Osteopontin Levels in an Asbestos-Exposed Population

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Abstract

Purpose: Serum osteopontin levels in patients with malignant mesothelioma have been reported to be higher than in healthy subjects. This study assessed serum osteopontin levels in an asbestos-exposed population to test whether nonmalignant asbestos-related disorders could influence osteopontin levels.

Experimental Design: This cross-sectional study evaluated serum osteopontin levels in 525 male subjects. Subjects were classified into six different diagnostic groups, including asbestosis (n = 23), silicosis (n = 20), diffuse pleural thickening (n = 110), asbestosis and diffuse pleural thickening (n = 13), pleural plaques (n = 142), and healthy subjects with a history of asbestos exposure (n = 217).

Results: Mean serum osteopontin levels differed among the six groups (P < 0.0001). Mean osteopontin values of the healthy individuals exposed to asbestos were significantly different from that of subjects with asbestosis (P < 0.001) and diffuse pleural thickening (P < 0.001). There was a significant difference in mean serum levels of osteopontin in healthy individuals exposed to asbestos (n = 217) compared with the group mean of all subjects with asbestos-related disorders (n = 288; P < 0.0001).

Conclusions: Our results suggest that osteopontin levels are elevated in subjects with asbestos-related disorders without malignant mesothelioma. These data indicate that osteopontin, although reported to be useful for detecting malignant mesothelioma in asbestos-exposed individuals, may be influenced by nonmalignant processes.

Many studies have confirmed that exposure to asbestos causes malignant mesothelioma, a highly aggressive, fatal, and rare cancer (1–4). However, only a small number of people exposed to asbestos will develop malignant mesothelioma, and the majority will have benign asbestos-related pleural disorders including pleural plaques and diffuse pleural thickening. The latency period between first exposure to asbestos and the development of malignant mesothelioma has a wide range, but the average period is 15 to 40 years (5, 6). The median survival time after diagnosis is <18 months (7–9). Worldwide, the incidence of malignant mesothelioma has continued to increase (6, 10, 11). Malignant mesothelioma is difficult to diagnose in its early stages, and there are no satisfactory treatments that control this disease. Early detection might allow use of combination therapy to control or eradicate this neoplasm.

Currently, there is considerable interest in the use of biomarkers for the early detection of several malignancies including malignant mesothelioma (12). Recent studies have shown that osteopontin and soluble mesothelin-related protein hold promise in detecting malignant mesothelioma, perhaps at an early stage (13–16). Osteopontin is a tumor-associated glycoprotein that regulates cell-matrix interactions and cellular signaling through binding with an integrin and the CD44 receptor (17, 18), and has been reported to be useful for detecting malignant mesothelioma, perhaps at an early stage (14, 16, 19–21). One retrospective study by Pass et al. (14) found that the mean serum osteopontin levels in a population with pleural malignant mesothelioma (n = 76) were significantly elevated in comparison with asbestos-exposed (n = 69) and non–asbestos-exposed (n = 45) control subjects without malignant mesothelioma. As an indicator of malignant mesothelioma, osteopontin levels in stage I mesothelioma had a sensitivity of 84.6% and a specificity of 88.4% using a cutoff value of 62.4 ng/mL. A more recent study by Grigorou et al. (16) from France reported that the serum osteopontin levels in malignant mesothelioma patients (n = 96) were higher than in those of healthy asbestos-exposed subjects (n = 112). There was, however, no difference in serum osteopontin levels when comparing subjects with malignant mesothelioma (n = 96) with those who had pulmonary metastases from other carcinomas (n = 43) and benign pleural diseases associated with asbestos exposure (n = 33). The limitation of these studies is the underrepresentation of the benign asbestos-related diseases (14, 16), which may be clinically difficult to distinguish from malignant mesothelioma, particularly in its early stages. To evaluate osteopontin...
Translational Relevance

Serum osteopontin has been proposed as a screening tool for malignant mesothelioma. Early detection of malignant mesothelioma could theoretically lead to improved survival. However, little is known about other factors affecting osteopontin levels including benign asbestos-related disorders. We screened 525 asymptomatic asbestos-exposed men, and found a significant difference between mean osteopontin levels in healthy individuals exposed to asbestos compared with the group mean of subjects with asbestos-related disorders. This implies that osteopontin may be affected by nonmalignant processes. Caution should be exercised in using this test clinically until all potential confounding factors have been taken into account.

