Role of interleukin-6 (IL-6) in diagnosis of malignant pleural mesothelioma

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KEYWORDS
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Abstract  Aim of the work: To evaluate the role of interleukin-6 (IL-6) in diagnosis of malignant pleural mesothelioma by detecting its level in the pleural fluid and serum of the patient with malignant pleural mesothelioma in comparison with other causes of pleural effusion.

Materials and methods: This study was conducted on 44 patients (25 males and 19 females) with pleural effusions of different etiologies, at Banha University Hospital from April 2011 to December 2012. These patients were classified according to their final diagnosis into four groups: Group I: included 20 cases with malignant pleural effusions secondary to malignant pleural mesothelioma. Group II: included 7 cases with malignant pleural effusions secondary to metastatic adenocarcinoma. Group III: included 7 cases with tuberculous pleural effusions. Group IV: included 10 cases with transudative pleural effusions. IL-6 measured in both plasma and pleural fluid of selected patients and statistically analyzed.

Results: The mean values of pleural fluid IL-6 were higher among patients with malignant effusion (groups (I, II), respectively) than those with non-malignant (groups (III, IV)), these differences were statistically significant (p < 0.001). There was a significant increase in pleural fluid IL-6 levels in group (I) mesothelioma mean was (1627.4 ± 294.3) versus group (II) adenocarcinoma mean was (1501.1 ± 274.2) p < 0.05 being higher in group (I). There was no significant difference in the mean levels of serum IL-6 in malignant groups (I, II), versus benign groups (III, IV) but there was a high significant increase in pleural fluid IL-6 level in malignant groups (I, II) mean was (1590.7 ± 294.6) versus benign groups (III, IV) mean value was (1260.6 ± 145.5), p < 0.001 being higher in malignant groups.

Conclusion: Pleural fluid level of interleukin-6 can be used as diagnostic tool for malignant pleural mesothelioma (MPM). Pleural fluid level of interleukin-6 (IL-6) can be used in differentiating malignant from non-malignant effusions, pleural effusions secondary to malignant pleural mesothelioma (MPM) from those secondary to Adenocarcinoma.

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Introduction

Pleural effusions are often a diagnostic dilemma as the differential diagnosis is wide [1]. However, in about 5–10% of cases the effusions remain undiagnosed after the initial evaluation and 20% of these effusion are later proved to be malignant [2]. Cytokines are proteins with relatively low molecular weight that are secreted by cells in response to a variety of different stimuli and act as key mediators of the host response to various infections, inflammatory and immunologic challenges [3]. IL-6 is a pleiotropic cytokine stimulating a variety of cell types [4]. Mesothelioma cells and cell lines have been reported to produce IL-6 [5]. Since IL-6 is relatively stable and its concentrations can be measured in synovial or cerebrospinal fluid the measurement of IL-6 in the body fluid would be useful to determine the activity of inflammation in patient with complicated conditions [6,7]. IL 6 role as an anti-inflammatory cytokine is mediated through its inhibitory effects on TNF-alpha and IL-1, and activation of IL 10 [8]. IL 6 plays a central role in host defense against infection and tissue. IL 6 elicits cellular actions by binding to the membrane bundle IL 6 receptor [7].

Because IL 6 is an autocrine growth factor for many cell types, its hyperproduction has been associated with a variety of malignancies including plastocytoma, multiple myeloma, uterine cervical carcinoma and Kaposi’s sarcoma [9,10].

Patients and methods

This study was conducted on 44 patients with pleural effusions of different etiologies, at Banha University Hospital from April 2011 to December 2013 after approval of the study protocol by the Local Ethical Committee and obtaining written fully informed patients’ consent. These patients were classified according to their final diagnosis into four groups: Group I: malignant pleural effusions secondary to malignant pleural mesothelioma. Group II: malignant pleural effusions secondary to metastatic adenocarcinoma. Group III: tuberculous pleural effusions. Group IV: transudative pleural effusions.

