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PROINFLAMMATORY CYTOKINES : ARE THEY USEFULL IN DIFFERENTIAL DIAGNOSIS OF PLEURAL EFFUSIONS?

PROINFLAMATUAR SİTOKİNLER : PLEVRAL EFFÜZYONLARIN AYRIMLANMASINDA YARARLIMIDIR?

Çiğdem YENİSEY[1] Serir AKTOĞU[2] Sabri KALENCİ[3] F. Onur ERER[2]

[1]Adnan Menderes University, School of Medicine, Department of Biochemistry, Aydın
[2]and [3] Yenişehir Suat Seren Chest Hospital, Clinics of 4B and 9B, İzmir

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SUMMARY

Interleukin 1- beta (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF- α) were measured in pleural fluid from 128 patients with pleural effusion in order to evaluate the diagnostic utility of these cytokines. We studied 42 patients with malignant, 23 patients with parapneumonic, 38 patients with tuberculous, and 25 patients with transudative pleural effusion. Patients samples were taken applied to the Yenişehir Suat Seren Chest Hospital at the clinics 9B an 4B were diagnosed suffereing from pleural effusion. Cytokines were measured by chemiluminescent enzyme immunometric assay using BIODPC commercial kits via IMMULATE 2000 hormone analyzer. In exudative samples the difference between the pleural fluid and serum, IL-1β, IL-6, and TNF-α levels were found statistically important. Only, IL-6 levels were found dramatically different between serum and pleural fluid in transudative samples. Mean IL-6 and TNF-α levels were significantly higher in pleural fluid than in serum in all three groups of patients (malignant, parapneumonic, and tuberculosis). But, the highest mean level of IL-6 and TNF-α were found in tuberculous pleural fluid. In conclusion, low serum IL-6 and high pleural fluid IL-6 can distinguish transudates due to heart failure from exudates. Also, serum and pleural fluid IL-6 could be useful as a complementary marker in the differential diagnosis of two most common types of exudates (tuberculous and malignant) and TNF-α in the serum and pleural fluid could be useful confirmation differential diagnosis of tuberculous and malignant effusions.

ÖZET

Interlökin 1-beta (IL-1β), interlökin-6 (IL-6) ve tümör nekroz faktörü-alfa (TNF-α) , bu sitokinlerin tanısal kullanımını değerlendirmek amacı ile, 128 hastanın plevral sıvısında ölçüldü. Çalıştığımız hasta grubunda 42 hastanın malign, 23 hastanın parapnömanik, 38 hastanın tuberküloz ve 25 hastanın transudatif plevral effüzyonu vardı. Hasta örnekleri Yenişehir Suat Seren Göğüs Hastalıkları Hastanesinde 9B ve 4B kliniklerinde yatan hastalardan sağlandı. Sitokinler IMMULATE 2000 hormon otoanalizöründe BIODPC ticari kitleri yoluyla ölçüldü. Eksudatif serum ve plevral sıvı örnekleri arasındaki IL-1β, I L-6 ve TNF-α düzeyleri arasındaki fark istatistiksel açıdan önemli bulundu. Transüdatif örneklerde, plevra ve serum düzeyleri arasında sadece IL-6 düzeylerindeki fark önemli bulundu. IL-6 ve TNF-α'nın ortalama değerleri her üç grupta da plevral sıvılarda, seruma kıyasla daha yüksek ve anlamlı bulundu (malign, parapnömanik ve tüberküloz). Fakat, en yüksek IL-6 ve TNF-α düzeyleri tüberküloz grubunda saptandı. Souç olarak, düşük serum IL-6 ve yüksek plevral sıvı IL-6 düzeyleri kalp yetmezliği nedeni ile oluşan transüdaları, eksüdalardan ayırmaktadır. Aynı zamanda, serum ve plevral IL-6 en yaygın görülen iki tip eksudanın (tüberküloz ve malignant) tümüyle ayrılmasında kullanılabilir ve serum ve plevradaki TNF-α düzeyleri tüberküloz ve malign effüzyonların ayırıcı tanısının desteklenmesinde yararlı olabilir.

