

The Diagnostic Value of T. helper 1 Cytokines in Tuberculous and Malignant Pleural Effusions.

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الخلاصة

تعتبر إفادة الجذبة من العلامات السريرية لمرضى التدرن الرئوي وسرطان
والتميز بكونه من النوع اللمفاوي النم وذجي في كلا المرضين وأن تمييز هذه
العلامة في كلا المرضين أمر محتيج ذات الجذب التدرني بس زيادة الاسد تجابة
المناعية الناتجة من الخلايا اللمفاوية نوع T المساعدة الإمرال الذي يشجع على
قياسه واتجه هذه الخلايا ككشورات تشخيصية يؤهلها الغرض من قياسه
العوامل: IFN- γ و IL-12 و IL-18 في السائل الجنبيل 39 مصابا "بإفادة الجذب
منه" بسبب التدرن الرئوي و 29 مصابا "بسرطان الرئة" داسد تخدم
فحص ELISA لهذا الغرض. أظهرت نتائج الدراسة الحالية وجود ارتفاعا "مهما" من
الإحاطة في مسد تويات العوامل أعلاه في مرضى التدرن الرئوي مقارنة
معها في مرضى سرطان الرئة. ارتبطت مسد توى العامل IFN- γ مع مسد توى IL-
12 ارتباطا "قويا" ($r=0.71$) أعطت العوامل الثلاث أهمية تشخيصية حذيل حلات
بطريقة ROC الإحصائية وقد تميز العامل IFN- γ عن غيره في هذا الغرض. حسبت
أيضيل "القطع العليا للعوامل الثلاث في تمييز مرضى التدرن الرئوي عن مرضى
سرطان الرئة" من الدراسة الحالية أن هذه العوامل يمكن أن تخدم العملية
التشخيصية بين المرضين كما نوصي بدراسات أوسع تشمل نواتج الخلايا اللمفاوية T
المساعدة 2 لإكمال الصورة.

Abstract

The pleural effusion is an occasion clinical presentation of tuberculosis and lung cancer and clear differentiation of this condition is critical, as it appeared as typical lymphocytic pleural effusion in both diseases. There is a dominance of Th1 response in tuberculous pleurisy, a matter which encourages the evaluation of Th1 cytokines in order to reveal a possible diagnostic tool. Interferon-gamma (IFN- γ) as a major product of Th1, Interleukin-12 (IL-12) and (IL-18) as IFN- γ inducing cytokines were evaluated in 39 patient's pleural effusions (10 tuberculous and 29 lung cancer) using ELISA. Our results demonstrated significantly increased levels of IFN- γ , IL-12 and IL-18 in tuberculous pleural effusions compared with those of malignancies. Moreover, it was found that IFN- γ is strongly correlated ($r=0.71$) with IL-12. The Receiver-Operator-Characteristics (ROC) analysis explored great

areas under curves (AUCs) for the three cytokines suggesting diagnostic marking for tuberculous pleurisy differentiating it from malignant one. The most reliable cytokine in the diagnosis was the IFN- γ as it appeared at ROC of 0.93 followed by IL-12 and IL-18 which contribute to 0.80 and 0.71 ROC's, respectively. A larger study including more cytokines regarding Th1 and Th2 products is recommended.

Introduction

It is estimated that one third of the world's population is infected with *Mycobacterium tuberculosis* contributing to 8.4 million new cases in 1999⁽¹⁾. This pathogen is especially prevalent in developing countries causing 3 million deaths yearly as referred to by WHO reports ⁽²⁾. Tuberculous pleural effusions resulted from the delayed-hypersensitivity caused by specific proteins included in TB microorganism, a condition lead to the migration of subpleural caseous focus in the lung into the pleural space⁽³⁾.

Cytokines secreted by T helper cells against the infectious agents are of critical importance for the outcome of many diseases. T helper cell response can be divided into Th1 or Th2 based on the pattern of cytokine secretion, which in turn determine whether the predominant immune response is of cellular (Th1) or humoral (Th2) type. Th1 cells produce IFN- γ , IL-2 and Tumor Necrosis Factor- β (TNF- β), whereas Th2 produce IL-4 and IL-5 characteristically⁽⁷⁾. T cells that produced cytokine pattern distinct from the well-defined Th1/Th2 sets have been described and named Th0, Ths or Thr^(8,9).

In infection with *M. tuberculosis*, a Th1 type response has been shown to be protective⁽¹⁰⁾. The early studies of Shimokata *et al*, 1991 and 1982^(4,5), on tuberculous pleural effusions have shown high concentration of IFN- γ and that lymphocytes from such effusions produce IFN- γ after an *in vitro* stimulation with a purified protein derivatives (PPD)^(5,6). IFN- γ , a Th1 type cytokine, is essential in tuberculosis immunity, which is the single most important factor for macrophage activation⁽¹¹⁾. It has been found that IFN- γ is reliable marker of the presence of pleural effusions,

and this cytokine is produced in response to the induction role of IL-18 produced by activated macrophages in synergy with IL-12^(12, 13). There is no clear differentiation of tuberculous pleurisy from malignant pleurisy patients, in both, the pleural effusions are typically lymphocytic⁽¹⁴⁾. In this study, we planned to evaluate the levels of IFN- γ and IFN- γ inducing cytokines (IL-12 and IL-18) in tuberculous and malignant pleural effusions patients, in an attempt to shed light on Th1 role in localized TB compared with lung cancer and to evaluate the diagnostic importance of these pleural fluid markers in such pathological conditions.