Exclusion criteria

1. Any effusion due to undetermined cause or suspected to have more than one possible cause.
3. Patients already started any kind of treatment.

All patients were subjected to the following: (1) Full medical history and clinical examination, routine laboratory investigations, radiological examination (CXR & CT chest), abdominal ultrasonography, echocardiography whenever needed. (2) Tuberculin skin test and Sputum examination for acid fast bacilli (AFB) by Ziehl–Neelsen stain. 3-Fiber-optic bronchoscopy: for patients with suspected bronchogenic carcinoma where tissue biopsies or bronchoalveolar lavage (BAL) was sent for histopathological examination. Diagnostic thoracocentesis: was done to all patients for physical, chemical, bacteriological examination and cytological examination. Quantitative measurement of pleural fluid IL-6 and using ELISA technique. Pleural biopsies: were taken for all patients in groups (I, II, and III). Venous blood samples for quantitative estimation of serum IL-6. Quantitative measurement of IL-6 in serum and pleural fluid using ELISA kit supplied by KOMA BIOTECH INC. Catalog No. K03394.

Statistical analysis

The collected data were analyzed using SPSS version 16 soft ware. Chi square test ($$\chi^2$$), student “t” test and ANOVA were used as tests of significance. ROC curve was used to detect cutoff values of IL-6 with optimum sensitivity and specificity. Stepwise multiple regression analysis was done to detect the significant predictors of IL-6.

Results

The mean of age (in years) of group I was 65.9 ± 12.4, group II was 65 ± 9.8, group III was 53.1 ± 6.94 and group IV was 62 ± 8.5 years with male predominance 56.8%. The most common presenting complaints were shortness of breath ($$n = 42$$ cases, 95.5%) followed by cough that occurred in ($$n = 41$$ cases, 93%), then chest pain ($$n = 38$$ cases, 86.4%), fever ($$n = 12$$ cases, 27.3%) and hemoptysis ($$n = 6$$ cases, 13.6%). The classic radiographic picture was homogenous opacity with concave upper border rising toward the lateral chest wall it may be moderate sized effusions ($$n = 19$$ cases, 44.2%), while cases with massive effusion showed complete or near complete opacification ($$n = 15$$ cases, 34.9%), and mild effusion ($$n = 9$$ cases, 20.9%). Right side effusions were 27 cases, 62.8%, left-sided 11 cases 25.6% and bilateral 5 cases 11.6%. Tuberculin skin test was positive in 3 cases in group (I) mesothelioma representing 15%, and 7 cases in group (III) TB representing 100% and lastly all cases were negative in group (II adenocarcinoma, IV control). The mean levels of platelets was higher in mesothelioma (I) followed by TB effusion (III) and lastly adenocarcinoma (II) versus group (IV) and these was significance difference as $$p < 0.001$$.

The mean values of serum and pleural fluid protein levels were as the following: group (I) (4.47 ± 0.77 and 3.9 ± 0.82 g/dl), respectively, in group (II) (4.35 ± 0.76 and 4.7 ± 1.01 g/dl), respectively, in group (III) (3.94 ± 0.60 and 4.2 ± 0.50 g/dl) the ratios of PLF/Sptn were always more than 0.5 in the three exudative groups. While in group (IV) (4.8 ± 0.89 and 2.3 ± 0.48 g/dl), respectively, and the ratios of PLF/Sptn were always less than 0.5.

The mean values of serum and pleural fluid LDH levels were as following: group (I)(109.9 ± 16.7 and 1107.6 ± 296.8 U/L), respectively, in group (II) (117.0 ± 12.9 and 927.5 ± 388.2 U/L). In group (III) (102.0 ± 11.8 and 805.8 ± 330.8 U/L), respectively, and the ratios of FLDH/SLDH were more than 0.6 and the absolute values of FLDH were more than 200 IU/L and more than 2/3 of the upper limit of the serum in the three exudative groups. While in group (IV) control, the mean levels was (276.6 ± 35.9 and 194.2 ± 29.7 IU/L), respectively, and the ratio of FLDH/SLDH was less than 0.6 and the absolute values of FLDH were less than 200 IU/L and less than 2/3 of the upper limit of normal serum.