Yazışma adresi : Çiğdem YENİSEY, Adnan Menderes University, School of Medicine, Department of Biochemistry, AYDIN - TÜRKİYE
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INTRODUCTION

The development of inflammation in the pleura results in an increased vascular permeability leading to pleural fluid accumulation. This pleural fluid is enriched in proteins, inflammatory cells, and mediators (1,2). The diagnosis and management of pleural effusion remains a clinical challenge. Although a variety of simple laboratory tests are in use, a number of cases remain undiagnosed or the "diagnosis" is based on clinical evidence alone. This is especially true regarding the differentiation of exudates of various causes (3-5). Tuberculous pleurisy (TBP) is a common cause of pleural effusions in areas with high disease prevalence. The diagnosis of TBP is sometimes encountered. TBP represents largely an immunological reaction in which a repertoire of cytokines is intimately involved in the pathogenesis. These include especially interleukin (IL) IL-β, IL-6, IL-8, tumor necrosis factor-alpha (TNF-α), and interferon gamma (IFN-γ) (6).

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 (7-9). Cytokines are thought to exert their effects by binding to specific receptors on the surface of the cell, although cytokines may in some instances have direct membrane effects (10).

Cytokine-producing cells and cytokines have been reported in pleural effusions from patients with malignant diseases, tuberculosis, and empyema (11-16). IL-1β is an immunoregulatory cytokine with an essential role T-cell activation. It has been reported that IL-1β levels are increased in tuberculous pleural effusion and in malignant pleural effusion after pleurodesis (17,18). IL-6 is a multifunctional cytokine secreted by lymphoid and and non-lymphoid cells that regulates B-cell and T-cell function and is a potent inducer of the acute-phase protein response (6-9,19,20). IL-6 has long been regarded as a proinflammatory cytokine induced by lipopolysaccharide along with TNF-α and IL-1β (7). IL-6 is often used as a marker for systemic activation of proinflammatory cytokines and has been found to be elevated in malignant pleural effusion, especially after pleurodesis (19,21). However, conflicting results have been reported in distinguishing malignant from tuberculous pleural effusion by IL-6 levels (16,22,23). TNF-α is secreted by activated macrophages, monocytes, and many other cells, including B cells, T cells, and fibroblast. Increased levels of TNF-α have been found both in tuberculous and malignant pleural effusion. A possible source for the increased production of this inflammatory mediator could be the monocytes or the macrophages that are found in the pleural fluid and (14,15,19,22,24). The aim of the study was to measure IL-1β, IL-6, and TNF-α in both pleural fluid and serum of patients with malignant, tuberculous, or parapneumonic pleural effusions in order to investigate the usefulness of these cytokines in differential diagnosis and also to compare with transudate pleural effusions.

MATERIAL AND METHOD

Patients

We studied prospectively 128 consecutive patients with pleural effusion who were hospitalized for management of pleurale effusion enrolled into the study. Their mean ± SD age was 53 ± 11 years (range 15 to 80 years). The criteria of Light et al (25) were applied to differentiate transudates from exudates: 103 patients had exudates and 25 patients had transudates (due to congestive heart failure).

Forty two exudates were considered malignant, since malignant cells were detected on cytologic examination of the pleural fluid or biopsy specimens. A pleural effusion was considered parapneumonic when there was an acute febrile illness, with purulent sputum and pulmonary infiltrates, in the absence of malignancy or other diseases causing exudate and neutrophilia in pleural fluid. Tuberculous pleural effusion was diagnosed in patients by positive culture findings for Mycobacterium tuberculosis or a pleural biopsy specimen showing typical epithelioid cell granulomas (2,3,25). The protocol was approved by the hospital ethical committee, and all subjects gave their consent.

Method

After the thoracentesis of pleural fluid, a specimen was subjected to routine biochemical analsis includind tests for total protein, glucose, and lactate dehydrogenase. A second sample was added to a tube containing EDTA for differential cell counting. Bacterial cultures and cytologic examinations were performed on all pleural effusions. These studies were performed in the laboratories of Suat Seren Chest Hospital in İzmir Yeni-şehir. Cytokines measurements were performed in the biochemistry and hormone laboratories of Adnan Men-deres University Medice Faculty Hospital. For the cytokine measurements, pleural fluid was immediately centrifuged at 4000 rpm for 10 minutes at 4 ºC. Then pleural samples were kept at – 85 ºC and the determination was performed in each week after collected the samples. In addition , blood samples were drawn for serum analysis and processed in the same way as the pleural fluid. Cytokine concentrations were done using with the commercial BIODPC (Products Corporation, Los Angeles, CA, USA) kits (cat. No: for LKL11 for IL-1β; cat. No: LK6P1 for IL-6; cat. No: LKNF1 for TNF-α) by IMMULATE hormone autoanalyzer.

