Biochemical Study on Pleural Effusion Fluid in Tuberculous and non-Tuberculous in Iraqi Patients

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Abstract

Seventy exudative lymphocytic pleural fluid specimens of patients with suspected tuberculous pleural effusion submitted to the National Reference Laboratory of tuberculosis/Baghdad from October 2012 to February 2013. These effusions were due to tuberculosis pleuritis (n=12) and non-tuberculosis pleuritis (n=58). The following parameters were analyzed: protein concentration, glucose concentration, lactate dehydrogenase (LDH) concentration and adenosine deaminase activity (ADA). As a result, the protein concentration was higher in TPE patients (8.80 ± 0.89 g/dl) than it's concentration in non-TPE patients (7.61 ± 0.54 g/dl), as well as LDH concentration was (3366.58 ± 284.28 U/L) in TPE patients and (3024.12 ± 116.84 U/L) in non-TPE patients and ADA activity was higher in the TPE patients (226.05 ± 16.90 U/L) than (153.06 ± 9.37 U/L) in non-TPE too. Whereas glucose was the unique parameter that it's concentration is lower in TPE patients than it's concentration in non-TPE patients (27.23 ± 4.81 mg/dl) and (199.80 ± 18.51 mg/dl) respectively. As a conclusion, the combination of the two parameters, protein level > 5 g/dl and glucose level < 60 mg /dl may be diagnostic for tuberculous pleural effusion. The higher level of ADA, greater the chance of the patient having TB while lower the level lesser the chance of the patient having TB. LDH measurement is a sensitive, but rather non-specific inflammatory marker.

Key words: pleural effusion, tuberculous pleural effusion, biochemical analysis of pleural fluid

دراسة كيميائية حياتية لسائل غشاء الجنب الالتهابي التدرني و غير التدرني في المرضى العراقيين

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الخلاصة

شملت الدراسة جمع 70 عينة من سائل الجنب من المرضى الوافدين إلى المختبر المرجعي للتدرن/ بغداد للفترة 2012/10/1 –2013/2/1 و كان عدد المصابين بسائل غشاء الجنب الالتهابي التدرني (12) و عدد المصابين بسائل غشاء الجنب الالتهابي غير التدرني (58). و تم قياس المؤشرات الكيميائية الاتية: تركيز البروتين, تركيز الكلوكوز , انزيم LDH و انزيمADA . وجد تركيز البروتين في مرضى التدرن اعلى (8.80±

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0.89 غم/ ديسي لتر) من تركيزه في مرضى غير التدرن (7.61± 0.54 غم/ ديسي لتر) و كذلك تركيز انزيم LDH حيث كان (3366.58 ± 284.28 وحدة / لتر) في مرضى التدرن و (3024.12± 116.84 وحدة / لتر) في مرضى غير التدرن. و انطبق هذا ايضا على انزيم ADA الذي كان تركيزه (226.05 ± 16.90 وحدة/ لتر) في مرضى التدرن و (153.06± 9.37 وحدة/ لتر) في مرضى غير التدرن. اما سكر الكلوكوز فقد كان المؤشر الوحيد بتركيز اقل في مرضى التدرن من تركيزه في مرضى غير التدرن (27.23 ± 4.81 ملغم/ ديسي لتر) و (199.80± 18.51 ملغم/ ديسي لتر) على التوالي. نستنتج, أن دمج المؤشرين, تركيز بروتين > 5 غم/ ديسي لتر و تركيز سكر الكلوكوز < 60 ملغم/ ديسي لتر قد يعد تشخيص لسائل الجنب التدرني و كلما زاد تركيز انزيم ADA يزيد من احتمالية حدوث التدرن في المرضى و العكس صحيح. اما انزيم LDH فبلرغم من حساسية قياسه الا انه مؤشر غير نوعي.

Introduction

Tuberculosis (TB) remains a leading public health problem worldwide [1], with an estimated 9 million new cases and 2 million deaths every year. In the majority of cases, the disease affects the lungs, but there are also not negligible numbers of cases (about 15%) with extrapulmonary involvement in low-incidence countries. There are even higher rates in high-incidence countries. Iraq has been identified as middle-TB burden country in the world. Iraq contributes to 3% of the total cases in the Middle East region and considered among the nine high TB burdened countries in the region. It is estimated that more than 3100 patients die due to TB annually [2]. Pleural TB is the second most frequent form of extrapulmonary TB and the most frequent cause of exudative pleural effusions in areas with a high prevalence of HIV infection. Patients present at all ages with uni- or bilateral pleural effusion and acute to subacute onset of chest pain, fever, weight loss, breathlessness, and cough. The diagnostic workup includes pleural fluid aspiration and pleural biopsy, as pleural fluid smear and culture are often negative due to the paucibacillary nature of pleural TB [3].

