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Th1 and Th2 Cytokine Profiles in Malignant Pleural Effusion

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ABSTRACT

Background: The alteration of Th1 and Th2 cytokine levels is the subject of controversy in pleural effusions caused by malignancy, a situation that favors a Th2 immune response. **Objective:** To examine the different levels of IL-4 and IL-10 (Th2 cytokines), and IL-2 and interferon- γ (IFN- γ) (Th1 cytokines) in malignant and non-malignant pleural effusions. **Method:** The cytokine levels in pleural fluid of 62 patients with malignant pleural effusion (44 with lung cancer and 18 with extrathoracic tumors), 8 with tuberculous and 8 with congestive heart failure pleural effusion were analysed using enzyme-linked immunosorbent assays. **Results:** IL-2 was below the detectable concentration of the assay. A significant decrease in IFN- γ level was observed in malignant but not in congestive heart failure cases compared to tuberculous cases. IL-10 levels were higher in malignant and tuberculous pleural effusions than in congestive heart failure pleural effusions, however, this difference did not reach the significant level. IL-4 levels were also increased non-significantly in lung cancer pleural effusions compared to the other groups. **Conclusion:** Our results show a wide variation in IL-4, IL-10, and IFN- γ levels in malignant pleural effusions, a pattern which was not convincing enough to differentiate the cause of effusion.

Keywords: IFN- γ , IL-4, Lung Cancer, Pleural Effusion

INTRODUCTION

Pleural effusions, accumulation of excess fluid in the pleural space, are classified into transudates and exudates. Transudative pleural effusions are usually caused by systemic diseases that increase pressure filtration. In contrast, exudative pleural effusions usually reflect the existence of a primary pleural disease. Congestive heart failure, cirrhosis and renal failure are the most common causes of transudative pleural effusions, but bacterial pneumonia and cancer, generally lung and breast, are the most common causes of exudative pleural effusions. The differentiation between transudative and exudative pleural effusions are partly achieved by the 4 Light's criteria, in which, i) pleural

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fluid protein/serum protein >0.5 , ii) pleural fluid lactate dehydrogenase/serum lactate dehydrogenase >0.6 , iii) pleural lactate dehydrogenase >200 IU, and iii) pleural fluid lactate dehydrogenase $>$ two-third times normal upper limit for serum, are suggestive criteria for an exudative pleural effusion. Once an exudative effusion was diagnosed, invasive diagnostics might be required for establishing a malignant pleural effusion (1). In this respect, discovery of sensitive and specific soluble biomarkers in body fluids that are easy to obtain is under intensive research. Cytokines are secretory molecules that are produced by immune and non-immune cells. They have attracted much attention as diagnostics, prognostics and therapeutics in cancer due to their measurability in body fluids (2,3).

In contrast to T helper (Th1) cytokines, it is generally accepted that cancer cells benefit from Th2 cytokines, of which, interleukin (IL)-4 and IL-10 are the most prominent. IL-4 is mainly produced by Th2 lymphocytes, mast cells and basophils. Its production by cancer cells was detected in lymphoma cells and pancreatic cancer cells. IL-10 is produced by a wide range of immune cells and non-immune cells (e.g., cancer cells). Both cytokines can promote different aspects of tumorigenesis by immunological mechanisms, such as reduction of Th1 cytokines and impairment of antigen presentation, and non-immunological mechanisms, such as protection of cancer cells from apoptosis (4,5). IL-2, or T cell growth factor, and interferon- γ (IFN- γ) are produced in Th1 pathway. IL-2 induces T lymphocytes to produce IFN- γ , the signature mediator of a Th1 response. IFN- γ plays mandatory roles in promoting innate and adaptive immune responses against tumors and intracellular infections, such as tuberculosis (2,4).