The reference value for IL-1β was <5.0 pg/mL for healthy controls and also low limit of detection (LLD) for this test was 5.0 pg/ mL. The reference range for IL-6 was (<5-11.3 pg/mL) and also LLD was 5.0 pg/mL. The reference range for TNF-α was (0-8.1 pg/mL) and also LLD was nondetectable. To note that 99% of healthy samples yielded results that were below 5.0 pg/mL or nondetectable in given procedure for IL-1β, IL-6 and TNF-α determination. In addition, in both three tests the upper limit detection was 1000 pg/mL. If exceed this value, we diluted samples with sample diluent and determined again. 500 µl sample was put into sample cuvettes for IL-1β, IL-6, and TNF-α determination. Approximately,100 µl sample was taken by probe automatically for cytokine determination.

Statistical Analysis

Values are expressed as mean ± sem (mean ± standart error mean). Statistical comparison of the mean values of different groups was performed using the nonparametric Mann-Whitney test. In the same group the statistical comparison was performed Wilcoxon test. p value < 0.05 was considered statistically significant. All analyses were performed using SPSS (10.0) version.

RESULTS

Exudates vs Transudates

Table 1 shows the values of IL-1β, IL-6, and TNF-α in and serum of patients with transudative and exudative pleural effusions. For every cytokine tested pleural fluid levels were found higher considering serum levels. But only, in exudative samples the difference between the pleural fluid and serum, IL-1β, IL-6, and TNF-α levels were found statistically important. To note that, IL-6 levels were found dramatically different between serum and pleural fluid both exudative and transudative samples (Table 1).

It is important that only in transudates all samples IL-1β serum levels were found <5.0 pg/mL. So, we accepted the results found <5.0 pg/mL as 5.0 pg/mL. Because as mentioned above in this method LLD for this test was 5.0 pg/ml. Interestingly, when compared the different groups cytokines levels, we saw that only transudative pleural fluid and exudative serum IL-6 levels were found statistically significantly higher their corresponding samples. All pleural IL-6 values were found higher 1000 pg/mL in transudative samples, which is the highest detection limit of this assay. The fact that IL-6 values best presented the differences between pleural and serum, in order to evaluate the distinguishing exudates from transudates, we used the ratio of pleural IL-6/serum IL-6. The mean ± sem value of eksudates (41.5 ± 8.2) and transudates (90.0 ± 32.0) and the median value were found as 15.97 and 55.24, respectively. When compared the mean values, the p value was found 0.037

Table 1. Values of IL-1β, IL-6, and TNF-α in Pleural Fluid and Serum of Patients With Transudative or Exudative Pleural Effusions*

Cytokines	IL-1β (pg/mL) Range	IL-6 (pg/mL) Range	TNF-α (pg/mL) Range
Transudates			
Pleural fluid	10.1 ± 4.23 (5.0 – 43.7)	1322 ± 65.2 [a] (1120 – 1620)	131 ± 73.7 (6.2 – 613)
Serum	5.0 ± 0.0 (<5.0)	49.3 ± 24.9 (5.0 – 244)	69.0 ± 37.0 (5.3 – 113)
Median (pleura)	5.0	1370	44,85
Median (serum)	5.0	24.8	30,05
P value			
(within group)			
Exudates			
Pleural fluid	51.5 ± 17.3 (5.0 – 502)	917 ± 96.3 (13.0 – 1790)	195 ± 43.2 (12.6 – 980)
Serum	9.06 ± 1.93 (5.0 -69.0)	87.6 ± 31.1[b] (5.0 – 890.0	42.7 ± 13.9 (4.8 – 542)
Median (pleura)	8.9	1190	79.6
P value	5.0	32.65	22.6
(within group)	< 0.05	< 0.000	< 0.01