Materials and Methods

Study population. This study population was a part of a prospective cohort study conducted at the Workers’ Compensation (Dust Diseases) Board (DDB) of New South Wales, Australia (Park et al., in press). The DDB is a statutory authority that provides compensation to workers with dust diseases employed in New South Wales. An award is made after the diagnosis has been established by the DDB Medical Authority, a panel of three respiratory physicians specializing in occupational lung disease. Detailed clinical and pathologic information is available to the Medical Authority. Lifetime occupational histories are collected. The DDB regularly screens a large number of workers with previous asbestos exposure for potential respiratory disease. Asbestos-exposed workers and workers with other dust exposures attending the DDB from January to November 2006 for a routine examination for screening and compensation purposes were invited to participate in the study. The routine examination included a standardized questionnaire, radiology, lung function, and a clinical examination by a thoracic physician. Subjects were consecutively recruited, and if they agreed to participate, they gave signed informed consent followed by blood collection. The study was approved by the Human Research Ethics committee of St. Vincent’s Hospital, Sydney, Australia. Participants were not compensated for their participation. Respiratory symptoms and physical examination were recorded from a physician-administered clinical card. A chest radiograph was mandatory, and chest computed tomography scan was done if clinically indicated. For study purposes, the presence or absence of asbestos-related or other diseases was classified according to the determination of the Medical Authority comprising three respiratory physicians. Classification of such disorders was set up before the study was initiated, and categorization was done according to the American Thoracic Society diagnostic criteria (22).

Analysis of osteopontin. Serum samples were stored at -80°C until further analysis. Serum osteopontin concentrations were measured by a specific human osteopontin ELISA kit (Immunobiological Laboratories) according to the manufacturer’s instructions, and results were expressed in ng/mL. The limit of detection of the assay was 3.33 ng/mL. Further dilutions were prepared as necessary to bring samples within the dynamic range. All samples were coded for a blind analysis, and each serum was determined in duplicate. We used a cutoff level of osteopontin for abnormality of ≥62.4 ng/mL (14).

Statistical analysis. Data are expressed as mean ± SD. The osteopontin level below the limit of detection was assigned a value of 1.67 ng/mL (limit of detection/2) for the purpose of statistical analysis. We compared estimated osteopontin levels among groups using ANOVA. The Bonferroni correction was applied for multiple comparisons in post hoc and Student’s t tests. A two-tailed P value < 0.05 was considered significant. All statistical analyses were done in GraphPad Prism (Version 4, Graphpad Software).

Results

Demographics. A total of 525 male participants were recruited. Demographic details are shown in Table 1. The mean (±SD) age of the participants was 66.9 (±10.1) years, and 8.0% (n = 42) participants reported current cigarette smoking with 56% (n = 294) being ex-smokers. The mean body mass index (BMI) for the entire cohort (n = 525) was 28.3 ± 4.2 kg/m², with 52.2% (n = 274) of subjects classified as overweight (BMI = 25.0-29.9) and 28.6% (n = 150) as obese (BMI >30). Subjects were classified into six diagnostic groups: asbestosis (n = 23), diffuse pleural thickening (n = 110), asbestosis and diffuse pleural thickening (n = 13), pleural plaques (n = 142), silicosis (n = 20), and the otherwise healthy but asbestos-exposed population (n = 217).

Serum osteopontin values. Fifty-five (10.5%) of the serum samples were below the limit of detection of the osteopontin levels in this study, including 2 subjects with silicosis, 7 with diffuse pleural thickening, 14 with pleural plaques, and 32 healthy subjects. Mean serum osteopontin levels differed significantly among the six diagnostic groups (P < 0.0001; Fig. 1). Mean (±SD) serum osteopontin levels did not differ between the healthy population exposed to asbestos (24.1 ± 25.6 ng/mL) and those with silicosis (20.9 ± 16.6 ng/mL; P > 0.05). Mean (±SD) serum osteopontin levels differed in those diagnosed with asbestos-related disorders: asbestosis (70.2 ± 41.5 ng/mL), diffuse pleural thickening (46.2 ± 61.5 ng/mL), asbestosis and diffuse pleural thickening (26.4 ± 17.2 ng/mL), and pleural plaques (32.4 ± 43.4 ng/mL). The mean osteopontin values of the healthy individuals exposed to asbestos were significantly different from that of subjects with asbestosis (P < 0.001) and diffuse pleural thickening (P < 0.001). There was also a significant difference in mean osteopontin level among the groups with asbestosis and silicosis (P < 0.01), subjects with asbestosis and diffuse pleural thickening (P < 0.05), and pleural plaques (P < 0.001).

