensitivity, specificity, positive and negative predictive value (PPV and NPV, respectively) and diagnostic accuracy were calculated. Survival was defined as the period between the date of the pathological diagnosis and death. For patients who did not die, this duration was defined as the period between the time of diagnosis and January 2014. Univariate survival analysis was performed using the Kaplan-Meier method in patients with MM. Afterwards, the possible factors identified with univariate analyses were entered into the Cox Regression analysis, to determine independent predictors of survival. P values <0.05 were considered statistically significant.

Results

Patient characteristics

Baseline characteristics for the 43 patients (primary involvement: 39 pleural, 4 peritoneal mesothelioma) are summarized in Table 1.

Diagnostic evaluation

When MM patients and healthy controls were compared, there was no significant difference among age, gender, history of smoking and BMI. However, serum fibulin-3 levels [mean (±SD) 90.3±42.1 and 17.8±12.7 ng per mililiters, respectively] differed significantly (p<0.001). The ROC curve was generated to analyze the

diagnostic values of serum fibulin-3 (Figure 1). The results showed (Table 2) that the best cut-off point for serum fibulin-3 was 36.6 ng/ml with an AUC (area under the curve)=0.976, sensitivity=93.0%, and specificity=90.0.

Survival analyses

Parameters of the clinical and laboratory that we expected to be associated with prognosis were used in univariate analysis. We found that significant poor prognostic factors were advanced stage, high WBC, high platelet count, high CRP (p<0.05 for each variable). No associations were found between MM prognosis and age, gender, asbestos exposure and duration of exposure,

Table 1. Characteristics of the Malignant Mesothelioma Patients

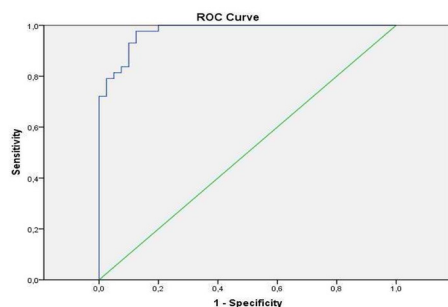|	
Age (mean±sd, years)	61.7±12.2
Age group (≤60/>60 years)	19/24
Gender (female/male)	22/21
BMI (mean±sd, kg/m^2)	24.8±4.2
Asbestos exposure (n,%)	36 (83.7)
Duration of asbestos exposure (mean±sd, years)	27.7±18.7
History of smoking (n,%)	20 (46.5)
Duration of symptoms (months)	5.9±6.4
Laboratory results (mean±sd)	
Hemoglobin concentration (g/dl)	12.5±1.9
White blood cell count (x10^3/μl)	9.2±3.7
Platelet count (x10^3/μl)	346.8±174.6
Serum albumin level (g/dl)	3.2±0.6
Lactate dehydrogenase (U/l)	238.0±95.3
C-reactive protein (mg/l)	45.1±50.2
Erythrocyte sedimentation rate (mm/h)	33.4±16.8
Levels of fibulin-3 (ng/ml)	90.3±42.1
Diagnostic method (n, operative/nonoperative)	23/20
Histopathological type (n,%)	
Epithelial	30 (69.8)
Others	13 (30.2)
Stage (n,%) (n=39)	
Stage I-II	18 (46.1)
Stage III-IV	21 (53.9)
Performance status (Karnofsky) (n,≤60/>60%)	9/33
Treatment (n,%)	
Best supportive care	21(49)
Chemotherapy	15(35)
Multimodal therapy	7(16)
Survival time (mean±sd, months)	9.6±9.5
Status (n,%)	
Alive	20(46.5)
Dead	23(53.5)

*BMI, Body mass index

Figure 1. ROC Curve to Analyze the Diagnostic Values of Serum Fibulin-3

Table 2. Validity of Different Serum Fibulin-3 Levels for the Diagnosis of the Mesothelioma

Cut-off point	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
30.1	97.7	87.5	97.2	89.4	92.8
36.6	93.0	90.0	92.3	90.9	91.6
48.8	81.4	95.0	82.6	94.6	87.9