*Data are presented as mean ± sem

[a] p< 0.000 as compared with exudates

[b] p<0.05 as compared with transudates

Comparison of Various Types of Exudates

Table 2 shows the values of IL-1β, IL-6, and TNF-α in the pleural fluid and serum of patients with malignant, parapneumonic, or tuberculous exudates. Actually, the considerable difference was found between pleural fluid and serum IL-1β level in parapneumonic group, but this was not statistically important. In malignant, IL-1β levels in pleural fluid were not significantly different from those in serum. Only, a significant differences was found between pleural and serum levels in tuberculous for three cytokines. Mean IL-6 and TNF-α levels were significantly higher in pleural fluid than in serum in all three groups of patients. But, the highest mean level of IL-6 and TNF-α were found in tuberculous pleural fluid. It is important that we diluted all pleural samples of tuberculous patients to find actual value of IL-6, the fact that all samples were exceed 1000 pg/mL which is the highest detection limit of this assay. It is important that, as compared the groups, in tuberculous pleural fluid and serum IL-6 levels both of them were found statistically significantly higher than corresponding malignant samples.

Table 2. Serum and Pleural Fluid Values of IL-1β, IL-6, and TNF-α in Patients With Malignant, Parapneumonic, and Tuberculous Pleural Exudates*

Cytokines	IL-1β (pg/mL) Range	IL-6 (pg/mL) Range	TNF-α (pg/mL) Range
Malignant			
Pleural fluid	6.31 ± 0.67 (5.0 – 15.8)	527 ± 122 (13.0 – 1450)	189 ± 65.3 (12.6 – 1150)
Serum	8.53 ± 2.03 (5.0 – 38.1)	31.8 ± 5.42 (5.0 – 77.3)	28.7 ± 4.6 (6.1 – 78.6)
Median (pleura)	5.0	260.3	82.7
Median (serum)	5.0		
P value			
(within group)			
Parapneumonic	72.3 ± 25.3[a] (5.0 – 320)	1457 ± 64.7[b] (1100 – 1920)	254 ± 74.9 (18.0 -980)
Pleural fluid	11.1 ± 4.53 (5.0 – 69.0)	64.3 ± 30.3[c] (6.5 – 442)	69.0 ± 37.0[d] (4.8-542)
Serum	35.35	1465	139.5
Median (pleura)	5.0	29.45	24.15
P value	< 0.05	< 0.000	<0.05 (virgülü nokta yaptım)
(within group)			

∗Data are presented as mean ± sem

[a]p< 0.000, bp< 0.01, cp< 0.05, and dp< 0.05 as compared with malignant

DISCUSSION

In this prospective study, IL-1β, IL-6, and TNF-α were measured in pleural fluid and serum in 128 patients with pleural effusions. Pleural effusion is most frequently seen during the course of pneumonias. Shimokata et al (26) analyzed IL-1β levels in pleural fluid of tuberculous and neoplastic etiologies, they found no significant difference between the groups. This finding was supported our results. Also, Agrenius et al (24) measured IL-1β in pleural fluid from 13 patients with malignant pleural effusion. They observed detectable levels of pleural IL-1β in all pleural samples. On the contrary, except two samples we found <5.0 pg/mL in 40 malignant pleural samples for IL-1β. In our study, mean value of pleural fluid IL-1β obtained exudative patients was higher than those found in transudative effusions, due to sem value higher so this difference was found no statistically important.The results of the study clearly showed that the mean levels of all three measured cytokines were significantly greater in the pleural fluid of patients compared with serum. This is probably due to local production of the cytokines by inflammatory cells that have accumulated in the pleural cavity, although stimulated mesothelial cells may also contribute to the higher cytokine levels in pleural exudates. Our results supported the data obtained Silva-Mejias et al (27). All of the IL-1β levels in serum were below <5.0 pg/mL in patients with transudate than in those with exudates. In addition, all of the IL-6 levels were found above strictly 1000 pg/mL which is the highest detection limit of this assay. This finding could have significant clinical utility in distinguishing transudates from exudates by measuring serum cytokine IL-1β and pleural IL-6 levels (Table 1). Obviously, further studies on a larger patient population are needed to verify this observation. Serum IL-6 levels have been extensively studied in several clinical syndromes (28-31). Although, a high serum level of IL-6 is not a universal marker of systemic inflammation (31), elevated IL-6 levels in combination with the activation of other cytokines in serum have been found to be related to a systemic inflammatory response and poor prognosis in several pneumonia, sepsis and ARDS studies (29,32).