There was a significant difference in mean serum levels of osteopontin in the healthy individuals exposed to asbestos (n = 217) when compared with the group mean of all subjects with asbestos-related disorders (n = 288; 24.1 ± 25.6 and 40.4 ± 51.3 ng/mL, respectively; P < 0.0001; Fig. 2). When
serum osteopontin levels were compared with demographic variables, serum osteopontin levels in our cohort was positively associated with age (P < 0.0001) but not significantly correlated with BMI (P = 0.1850) or smoking status (P = 0.2604).

Discussion

The challenge in improving survival in patients with asbestos-related carcinoma of the bronchus lies in early detection of disease and surgical resection or combined treatment, and this may also apply to malignant mesothelioma (23). Early detection of these tumors is not easy, and radiologic surveillance is imperfect (24). To diagnose malignant mesothelioma at an early stage, a combination of screening processes including biomarkers and conventional methods such as chest radiology and computed tomography or positron emission tomography and computed tomography may be a practical approach. Examples of promising biomarkers reported recently are osteopontin and soluble mesothelin-related protein (13–16). Therefore, it is important to consider whether these biomarkers might be useful in a prospective surveillance program in a high-risk population. Osteopontin probably has a lower diagnostic accuracy than soluble mesothelin-related protein in the detection of malignant mesothelioma, and combining osteopontin and soluble mesothelin-related protein seems to have no greater accuracy for diagnosing malignant mesothelioma than using soluble mesothelin-related protein alone (16). Osteopontin has been reported to have low specificity for malignant mesothelioma, but it has been suggested as useful for disease monitoring (16, 25).

Our study population had a history of documented occupational asbestos exposure, and most of the participants had asbestos-related disorders. Thus, they represent the population most at risk of development of malignant mesothelioma. We found that the mean serum levels of osteopontin in the six diagnostic groups were significantly different (P < 0.0001). This differs from the study by Pass et al. (14), which reported that the mean serum level of osteopontin was not significantly different in a population with and without asbestos exposure (P = 0.06; n = 114) and that osteopontin levels were not affected by the presence of pleural plaques (P = 0.88). However, this research group did find that the mean serum osteopontin level in subjects with asbestos was higher than subjects without asbestos (P = 0.004), and that mean serum osteopontin levels in subjects with both asbestos and pleural plaques (n = 10) were higher than those in subjects with asbestos (n = 10), pleural plaques (n = 43), and healthy subjects with asbestos exposure (P = 0.004; n = 6). The likelihood explanation for the difference between Pass’ study and ours is a type II error in the former due to a smaller sample size. Another recent study by Grigoriu et al. (16) found that healthy subjects with a history of asbestos exposure (n = 112) had a lower serum level of osteopontin than 43 patients with pleural metastasis of adenocarcinomas (P = 0.001), 33 patients with benign pleural lesions associated with asbestos exposure or having both pleural plaques and diffuse pleural thickening (P = 0.006), and 96 patients with malignant mesothelioma.