Subjects and Methods

Subjects

A total of 89 pleural effusion samples were investigated in the laboratories unit of Shahid Adnan teaching hospital for specialist surgeries, Baghdad, Iraq during the period Feb. 2004 to July 2005. These samples were kindly collected by professional seniors using diagnostic thoracentesis. The samples returned to patients with pleural effusions due to various pathological conditions.

Of the 89 samples above, 39 were identified as lymphocytic exudative effusions based on the more than 50% ratio lymphocyte content predicted by WBC's differential count^(15, 16). Ten of the lymphocytic exudative effusions were of tuberculous patients group based on positive Acid Fast Bacilli (AFB) of the effusion and/or the sputum, in addition to the clinical feature and X-ray investigations. The reminder 29 samples represented malignancies group of patients with lung cancers as revealed by cytologic examination of the pleural fluid which supported the clinical signs and X-ray investigations.

Methods

The heparinized (10 U/ml) 39 lymphocytic pleural effusions to be studied, have been passed through a sterile metallic mesh before centrifuged 378g for 5 minutes by cold centrifuge (4°C).

The supernatants then aliquoted, and preserved deeply-frozen till cytokines assays could be carried out.

Primarily, the pleural effusions protein, Lactate Dehydrogenase (LDH), serum protein, and serum LDH were measured just after thoracentesis and pleural effusions processing. Enzyme-linked Immunosorbent Assays (ELISA) for IFN- γ , IL-12, and IL-18 were conducted using kits (MBL, Tokyo, Japan) kindly supplemented by the central health laboratories, Baghdad, Iraq. Procedures have performed in accordance with manufacturer instructions. The three cytokines have assayed in thawed aliquots of preserved samples of pleural effusions.

Statistical analysis: Data obtained were analyzed by professional statistician. The differences among pleural effusions were submitted for Mann-Whitney test, expressed as median and range, the significant differences was under 0.5 p value. Pearson's correlation test used to calculate the correlations. Receiver-Operator-Characteristics Curves (ROC) were constructed for the determination of optimal cut off point based on the highest likelihood ratio. The diagnostic value for each cytokine was demonstrated in the area under curves (AUC's)⁽²²⁾.

Results

The age, sex, of both patients groups are depicted in table 1, in which, the pleural protein estimated by (g/dl) and pleural lymphocytes/neutrophils ratio revealed significant differences between the TB and lung cancer patients. However, the LDH level in pleural effusions and sera has shown to be insignificantly differed and so do the serum protein. All of the ten tuberculous pleurisy specimens were AFB-positive, the patients with malignancies (n=29) all had adenocarcinoma as resulted in biopsy exam.

Table 1: The general characteristics, sera, pleural effusions of patient's study groups.

Parameters	Tuberculosis patients (n = 10)	Lung cancer patients (n = 29)	P
Patient characteristics			
Sex (male/female)	7/3	16/13	
Age (yr)	56 (43–66)	53 (45–80)	NS*
Pleural Effusion			
Protein (g/dL)	5.1 (3.5–7.0)	4.2 (2.5–6.1)	<.05
Lctatedehydrogenas(IU/L)	350 (134–798)	366 (71–2280)	NS
Lymphocytes/Neutrophils (%)	90 (75–94)/ 2 (1–12)	65 (48–90)/ 10 (2–40)	<.05
Serum			
Protein (g/dL)	6.8 (5.4–8.1)	6.2 (4.8–7.6)	NS
Lctatedehydrogenas (IU/L)	266 (101–522)	259 (110–668)	NS

*NS = Not significant, data expressed as mean (range.)

The cytokine's levels represented in table 2 revealed that the three cytokine's concentrations in tuberculous pleural effusions were greater than those in malignancies, results which contribute to significant differences. The median concentration of IFN- γ , IL-12 and IL-18 in TB patients estimated by pg/ml were 1155, 1245 and 701, respectively compared with those in lung cancer group; 33, 398 and 220, respectively.

Table 2: The cytokine median (range) concentrations in tuberculous and lung cancer patient's pleural effusions.

