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ORIGINAL ARTICLE

Differential diagnostic efficiency of T cells subsets versus interferon-gamma, tumor necrosis factor-alpha and adenosine deaminase in distinguishing tuberculous from malignant pleural effusions

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KEYWORDS

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Abstract *Background:* The differential diagnosis of tuberculous and malignant pleural effusion (PE) is extremely difficult and continues to pose clinical challenges.

Aim of the study: To evaluate the utility of pleural fluid interferon gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), adenosine deaminase (ADA) levels with T cells subsets in differential diagnosis of malignant (MPE) and tuberculous pleural effusions (TPE).

Methods: Forty patients with pleural effusion (20 tuberculous and 20 malignant) were included in the study. The percentages of CD3+ lymphocytes, CD4+ lymphocytes and Treg (CD4+ CD25+) cells in pleural effusion from patients with tuberculous and malignant PE were determined by flow cytometry. The concentrations of IFN-γ, TNF-α, and ADA were simultaneously determined in pleural fluids by enzyme linked immunosorbent assay and colorimetric methods.

Results: IFN-γ, TNF-α and ADA concentrations were significantly higher in TPE than MPE (2.26 ± 1.62 vs. 0.3 ± 0.20 IU/ml: $P < 0.0001$, 122.45 ± 47.69 vs. 35.03 ± 31.88 pg/ml: $P < 0.0001$ and 84.22 ± 41.47 vs. 23.19 ± 17.93 U/l: $P < 0.0001$ respectively). T-cells markers (CD3+ T-cells, CD4+ T-cells and T reg cells) were significantly higher in TPE than MPE (76.46% vs. 65.29%; P 0.004, 51.21% vs. 43.50%; P 0.044 and 14.60% vs. 12.43%; P 0.032

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respectively). CD3+ plus CD4+ as well as CD3+ plus CD4+ plus T reg combinations were all 100% specific for discriminating TPE from MPE. TNF-α plus IFN-γ, TNF-α plus ADA, as well as IFN-γ plus TNF-α plus ADA, were 100% specific for discriminating TPE from MPE. Furthermore, the specificity of combined-diagnostic value of IFN-γ, TNF-α and ADA with T cells subsets was >95%.

Conclusions: The combinations of pleural fluid IFN-γ, TNF-α and ADA levels and T cells subsets could effectively address the challenge of distinguishing tuberculous pleural effusion from malignant pleural effusion.

Introduction

Tuberculosis is a common disease in Egypt, with an incidence rate of 19/100,000 and 5530 new cases diagnosed in 2011 [1]. Tuberculosis typically attacks the lungs, but can also affect other parts of the body. Extra pulmonary infection sites include the pleura, the central nervous system, the lymphatic system, the genitourinary system, and the bones and joints [2].

TPE is caused by the rupture of a pulmonary sub pleural caseous focus which releases mycobacterium into the pleural cavity, thereby triggering an immune response involving mainly macrophages, CD4+ T lymphocytes, and the cytokines released by these cells (especially interleukin 1, interleukin 2, and interferon γ) [3].

It has been well documented that CD4 (+) T lymphocytes are the dominant leukocytes present in TPE. Traditionally, CD4 (+) T cells have been classified into two functionally distinct subsets, helper T-cell type 1 (Th1) and Th2 cells, based on their cytokine secretion profiles. Recently, regulatory T cells, Th17 cells, Th9 cells, and Th22 cells have been added to the 'portfolio' of this ever enlarging subset [4]. CD4+CD25+ T cell numbers in TPE were much higher than those in peripheral blood from patients with TPE and from healthy subjects, also CD4+CD25+ T cells infiltrating pleural space were regulatory T cells as they expressed high levels of Foxp3 transcription factor. Moreover, CD4+CD25+ T cells could potently suppress the proliferation of responding T cells [5], also it was found that CD4+CD25+ T-cell numbers in MPE were much higher than those in PE from patients with lung cancer without malignant effusion and higher than numbers in peripheral blood [6].

Various cytokines have been demonstrated in pleural fluid from patients with TB pleural effusions, interferon γ, which is released by activated CD4+ T cells is capable of activating macrophages, increasing their bactericidal capacity against *Mycobacterium tuberculosis* and is involved in granuloma formation. Several studies have found elevated concentrations of INF-γ in TB pleural effusions, which are related to increased production at the disease site by effector T cells. Another highly efficient marker is ADA, has been reported to accumulate in the pleural fluid of TB patients and can predict TPE with high sensitivity and specificity at 95% and 90% respectively [7], Also high concentrations of TNF-α in pleural fluid have been observed in several diseases, including tuberculosis [4].

This prompted us to conduct this study to assess the role of T Cells subsets, IFN-γ, TNF α and ADA in the differential diagnosis of TPE and MPE, by determining the best cutoff levels of pleural fluid T Cells subsets, IFN-γ, TNF α and ADA for differentiating between TPE and MPE.

Patients and methods

Patients' selection

Our study was carried out in the Departments of Chest, Medical Microbiology and Immunology and Clinical pathology, Faculty of Medicine, Sohag University during the period from November 2012 to December 2013.The study was approved by the research ethics committee and consents were obtained from patients in the study.

Collection of pleural fluid was carried out to patients who were indicated for thoracocentesis. Patients were subsequently included if the examinations of pleural effusion and/or biopsy specimens established a diagnosis of TPE or MPE. Twenty patients were shown to have tuberculous PE, as evidenced by the growth of *M. tuberculosis* from PE or by demonstration of granulomatous pleuritis on a closed pleural biopsy specimen in the absence of any evidence of other granulomatous diseases. Malignant PE was collected from 20 patients with newly diagnosed lung cancer. Histologically, among the patients with malignancies, 14 had adenocarcinoma, 3 had squamous cell carcinoma, 1 had Hodgkin Lymphoma and 2 had distant metastasis.

At the time of sample collection, none of the patients had received any antituberculosis therapy, anticancer treatment, corticosteroids, or other nonsteroid anti-inflammatory drugs.

Methods

All patients were subjected to:

Complete history taking; personal history and family history.
Thorough clinical evaluation.
Radiological evaluation (Chest X rays).
Complete blood count, serum biochemical tests.

Pleural fluid samples were obtained by intercostal needle aspiration. All pleural fluids were stained and cultured for the presence of bacteria and analyzed cytologically for the presence of tumor cells. Total and differential white cell counts, proteins, glucose and lactate dehydrogenase were determined in all pleural fluids. The pleural fluid samples were centrifuged at 2000 rpm for 10 min. ADA activity was