*PPV, Positive predictive value; NPV, Negative predictive value

Table 3. Kaplan-meier Survival Analysis of 43 Mesothelioma Patients

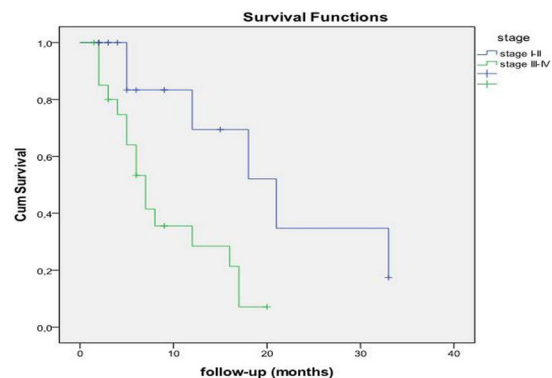| Variables		Median survival (months)	p*
Age group (years)	≤60	17.0	NS
	>60	7.0	
Gender	Female	12	NS
	Male	12	
Stage	I-II	21	0.004
	III-IV	7	
White blood cell count (x10^3/μl)	≤8.3	21	0.035
	>8.3	9	
Platelet count (x10^3/μl)	≤400.0	17	0.004
	>400.0	7	
C-reactive protein (mg/l)	≤50	17	0.029
	>50	9	

Figure 2. Multivariate Analysis Results for Advanced Stage Showing Increased Poor Prognosis

smoking history, initial symptoms, histopathologic subtype, performance status and serum levels of Hb, albumin, LDH, ESR and serum fibulin-3 (p>0.05 for each variable). Variables with p<0.05 in univariate analysis were used in the final model for multivariate analysis.

Prognostic factors affecting survival

Results of the univariate analyses revealed (Table 3) that advanced stage, high WBC, high platelet count, and high CRP were associated with poor MM prognosis. However, then the possible factors identified with univariate analyses were entered subsequently into the cox regression analysis, and only advanced stage remained as an independent predictor of survival (p=0.040). According to multivariate analysis results, advanced stage (Figure 2) increased poor prognosis 3.9 times.

Discussion

In this study, we found that levels of fibulin-3 in serum may aid in determining the diagnosis of mesothelioma.

However, it is not associated with their prognosis. Also, when the relationship between survival and parameters of the clinical and laboratory was investigated poor MM prognosis was associated with advanced stage.

It is well known that people have been exposed to environmental asbestos in some rural areas of the Southeast Anatolia Region of Turkey due to its geographical structure. So environmental asbestos exposure starts at birth and continues (Senyigit et al., 2000) Occupational asbestos exposures were more frequent in men, whereas environmental asbestos exposure was found to be nearly as frequent in men and women (Metintas et al., 2002). While pleural mesothelioma after occupational asbestos exposure mainly effects men (male to female ratio: 5 to 1), male/female ratio is much smaller in non-occupational mesothelioma patients (male to female ratio: 1 to 1) (Metintas et al., 2008; Teta et al., 2008). In our study results shown in Table 1, the ratio of male-to-female mesothelioma patients was approximately equal. Most of our patients declared to previously reside in rural areas and had environmental asbestos exposure for many years. Furthermore, there was no difference between sexes in the duration of exposure.

Historically, biomarkers have not turned out to be all that useful in predicting mesothelioma diseases (Creaney and Robinson, 2000). Gold standard for definitive diagnosis is still histopathological diagnosis, however, sometimes can be very difficult to achieve diagnosis (Ordonez, 2003). In other words, especially in such cases without histopathologic diagnosis, biomarkers provide additional contribution. Promising new biomarker studies are ongoing. In regards to diagnosis of MM, few studies have researched fibulin-3 in serum or plasma and in pleural effusions in order to distinguish mesothelioma patients from either healthy controls or patients with benign disease (Pass et al., 2012; Agha et al., 2014).