Cutokine conc. (pg/mL)	Tuberculosis patients (n = 10)	Lung cancer patients (n = 29)	P
IFN- γ	1155 (70–6000)	33 (2–112)	<0.0022
IL-12	1245 (401–3020)	398 (1–2200)	<0.003
IL-18	701 (33–1651)	220 (0–1648)	0.0015

In addition, a significant positive correlation in the pleural effusions of TB group was detected between IL-12 and IFN- γ concentrations ($r=0.71$, $p=0.2$) compared with week correlations found between IL-18 and IFN- γ ($r= 0.22$, $p=0.72$) and between

IL-18 and IL-12 levels ($r=0.2$, $p=0.81$). The interesting results were obtained when ROC constructed by blotting sensitivity against 1- specificity in order to create the areas under curves for each cytokine. As wider as this area for a given cytokine, as highest is the diagnostic value of it. Figure 1 shown that the most significant marker in the diagnosis of tuberculous pleuritis distinct it from malignant one is IFN- γ as it has an AUC (0.93) followed by IL-12 (0.80) and IL-18 (0.71). The optimal cut off values of each cytokine are ordered in table 3, at each pointed concentration or greater than it, the highest specificities, sensitivities and likelihood ratio of having TB rather than malignancies may be assumed. The highest likelihood ratio was that of IFN- γ (31.1) compared with those of IL-12 (15.4) and IL-18 (13.2) if these cytokines occurred at concentrations of (85.5, 1302.3 and 889 pg/ml, respectively).

Table 3: The diagnostic values of optimal cut off points of IFN- γ , IL-12 and IL-18 in tuberculous pleural effusions differentiating them from lung cancer pleural effusions.

Cytokine	Optimal Cutoff point (pg/mL)	Sensitivity	Specificity	Likelihood ratio
IFN- γ	85.5	0.90	0.97	31.1
IL-12	1302.7	0.66	0.95	15.4
IL-18	889	0.29	0.97	13.2

Discussion

More than half of the extrapulmonary TB cases are due to the involvement of the pleura and lymphocytic system. Tuberculous and malignant's pleural effusions are typical lymphocytic types⁽¹⁴⁾, and clear differentiation between patients with either condition is needed. The initial result in our study was the markedly increased concentration of

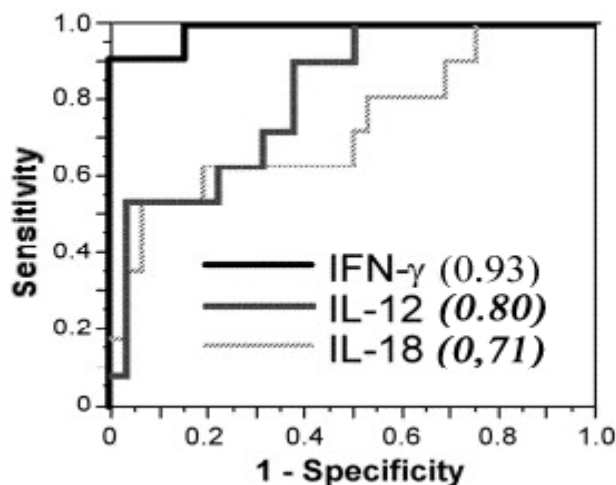


Figure 1: ROC analysis showing the areas under curves as diagnostic marks of the three cytokines for TB pleural effusions differentiating them from malignant pleural effusion.

IFN- γ and IFN- γ inducing cytokines, i.e., IL-12 and IL-18 in TB pleural effusions but not in malignant one, a suggestion of Th1 type of response in TB pleuritis. Sharma *et al*⁽¹⁷⁾, have referred to the important role played by cytokines in the pathogenesis of TB, and have reported a significance for IFN- γ and IL-12 in the induction of Th1 response in patients with TB. They observed elevated IFN- γ in pleural fluids and suggested enrichment of pleural space with Th1 cytokines in addition to the selective accumulation of IFN- γ ⁺ cells in pleural fluid⁽¹⁷⁾. The functional association of IFN- γ , IL-12 and IL-18 with Th1 type immune response (cellular immunity) has been studied by many authors.

In a Th1 response, IL-12 not only induces up-regulation of the IL-18 receptors on T cells, but also heightens their responsiveness to IL-18 and enhances IFN- γ production⁽¹⁸⁾. Robinson *et al*⁽¹⁹⁾ stated that IL-18 emerged as a novel cytokine acting in synergy with IL-12 to induce IFN- γ production in Th1 and natural killer cells. The role of IL-18 in the production of IFN- γ is well-established, however, in our study, the statistical analysis for correlations indicated weak correlation between them, instead, close association of IL-12 with IFN- γ production has been observed. Such result may be reasoned by the small-sized sample of our study. Moreover, Zhang and colleagues have found

that the stimulation of pleural fluid cells with *M. tuberculosis* produced high levels of IL-12⁽¹²⁾. IL-12 is known to induce a Th1 response in undifferentiated CD4⁺-cells suggesting a crucial role for these cells at the morbid sites of tuberculosis.

Our observations are in consistency with those other who have reported elevated pleural fluid IFN- γ in TB pleural effusions^(20, 21). These studies are strongly suggest that the level of IFN- γ can be used as a diagnostic marker for TB. In our study, we stated that, although all of the three cytokines were found to have high AUCs, IFN- γ still the most powerful cytokine in the diagnosis of TB pleurisy differentiating it from pleurisy due to malignancy. In this study, a confirmatory conclusion of Th1 predominance in TB infection was extracted, and diagnostic values for IFN- γ , IL-12 and IL-18 were explored. It is the first trial in Iraq, as to our knowledge, and additional larger studies regarding Th2 products in similar patients are recommended.

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