In vitro studies have shown that peripheral blood and pleural effusion lymphocytes from lung cancer patients if stimulated with phytohemagglutinin produce higher amounts of Th2 cytokines (4,6). Although these in vitro tests are clues for understanding the lung cancer pathogenesis, in the case of diagnosis, concern is the simplicity of a test. Notably, direct analysis of body fluids using Enzyme Linked Immunosorbent Assay (ELISA) is of particular interest. Pleural space in malignant pleural effusions is infiltrated with lymphocytes (1). In this regard, Th1 and Th2 cytokine measurements using ELISA have been studied by several researchers, but with conflicting results (2,6-10). Accordingly, the aims of our study were to investigate the possible utilization of IL-4, IL-10, IL-2, and IFN- γ as diagnostic biomarkers for malignant pleural effusion, either effusion caused by intra-thoracic tumors or extra-thoracic tumors. Two control groups were also provided, one comprised of pleural effusion samples collected from congestive heart failure patients, and the other was collected from patients with tuberculosis, a disease favoring a Th1 skewed immune response.

MATERIALS AND METHODS

Pleural Effusion Samples. The study was approved by the Ethics Committee of Shiraz University of Medical Sciences. Pleural effusion samples were obtained from the patients who were referred to Faghihi hospital in Shiraz, Iran from 2009 to 2011. Study groups were 62 newly diagnosed patients with malignant pleural effusions, including 44 patients with pleural effusions caused by non-small cell lung cancer and 18 with metastatic pleural effusions from extra-thoracic tumors, 10 with tuberculosis, and 8 with congestive heart failure. The diagnosis of malignant pleural effusion was approved by either the detection of malignant cells in the pleural effusion drawn by thoracentesis or in

pleural biopsy specimens. Tuberculous pleural effusions were diagnosed based on positive mycobacterium tuberculosis culture from biopsy specimens. Congestive heart failure patients had transudative pleural effusion with no clinical or paraclinical evidence (e.g, blood test, chest X ray, and abdominal sonography) of any other diseases. None of the participants had taken radiotherapy, chemotherapy, steroid or nonsteroid anti-inflammatory drugs or any drug affecting the immune system at the time of sampling. The characteristics of patients including age and sex are displayed in Table 1. Pleural effusion samples were centrifuged at $1000 \times g$ for 15 min at $4^{\circ}C$, and the supernatants were stored at $-70^{\circ}C$ until use.

Table 1. Characteristics of patients with pleural effusion.

Characteristics	Lung cancer (n = 44)	Extra-thoracic tumor (n = 16)	Tuberculosis (n = 10)	Congestive heart failure (n = 8)
Mean age \pm SD (years)	65.5 \pm 15.1	64.5 \pm 15.0	64.5 \pm 15.2	65.5 \pm 13.3
Male/Female	24/20	9/7	6/4	5/3
Tumor histologic type*				
Adenocarcinoma	27	-	-	-
Squamous cell carcinoma	17	-	-	-

Quantification of Cytokines. Cytokines in the pleural fluid samples were quantified in duplicate using commercial ELISA kits according to the manufacturer's instructions. ELISA kits were purchased from eBioscience, USA. The minimum detectable limits of the kits were 1.3 pg/ml for IL-4, 1.0 pg/ml for IL-10, 9.9 pg/ml for IL-2, and 0.99 pg/ml for IFN- γ . Concentrations lower than the detection limits were reported as undetectable.

Data Analysis. The nonparametric tests of Mann-Whitney U or Kruskal Wallis test were employed to compare the differences in the levels of cytokines in the study groups. The data were analysed using SPSS software (version 11.5.0; SPSS, Chicago, IL, USA). Findings were considered statistically significant at a p value less than 0.05.

RESULTS

In the present study, using ELISA, we examined IL-4, IL-10, IL-2 and IFN- γ levels in malignant pleural effusions either due to lung cancer or due to extra-thoracic tumors, and compared them to those in pleural effusions caused by tuberculosis or cardiac heart failure.

We found that IL-2 levels in all study groups were below the detection limit of the kit.

Table 2 indicates the median and mean levels of IL-4, IL-10, and IFN- γ in pleural effusions of the study groups. The median and mean IL-4 levels were higher in lung cancer than in other groups. IL-10 level was higher in malignant and tuberculous pleural effusions than in cardiac heart failure pleural effusions. Analysis of these the cytokines among the four group using Kruskal Wallis test, a test for several independent samples, revealed a statistical significant difference only in IFN- γ levels among the groups (Table 2).