As regard glucose level in serum and pleural fluid the lowest mean level of pleural fluid glucose was found in tuberculous pleural effusion (52.5 ± 20.5) followed by pleural effusion secondary to mesothelioma (54.7 ± 28.9) then pleural effusion secondary to adenocarcinoma (68.3 ± 17.2) and lastly transudative effusion (72 ± 22.8).
There was no significant difference in the mean levels of serum IL-6 in exudative effusions (mean was 291.7 ± 100.9), versus transudative effusions (mean was 134.6 ± 20.95) $p < 0.01$. But there was a high significant increase in pleural fluid IL-6 level in exudative effusions (mean was 1521.6 ± 299.7) versus transudative effusions (mean was 1257.6 ± 155.7) $p < 0.01$ being higher in exudative effusions (Table 1, Fig. 1).

Also there were high significant increases in pleural fluid IL-6 level in group (I), (II), mean values were (1627.4 ± 294.3) (1501.1 ± 274.2), respectively, versus transudative effusions group (IV) mean value was (1257.7 ± 155.2) being higher in group (I, II) (Table 1, Fig. 1).

Also there was significant increase in pleural fluid IL-6 levels in group (I) mesothelioma mean was (1627.4 ± 294.3) versus group (II) adenocarcinoma mean was (1501.1 ± 274.2) $p < 0.05$ being higher in group (I) (Table 2, Fig. 2).

There was no significant difference in the mean levels of serum IL-6 in malignant groups (I, II), versus benign groups (III, IV) mean values were (282.5 ± 106.8 and 213.29 ± 107.44), respectively, $p < 0.045$, but there was a significant increase in pleural fluid IL-6 level in malignant group (I, II) mean was (1590.7 ± 294.6) versus benign groups (III, IV) mean value was (1260.6 ± 145.5), $p < 0.001$ being higher in malignant groups (Table 3, Fig. 3).

In the present study (Table 4, Fig. 4) using a cutoff point of 1426.4 pg/ml pleural fluid IL-6 can be used to differentiate pleural effusion secondary to malignant mesothelioma from pleural effusion secondary to metastatic adenocarcinoma with sensitivity 85%, specificity 83%, PPV 81%, NPV 87%, respectively.

In the present study (Table 5, Fig. 5) using a cutoff point of >1317.92 pleural fluid IL-6 can differentiate malignant pleural effusions from non-malignant pleural effusions with sensitivity 76.9%, specificity 70.6%, PPV 80%, NPV 66.7%, respectively.

### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Exudative effusion (N = 34)</th>
<th>Transudative effusion (N = 10)</th>
<th>Student “$t$”</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum IL-6</td>
<td>291.7 ± 100.97</td>
<td>134.6 ± 20.9509</td>
<td>4.8</td>
<td>&lt;0.001*</td>
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<tr>
<td>Pleural fluid IL-6</td>
<td>1521.6182</td>
<td>1257.6 ± 155.72182</td>
<td>2.7</td>
<td>0.01*</td>
</tr>
</tbody>
</table>

*Significant in comparison to TB effusion group.

*Significant in comparison to transudative effusion group.

### Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>Std. Dev</th>
<th>Minimum</th>
<th>Maximum</th>
<th>ANOVA</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesothelioma (I)</td>
<td>20</td>
<td>1627.4 ± 294.3</td>
<td>294.33609</td>
<td>900.60</td>
<td>1990.30</td>
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<td></td>
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<tr>
<td>Adenocarcinoma (II)</td>
<td>7</td>
<td>1501.1 ± 274.2</td>
<td>274.29342</td>
<td>1246.80</td>
<td>1884.30</td>
<td>6.8</td>
<td>0.001*</td>
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<tr>
<td>TB effusion (III)</td>
<td>7</td>
<td>1264.8 ± 141.2</td>
<td>141.75964</td>
<td>1006.10</td>
<td>1406.20</td>
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<td></td>
</tr>
<tr>
<td>Transudative effusion (IV)</td>
<td>10</td>
<td>1257.7 ± 155.2</td>
<td>155.72182</td>
<td>1000.10</td>
<td>1604.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
<td>1465.6 ± 292.65728</td>
<td>292.65728</td>
<td>900.60</td>
<td>1990.30</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significant in comparison to TB effusion group.

*Significant in comparison to transudative effusion group.
In the present study (Table 6) using a cutoff point of >7.5 as ratio between pleural and serum levels of Interleukin-6 can diagnose transudative effusion, if the ratio <7.5 can diagnose exudative effusion with sensitivity 100%, specificity 79.4%, PPV 58%, NPV 100%, respectively.

Discussion

Cytokine – producing cells and cytokines have been reported in pleural effusions from patients with malignant diseases, tuberculosis and empyema. Interleukin-6 is a multifunctional cytokine secreted by lymphoid and non lymphoid cells that regulates B-cell and T-cell function and is a potent inducer of the acute – phase protein response. IL-6 is often used as a marker for systemic activation of proinflammatory cytokines (Opal and DePalo) [3].

This study was done to assess the role of interleukin-6 in differentiation of pleural effusion secondary to malignant mesothelioma, metastatic adenocarcinoma and tuberculous infection, as well as to find out a minimal invasive tool for differentiating the above mentioned causes of pleural effusion.

In the present study (Table 6) using a cutoff point of >7.5 as ratio between pleural and serum levels of Interleukin-6 can diagnose transudative effusion, if the ratio <7.5 can diagnose exudative effusion with sensitivity 100%, specificity 79.4%, PPV 58%, NPV 100%, respectively. Also there was significant increase in pleural fluid IL-6 level in exudative effusions (mean was 1521.6 ± 299.7) versus transudative effusions (mean was 1257.6 ± 155.7) p < 0.01 being higher in exudative effusions.

Also there was high significant increase in pleural fluid IL-6 level in group (I), (II), mean values were (1627.4 ± 294.3) (1501.1 ± 274.2) respectively versus transudative effusions group (IV) mean value was (1257.7 ± 155.2) being higher in group (I, II) (Table 2, Fig. 2).

This results matched with the results of Akarsu et al. [11] who reported that a panel of interleukins including IL-6 could be used in pleural fluid exudates and transudate distinction where they detected that IL-6 levels were 1858.5 ± 363.1 pg/ml in the exudate groups and 656.5 ± 160.9 pg/ml in the transudate groups p < 0.01. Although, the significance of pleural fluid IL-6 in differentiating exudates from transudates came in agreement with the results of Xirouchaki et al. [10] who

<table>
<thead>
<tr>
<th>Cutoff value</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>AUC</th>
<th>95% CI (AUC)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum IL-6 (172.2)</td>
<td>100</td>
<td>41.7</td>
<td>58.8</td>
<td>100.0</td>
<td>0.44</td>
<td>0.25-0.64</td>
<td>0.53</td>
</tr>
<tr>
<td>Pleural IL-6 (1426.4)</td>
<td>85</td>
<td>83.6</td>
<td>81</td>
<td>87</td>
<td>0.81</td>
<td>0.66-0.95</td>
<td>0.001*</td>
</tr>
</tbody>
</table>
reported that pleural fluid IL-6 is an accurate mean of distinguishing exudates from transudates. But the significance of serum IL-6 did not come hand in hand with our results where they found significant difference between the mean values of serum IL-6 when compared transudates versus exudates being higher in exudates also our results regarding serum IL-6 did not match with the results of Hsieh et al. [12] who found significant difference between the mean levels of serum IL-6 in exudates compared to transudates being higher in exudates.