Pass et al. (2012) were the first to report that that they measured plasma fibulin-3 levels (from 92 patients with mesothelioma, 136 asbestos-exposed persons without cancer, 93 patients with effusions not due to mesothelioma, and 43 healthy controls), effusions (from 74 patients with mesothelioma, 39 with benign effusions, and 54 with malignant effusions not due to mesothelioma), or both (Pass et al., 2012). They found that plasma fibulin-3 levels did not vary according to age, sex, duration of asbestos exposure, or degree of radiographic changes and that plasma fibulin-3 levels were significantly higher in patients with pleural mesothelioma (105±7 ng/ml in the Detroit cohort and 113±8 ng/ml in the New York cohort) than in asbestos-exposed persons without mesothelioma (14±1 ng/ml and 24±1 ng/ml respectively). They found that elevated levels of fibulin-3 in the plasma had a high sensitivity and specificity (97 and 96 percent, respectively, at a cutoff value of 52.8 ng/ml of fibulin-3) in distinguishing patients with pleural mesothelioma from those with a history of asbestos exposure but without mesothelioma and from those with other malignancies or benign causes of pleural effusion (Pass et al., 2012). The findings of this study suggest that levels of fibulin-3 in plasma and effusions may aid in determining the diagnosis and prognosis of pleural mesothelioma.

Another study has confirmed the role of serum and pleural fibulin-3 in the diagnosis of pleural mesothelioma and its role in differentiating between malignant and benign pleural diseases (Agha et al., 2014). This study showed correlation between serum and pleural fibulin-3. It showed that using a cut-off point of serum fibulin-3 (18 ng/ml), with AUC of 0.931 (sensitivity 100%, specificity 77.8%) differentiation between MM and benign pleural effusion could occur.

Our results showed that the levels of serum fibulin-3 in mesothelioma patients were significantly higher than healthy subjects (90.3±42.1 and 17.8±12.7 ng per mililiters respectively). Also, the best cut-off point of serum fibulin-3 (36.6 ng/ml) had the highest sensitivity (sensitivity 93%, specificity 90%). In patients presenting with a suspected mesothelioma, high levels of fibulin-3 (>36.6 ng/ml) in serum indicate which patients need further invasive procedures. In our study, we found a diagnostic value of serum fibulin-3, but it has not contributed to estimating mesothelioma prognosis. Although our study levels of fibulin-3 in mesothelioma patients were not associated with the prognosis, this result may be due to the small sample size.

Prognostic scoring systems may provide useful information in MM. The two most widely used scoring systems were developed by the Cancer and Leukemia Group B (CALGB) and the European Organization for Research and Treatment of Cancer (EORTC). When jointly evaluated, these studies show that pleural (as compared to peritoneal or pericardial) involvement, age older than 75 years, male gender, poor performance status, chest pain, non epithelial histology, advanced stage, leucocytosis, thrombocytosis and elevated LDH have predicted poor survival (Edwards et al., 2000; Metintas et al., 2001).

Recently, elevated serum CRP levels in several malignant diseases including breast, ovarian, renal, and lung cancer have also been identified as negative prognostic indicators (Hefler et al., 2008; Pierce et al., 2009; Wang and Sun, 2009; O'Dowd et al., 2010). With respect to MM, two retrospective screening studies suggested CRP as potent prognostic marker. High levels of serum CRP have been associated with poor prognosis due to the role of chronic inflammation in the development of MM that has been well established (Tanrikulu et al., 2010; Nojiri et al., 2011). A major limitation of all of the staging systems is the difficulty in assessing the extent of disease prior to treatment. Early stage was considered among the important positive prognostic characteristics in some studies (Van Gelder et al., 1994; Rusch and Venkatraman, 1999). In other study, advanced clinical stage was related to survival (Komurcuoglu et al., 2014). In our study, the initial significant poor prognostic factors were advanced stage, high WBC, high platelet count, high CRP (p<0.05 for each variable). Later according to multivariate analysis the results showed only advanced stage as significant parameter.

The small sample size limited the study. Additionally, cancer-free patients with asbestos exposure and adenocarcinoma patients were not placed in separate groups.

In conclusion, we determined that real use for serum fibulin-3 was not for prognosis but for diagnosis in MM. Also advanced stage was associated with poor MM prognosis. Further studies comparing fibulin-3 with other common biomarkers for MM should be done in order to confirm the role of fibulin-3 in the diagnosis, treatment monitoring and evaluation of disease prognosis. Consequently, prospective studies with a larger sample size are needed to rigorously assess the diagnostic power of fibulin-3.

Acknowledgements

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