Table 2. Mean and median levels of IL-4, IL-10, and IFN- γ in pleural effusions caused by lung cancer, extra-thoracic tumors, tuberculosis, and congestive heart failure.

Cytokine level	Lung cancer (n=44)	Extra-thoracic tumors (n=16)	Tuberculosis (n=10)	Congestive heart failure (n=8)	P value ^a
IL-4 (pg/ml)					
Mean \pm SD	10.9 \pm 15.8	6.0 \pm 7.4	6.4 \pm 4.8	6.7 \pm 15.2	0.350
Median (range)	5.1 (Un ^b -79.0)	3.2 (Un ^b -24.8)	1.0 (Un-18.0)	Undetectable (Un-44.0)	
IL-10 (pg/ml)					
Mean \pm SD	21.6 \pm 19.4	32.4 \pm 44.7	28.1 \pm 25.4	12.9 \pm 8.5	0.539
Median (range)	15.5 (Un ^c -80.4)	17.7 (1.2-177.0)	22.2 (6.6-79.8)	12.0 (3.4-23.4)	
IFN- γ (pg/ml)					
Mean \pm SD	4.2 \pm 8.7	16.1 \pm 41.9	57.7 \pm 64.6	7.3 \pm 9.3	0.006
Median (range)	2.1 (Un ^d -55.6)	3.6 (Un ^d -172.4)	19.3(1.5-140.0)	5.0 (Un ^d -29.0)	

^a p values calculated using Kruskal-Wallis test.

^b Un= Undetectable, The sensitivity of ELISA was 1.3 pg/ml for IL-4.

^c Un= Undetectable, The sensitivity of ELISA was 1 pg/ml for IL-10.

^d Un= Undetectable, The sensitivity of ELISA was 0.99 pg/ml for IFN- γ .

Using the Mann-Whitney U test, a test for two independent samples, comparisons between lung cancer and tuberculous pleural effusions, as well as between extra-thoracic tumor and tuberculous pleural effusions revealed a significantly lower IFN- γ levels in malignant pleural effusions ($p=0.002$ for lung cancer vs. tuberculous, and $p=0.03$ for extra-thoracic tumor vs. tuberculous pleural effusions).

DISCUSSION

The alteration of Th1 cytokines, including IFN- γ and IL-2, and Th2 cytokines, including IL-4, IL-10 levels in malignant pleural effusions are the subject of controversy. For example, Ogawa et al. compared levels of IFN- γ and IL-2 in pleural effusions of 18 patients with tuberculous pleural effusion and 25 patients with malignant pleural effusion. They found that the level of IFN- γ , but not IL-2, was significantly lower in malignant

cases compared to tuberculous ones (8). In addition to IFN- γ , IL-2 showed a significantly lower level in patients with malignant pleural effusions compared to tuberculous pleural effusion in a report by Shimokata et al. in which the levels of these two cytokines were measured in the pleural fluid of 20 patients with tuberculous pleural effusions and of 20 patients with malignant pleural effusions (9). Sikora et al. tested pleural effusions from 44 (18 non-malignant and 26 malignant) patients for IL-10 levels and found a significant elevation of this cytokine in malignant pleural effusions as compared to non-malignant ones (10). This was not confirmed by other independent studies (2). The undetectable levels of IL-4 in pleural effusions and sera of patients with malignant pleural effusions have been reported (7). However this cytokine was associated with several types of cancer in our geographic area (5, 11), implying that it might be a cancer biomarker.