The explanation of not finding significant difference in serum IL-6 between exudates and transudates is that there was significant increase in pleural fluid IL-6 than serum levels was reported by Marie et al. [13] who mentioned that cytokines can be trapped by the surrounding cells in their environment, measurable levels of cytokines in biological fluids represent the “tip of the iceberg”. Also Hoheisel et al. [14] conclude that elevated levels of IL-6 in pleural effusions are due to compartmentalization at the site of active disease. While Kiropoulos et al. [15] related this finding to local production of this pro-inflammatory cytokine (IL-6).

In the present study (Table 2, Fig. 2) there was significant increase in pleural fluid IL-6 levels in group (I) mesothelioma mean was (1627.4 ± 294.3) versus group (II) adenocarcinoma mean was (1501.1 ± 274.2) $p < 0.05$ being higher in group (I), these results matched with results of Nakano et al. [16] who reported that level of pleural fluid IL-6 in patients with malignant pleural effusions secondary to mesothelioma were significantly higher than in patients with adenocarcinoma. These results did not match with those of Yamaguchi et al. [17] who reported that IL-6 were elevated in effusions secondary to metastatic adenocarcinoma with mean values of 2970.5 pg/ml.

In the present study (Table 3, Fig. 3) there was no significant difference in the mean levels of serum IL-6 in malignant groups (I, II), versus benign groups (III, IV) mean values were (282.5 ± 106.8, and 213.29 ± 107.44), respectively, $p < 0.045$, but there was a high significant increase in pleural fluid IL-6 level in malignant groups (I, II) mean was (1590.7 ± 294.6) versus benign groups (III, IV) mean value was (1260.6 ± 145.5), $p < 0.001$ being higher in malignant groups.

These results came in agreement with those of Yamaguchi et al. [17] who reported that marked elevation of IL-6 was found in all of malignant pleural effusions. It ranged from 77.5 to 54.1 pg/ml. being far above the increased levels in the paired serum. Also Alexanderakis et al. [18] reported that pleural fluid concentrations of IL-6 were higher in malignant effusion group A versus group B (non malignant effusion) and group C (transudative effusion) $p < 0.01$.

In the present study (Table 4, Fig. 4) using a cutoff point of 1426.4 pg/ml pleural fluid IL-6 can be used to differentiate pleural effusion secondary to malignant mesothelioma from pleural effusion secondary to metastatic adenocarcinoma with sensitivity 85%, specificity 83%, PPV 81%, NPV 87%, respectively.

In the present study (Table 5, Fig. 5) using a cutoff point of >1317.92 pleural fluid IL-6 can differentiate malignant pleural effusions from non-malignant pleural effusions with sensitivity 76.9%, specificity 70.6%, PPV 80%, NPV 66.7%, respectively.

In the present study (Table 6) using a cutoff point of >7.5 as ratio between pleural and serum levels of Interleukin-6 can diagnose transudative effusion, if the ratio <7.5 can diagnose exudative effusion with sensitivity 100%, AUC 0.925 $p < 0.001$.

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>AUC</th>
<th>PPV</th>
<th>NPV</th>
<th>95% CI (AUC)</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;7.5</td>
<td>100%</td>
<td>79.4%</td>
<td>0.925</td>
<td>100%</td>
<td>58.8%</td>
<td>0.85–1.0</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Ratio >7.5 can diagnose transudative effusion with sensitivity 100%, AUC 0.925 $p < 0.001$.
Ratio <7.5 can diagnose exudative effusion.
Conclusions

Pleural fluid level of interleukin-6 can be used as diagnostic tool for malignant pleural mesothelioma (MPM). Pleural fluid level of interleukin-6 (IL-6) can be used in differentiating malignant from non malignant effusions, pleural effusions secondary to malignant pleural mesothelioma (MPM) from those secondary to Adenocarcinoma.

Conflict of interest

No conflict of interest.

References