Pleural effusion is one of the common complications of primary tuberculosis or in conjunction with pulmonary infiltrate typical of post primary tuberculosis [4]. The inner surface of the chest wall and the surface of the lungs are covered by the parietal and visceral pleural, respectively, with a potential space of 10-24 μm between the two pleural surfaces. This space is normally filled with approximately 1 ml of fluid, representing the balance between (1) hydrostatic and oncotic forces in the visceral and parietal pleural vessels and (2) extensive lymphatic drainage. Pleural effusions result from disruption of this balance and large amounts of fluid can accumulate in the pleural space under pathologic conditions. The first step in the diagnosis is to determine whether the effusion is a transudate or an exudate [5]. An exudative effusion is diagnosed if the patient meets Light's criteria [6].

A basic difference is that transudates, in general, reflect systemic disorders, whereas exudates usually signify underlying local (pleuropulmonary) disease. The definitive diagnosis of tuberculous pleural effusion requires the presence of granulomas in pleural tissue or a stained acid fast bacilli or positive culture from the pleural tissue or pleural fluid. The biochemical analysis of pleural fluid is important in the differential diagnosis of pleural effusion. Basing on protein and LDH levels in serum and pleural fluid in differentiation exudate from transudate pleural fluid basing on Light's criteria [7].

The protein concentration in pleural tuberculosis is frequently higher than 5 g/dl and this value is proposed as a cutoff for diagnostic presumption [8]. The protein-rich exudate was a common feature of the pleural fluid in tuberculosis patients [9]. LDH level is not specific in the differential diagnosis since it is elevated in tuberculosis and non-tuberculosis patients [7-10]. The glucose level which is less than 60 mg/dl in not specific because is also suggests malignant effusion and lupus pleuritis [7]. The sensitivity of ADA in the diagnosis of tuberculous pleural effusion is 87% and specificity is 89% and the elevation in pleural fluid ADA level predict TPE [7]. The sensitivity, specificity and accuracy of ADA in the diagnosis of TPE increase in regions with high prevalence of TB and low prevalence of HIV [10].

A local study found that ADA measurement is considered as diagnostic marker and found that there is significance difference between tuberculosis and non- tuberculosis patients [11]. The purpose of this study is to diagnose tuberculosis in patients with pleurisy basing on the biochemical content of pleural fluid which includes total protein, glucose, lactate dehydrogenase (LDH) and adenosine deaminase (ADA).

Materials and methods

Specimens

All pleural fluid specimens that were submitted to the Iraqi National Reference Laboratory for tuberculosis (NRL)/ Baghdad from October 2012 to February 2013 were included in the study. 70 specimens were tested, 12 specimens of tuberculosis pleural effusion patients and 58 specimens of non-tuberculous pleural effusion patients.

Total protein: the total protein activity of pleural fluid was measured according to the instruction of a commercial kit (test kit No.51013002) obtained from AGAPPE diagnostic Switzerland Gmbh /Germany. The colorimetric determination of total protein based on the principle of the Biuret reaction (copper salt in an alkaline medium). Protein in plasma or serum sample forms a blue colored complex when treated with cupric ions in alkaline solution. The intensity of the blue color is proportional to the protein concentration.

Glucose: Glucose activity of pleural fluid was measured according to the instruction of a commercial kit (test Kit No.51406001) obtained from AGAPPE diagnostic Switzerland Gmbh /Germany. The enzymatic colorimetric determination of glucose according to the following reaction:

$$\text{Glucose} + O_2 \xrightarrow{\text{Glucose Oxidase}} \text{Gluconic acid} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + \text{4-Aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Red Quinonimine} + 4H_2O$$

Lactate dehydrogenase (LDH): Lactate dehydrogenase (LDH)

Activity of pleural fluid was measured according to the instruction of a commercial kit (test Kit No. 1141010) obtained from Linear chemicals. S.L/Spain. Lactate dehydrogenase (LD/LDH) catalyzes the reduction of pyruvate to lactate in the presence of reduced nicotinamide adenine dinucleotide (NADH) at pH 7.5. The reaction is monitored kinetically at 340 nm by the rate of decrease in absorbance resulting from the oxidation of NADH to NAD^+ proportional to the activity of LD present in the sample according to following reaction:

$$\text{Pyruvate} + \text{NADH} + H^+ \rightarrow \text{L-Lactate} + NAD^+$$

Adenosine deaminase: The enzyme activity was measured according to Giusti method [12]. Adenosine deaminase (ADA) a predominant T-lymphocyte enzyme catalyses the conversion of adenosine to inosine, a stage of purine metabolism, as in the following equation:

$$\text{Adenosine} + H_2O \xrightarrow{\text{ADA}} \text{Inosine} + NH_3$$

$$NH_3 + \text{sodium hypochlorite} + \text{phenol} \rightarrow \text{blue indophenols}$$

So, the ammonia released from the hydrolysis of adenosine in the presence of enzyme which converts it to blue indophenols in the presence of sodium hypochlorite with phenol in alkaline medium. The catalytic factor in the reaction is sodium nitroprusside and the reaction stops by adding phenol nitroprusside solution.