Our study evaluated the possible significant alteration of IFN- γ , IL-2, IL-4, and IL-10 in pleural effusions caused by malignancy (either lung cancer or extra-thoracic tumors), a situation favouring Th2 responses. Our control samples were obtained from pleural effusions caused by tuberculosis (a Th1 favouring disease) or cardiac heart failure. IL-2 was below the detection limit of the kit. A significant lower level of IFN- γ in pleural effusions caused by lung cancer or extra-thoracic tumors was observed compared to the level in pleural effusions caused by tuberculosis. However, the maximum level of IFN- γ was detected in a malignant pleural effusion case, reflecting the wide variation among patients. Some insignificant alterations were observed in IL-4 and IL-10 levels. IL-4 level was higher in lung cancer pleural effusions compared to other groups. IL-10 was higher in malignant and tuberculous pleural effusions than in cardiac heart failure effusions (Table 2). Active production of IL-10 in pleural effusions has been previously reported in effusions produced by tuberculosis as well as those caused by malignancies (2,7).

Cytokines have successfully been used to boost the immune response against cancer (1). Despite insignificant alterations of IL-4, IL-10 and IFN- γ in malignant pleural effusions compared to cardiac heart failure pleural effusions, a portion of malignant cases had a marked alteration in the levels of these cytokines, as endorsed by standard deviations greater than means of some cytokines in malignant cases (Table 2). Standard deviations greater than means were previously reported for IL-10 and IFN- γ in malignant pleural effusion cases (2). Whether in these subgroups of the patients cytokine levels can be a guidance for therapy need more investigations.

In conclusion, we found a significant decrease of IFN- γ in malignant pleural effusions compared to the tuberculous ones. IL-10 and IL-4 were insignificantly increased in lung cancer pleural effusions compared to cardiac heart failure effusions. There was a patient to patient difference in the levels of these cytokines.

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REFERENCES

- 1 Heffner JE, Klein JS. Recent advances in the diagnosis and management of malignant pleural effusions. *Mayo Clin Proc.* 2008; 83:235-50.
- 2 Chen YM, Yang WK, Whang-Peng J, Tsai CM, Perng RP. An analysis of cytokine status in the serum and effusions of patients with tuberculous and lung cancer. *Lung Cancer.* 2001; 31:25-30.
- 3 Samsami Dehaghani A, Shahriary K, Kashef MA, Naeimi S, Fattahi MJ, Mojtahedi Z, et al. Interleukin-18 gene promoter and serum level in women with ovarian cancer. *Mol Biol Rep.* 2009; 36:2393-7.
- 4 Mocellin S, Wang E, Marincola FM. Cytokines and immune response in the tumor microenvironment. *J Immunother.* 2001; 24:392-407.
- 5 Mojtahedi Z, Khademi B, Yehya A, Talebi A, Fattahi MJ, Ghaderi A. Serum levels of interleukin-4 and interleukin-10 in head and neck squamous cell carcinoma. *J Laryngol Otol.* 2012; 126:175-9.
- 6 Mantovani G, Macciò A, Pisano M, Versace R, Lai P, Esu S, et al. Tumor-associated lympho-monocytes from neoplastic effusions are immunologically defective in comparison with patient autologous PBMCs but are capable of releasing high amounts of various cytokines. *Int J Cancer.* 1997; 71:724-31.
- 7 Chen YM, Yang WK, Whang-Peng J, Kuo BI, Perng RP. Elevation of interleukin-10 levels in malignant pleural effusion. *Chest.* 1996; 110:433-6.
- 8 Ogawa K, Koga H, Hirakata Y, Tomono K, Tashiro T, Kohno S. Differential diagnosis of tuberculous pleurisy by measurement of cytokine concentrations in pleural effusion. *Tuber Lung Dis.* 1997; 78:29-34.
- 9 Shimokata K, Saka H, Murate T, Hasegawa Y, Hasegawa T. Cytokine content in pleural effusion. Comparison between tuberculous and carcinomatous pleurisy. *Chest.* 1991; 99:1103-7.
- 10 Sikora JJ, Dworacki GT, Kaczmarek MT, Jenek RE, Zeromski JO. Immunosuppressive mechanisms in the microenvironment of malignant pleural effusions. *Cancer Detect Prev.* 2004; 28:325-30.
- 11 Satyam A, Singh P, Badjatia N, Seth A, Sharma A. A disproportion of TH1/TH2 cytokines with predominance of TH2, in urothelial carcinoma of bladder. *Urol Oncol.* 2011; 29:58-65.