**Table 1. Characteristics of participants**

<table>
<thead>
<tr>
<th></th>
<th>Overall distribution (n = 525)</th>
<th>Healthy exposed to asbestos (n = 217)</th>
<th>Silicosis (n = 20)</th>
<th>Asbestosis (n = 23)</th>
<th>Diffuse pleural thickening (n = 110)</th>
<th>Asbestosis / Diffuse pleural thickening (n = 13)</th>
<th>Pleural plaques (n = 142)</th>
</tr>
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<tbody>
<tr>
<td>Age, mean y (± SD)</td>
<td>66.9 (±10.1)</td>
<td>61.1 (±10.4)</td>
<td>70.7 (±6.4)</td>
<td>72.7 (±6.9)</td>
<td>71.9 (±6.9)</td>
<td>73.8 (±6.0)</td>
<td>69.7 (±8.6)</td>
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<tr>
<td>BMI, kg/m², n(%)</td>
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<td>&lt;18.5</td>
<td>0.4 (2)</td>
<td>0.5 (1)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.7 (1)</td>
</tr>
<tr>
<td>18.5–24.9</td>
<td>18.9 (99)</td>
<td>20.3 (44)</td>
<td>15.0 (3)</td>
<td>13.0 (3)</td>
<td>11.8 (13)</td>
<td>15.4 (2)</td>
<td>23.9 (34)</td>
</tr>
<tr>
<td>25–29.9</td>
<td>52.2 (274)</td>
<td>48.9 (106)</td>
<td>60.0 (12)</td>
<td>65.2 (15)</td>
<td>53.6 (59)</td>
<td>69.2 (9)</td>
<td>51.4 (73)</td>
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<tr>
<td>≥30</td>
<td>28.6 (150)</td>
<td>30.4 (66)</td>
<td>25.0 (5)</td>
<td>21.7 (5)</td>
<td>34.6 (38)</td>
<td>15.4 (2)</td>
<td>23.9 (34)</td>
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<tr>
<td>Smoking status, n(%)</td>
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<tr>
<td>Never smoker</td>
<td>36.0 (189)</td>
<td>45.6 (99)</td>
<td>40.0 (8)</td>
<td>21.7 (5)</td>
<td>21.8 (24)</td>
<td>15.4 (2)</td>
<td>35.9 (51)</td>
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<tr>
<td>Ex-smoker</td>
<td>56.0 (294)</td>
<td>43.3 (94)</td>
<td>60.0 (12)</td>
<td>69.6 (16)</td>
<td>73.6 (81)</td>
<td>84.6 (11)</td>
<td>56.3 (80)</td>
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<tr>
<td>Current smoker</td>
<td>8.0 (42)</td>
<td>11.1 (24)</td>
<td>0 (0)</td>
<td>8.7 (2)</td>
<td>4.6 (5)</td>
<td>0 (0)</td>
<td>7.8 (11)</td>
</tr>
</tbody>
</table>

**Fig. 1.** Osteopontin (OPN) levels (ng/mL) in healthy individuals exposed to asbestos, and who have silicosis, asbestosis, diffuse pleural thickening (DPT), combined asbestosis and DPT, and pleural plaques alone (PPs). Horizontal scale bars denote mean level. There were significant differences among the groups (ANOVA, P < 0.0001).
values were seen in those with asbestosis, it is tempting to speculate that the levels of osteopontin could be related to the degree of asbestos exposure. However, occupational exposure histories sufficiently detailed to enable asbestos exposure quantification were not collected in our study, so we cannot confirm this possibility. Our results suggest that benign pleural disease is associated with activation and/or secretion of osteopontin into the circulation. This is biologically plausible in view of the known role of osteopontin in extracellular matrix signaling and in laying down scar tissue. Alternatively, it is also possible that this finding is due to confounding, because other factors such as the presence of atherosclerosis are known to increase osteopontin levels. This suggestion is supported by the fact that osteopontin levels were also associated with age in our study ($P < 0.001$).

We found that 72 subjects (13.7%) had an elevated osteopontin level (≥62.4 ng/mL) using the cutoff point established in the study by Pass et al. (14). These subjects consisted of 20 healthy subjects with a history of asbestos exposure, 1 with silicosis, 11 with asbestosis, 21 with diffuse pleural thickening, and 19 with pleural plaques. None had evidence of malignant mesothelioma as judged by plain chest radiography and computed tomography, but one was subsequently found to have lung cancer. This individual had a raised osteopontin level at 117.6 ng/mL. Four participants died during the study period, one of lung cancer and one of metastatic carcinoma of the pancreas. The subject with lung cancer had an osteopontin level of 3.6 ng/mL, whereas that of the subject with carcinoma of the pancreas had one of 41.1 ng/mL. The other two deaths were not attributable to dust disease or malignancy.

In summary, our study examined the effect of nonmalignant asbestos-related disorders on circulating osteopontin levels in order to evaluate osteopontin as a potential screening tool in a high-risk population. Of 525 subjects, 72 (13.7%) had a level which was higher than the estimated cutoff point (≥64.2 ng/mL) reported by Pass et al. (14). However, none had evidence of malignant mesothelioma as judged by plain radiography or computed tomography, but one had lung cancer. It differs from previous reported studies in that our subjects were not attending hospital (14, 16), and were thus less likely to have symptomatic disease. Our data have shown that osteopontin levels are higher in subjects with nonmalignant pleural disease and that its utility in detecting malignant mesothelioma in this population is likely to be limited.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Acknowledgments**

We are grateful to all the subjects whose participation enabled us to complete the study.

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