Xirouchaki et al (19) and Yokohama et a (23) reported that IL-6 in pleural fluid was higher in the exudative group rather than transudate. Also, Alexandrakis et al (22) mentioned that they didn't find significantly difference serum and pleural fluid IL-6 levels between exudate and transudate. Also, when compared pleural IL-6/serum IL-6 ratios between exudates and transudates, we saw that in transudates the mean value of this ratio greater than exudates and this was statistically important.

In comparing the three types of exudates, only in tuberculous pleural IL-1β, IL-6, and TNF-α levels were found statistically higher than their serum levels, especially mean IL-6 level was found dramatically different. As in the transudates, strictly all of the IL-6 values in pleural fluid obtained tuberculous patients were exceed 1000 pg/mL, which is the highest detection limit of the assay. While the criteria of Light et al (3,25) are very accurate in distinguishing transudates from exudates, a comparable test for distinguishing exudates of various etiologies is still lacking. In this respect, the level of IL-6 in pleural fluid and in serum taken from tuberculous could be a useful complementary index in the differential diagnosis of the malignant and tuberculous, which is so important the treatment of the patient. The observation that the IL-6 level was higher in inflammatory than in malignant exudates could reflect a different response of the immune system in the pleural space. Since IL-6 mainly regulates the function of B cells and T cells, it is assumed that those cells are not primarily involved in the response to the invasion of malignant cells in the pleural cavity. In contrast, the very high pleural fluid to serum ratio of IL-6 in patients with tuberculous pleural effusions probably reflects an enhanced local immune response of the above-mentioned cells. Hirano et al (33) reported that T lymphocytes obtained from the pleural fluid of patients with tuberculous pleural effusions produced IL-6 when stimulated with PPD. Activated T lymphocytes could therefore be responsible for the local IL-6 production in the pleural fluid of patients with tuberculous pleural effusions (13,34). Although the data in this study suggest that IL-6 is lower in malignant than in tuberculous pleural effusions, the final diagnosis of a malignant effusion should still be based on positive cytologic and/or histologic examination. Similarly, the diagnosis of tuberculous pleural effusion should be based on a positive stain result for acid-fast bacteria, positive culture finding for mycobacteria, and/or pleural biopsy showing typical granulomas.

Our results showed that TNF-α, an important regulator of the immune response, was increased in pleural fluid both malignant and inflammatory exudates, compared to transudates. However, a considerable overlap of TNF-α levels was observed among the three types of pleural exudates, so only it was found low statistically significantly differences ($p<0.05$) as compared serum levesl of tuberculous and malignant. These results are in accordance with those reported by Soderblom et al (35) showing that TNF-α is not a good marker to separate tuberculous from malignant or parapneumonic pleural fluids. However, our results are in disagreement with those of Barnes et al (14) and Kim et al (36) who found that TNF-α levels in pleura were higher in tuberculous than in nontuberculous pleural effusions. This discrepancy may be due to the inhomogeneity of the patients included in their nontuberculous group. The fact that in our study TNF-α was elevated in a similar fashion in all three types of exudates could mean that this proinflammatory cytokine plays a significant role in the upregulation of the immune system of the pleura.

In summary, the observations of this study are: (1) low serum IL-6 and high pleural fluid IL-6 can distinguish transudates due to heart failure from exudates; (2) serum and pleural fluid IL-6 could be useful as a complementary marker in the differential diagnosis of two most common types of exudates (tuberculous and malignant); and (3) TNF-α in the serum and pleural fluid colud be useful confirmation differential diagnosis of tuberculous and malignant effusions; (4) also, it can be easy to detect by common methods exudates from transudates, pleural IL-6/serum IL-6 ratios may be use to confirm the diagnosis.

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