Statistical analysis

The Statistical Analysis System- SAS [13] was used to effect of difference factors in study parameters. Least significant difference-LSD test was used to compare between means in this study.

Results and discussion

Pleural fluid analysis of patients with tuberculous and non-tuberculous pleural effusions as illustrated in table-1.

Table 1- biochemical analysis of tuberculous and non-tuberculous pleural effusion patients.

Parameter	Non-tuberculous pleural effusion patients	Tuberculous pleural effusion patients	LSD Value
Protein (g/dl)	7.61 ± 0.54	8.80 ± 0.89	2.512 NS
Sugar (mg/dl)	199.80 ± 18.51	27.23 ± 4.81	81.790 *
LDH (U/L)	3024.12 ± 116.84	3366.58 ± 284.28	573.27 NS
ADA (U/L)	153.06 ± 9.37	226.05 ± 16.90	43.972 *
* ($P<0.05$), NS: Non-significant.			

In determining the differential diagnosis of a pleural effusion, it is important to classify the fluid as either a transudate or an exudate. The differential diagnosis of transudate is limited and the clinical diagnosis usually is apparent from the history and physical examination. Exudative pleural effusions have many more diagnostic possibilities and occur most commonly when there is an inflammatory process involving the pleura. Using Light's criteria [6] that fluid is considered exudates if any of the following apply:

• Ratio of pleural fluid to serum protein greater than 0.5g/dl
• Ratio of pleural fluid to serum lactate dehydrogenase (LDH) greater than 0.6 U/L
• Pleural fluid LDH greater than two thirds of the upper limits of normal serum value.

In the present study the upper limit was 414U/L, so the LDH value of pleural fluid must be greater than 276 U/L in order to classified as exudate, in the present study all the pleural fluid specimens diagnosed as exudates applying the Light criteria. The protein concentrations in tuberculous pleural effusion patients (TPE) were higher than those of non-TPE patients table 1 and these results agree with the fact that the pleural fluid protein level of TB patients frequently exceeds 5 g/dl and this finding suggests tuberculous pleuritis [14]. The elevation in total protein in TB patients can be interpreted that it is belongs to the hypersensitivity reaction which plays a large role in the pathogenesis of tuberculous pleural effusion which increases the permeability of the pleural capillaries to protein and then the increased protein levels in the pleural fluid result in a much higher rate of pleural fluid formation [15].

The glucose concentrations in TPE patients were lower than those of non-TPE patients table 1and these results agree with the fact that low pleural fluid glucose which is < 60 mg/dl suggests tuberculous pleuritis [16]. The mechanisms responsible for low pleural fluid glucose level in tuberculous pleural effusion appear to be a combination of enhanced glycolysis by pleural fluid cells, bacteria and pleural tissue in conjugation with an impairment to transport of glucose from blood to pleural fluid [15, 17]. The lactate dehydrogenase (LDH) concentrations in TPE patients were higher than those of non-TPE patients table 1and this results agree with the fact that the LDH > two thirds of upper limits of normal for serum LDH (normal value is 202-414 U/L) which found in any condition causing an exudates. Furthermore, the very high levels of pleural fluid LDH (> 1,000 U /L) typically are found in patients with complicated parapneumonic pleural effusion and in about 40 percent of those with tuberculous pleurisy [16]. However, total lactate dehydrogenase activity in the pleural fluid is of little value in the discrimination between various types of exudative effusion [18].

The high activity of LDH can be used as indicators for disturbances of the cellular integrity as tissue breakdown releases LDH induced by pathological conditions such as hemolysis, cancer and so on. The high level is suggestive a great extent of pleural diseases or the presence of blood in the pleural cavity. The adenosine deaminase (ADA) concentrations in TPE patients were higher than those of non-TPE patients and these results agree with the fact that tuberculous pleural effusion patients have level of ADA activity > 40 U/L [15]. The cause of increased ADA activity in tuberculous pleural effusions is still uncertain. One of the suggestions that ADA is primarily concerned with the proliferation and differentiation of T-lymphocytes [21], the pleural effusion ADA activity is thought to reflect the cellularity of activated T-lymphocytes in the pleural compartment. The increased ADA activity might be due to the T-cell population of tuberculous pleural effusions being more immature and reactive than those of other effusions on account of the cellular immune response to mycobacterial antigen. ADA activity usually elevated in tuberculosis, in this situation, the activity of the enzyme in generally higher than 40 U/L, although similar levels are observed in lymphocytic pleural effusions other than tuberculosis. However, the level of ADA below 40 U/L excludes the diagnosis of tuberculosis pleural effusion [22]. The results of ADA analysis agreed with local study results [11] that the measurement of ADA is thus likely to be a useful diagnostic tool for tuberculosis. The results of ADA assays should be interpreted in parallel with clinical findings and the results of conventional tests [23]. The results of protein analysis were lower than the results of [7] but these results were higher than the results of [8, 9] and the results of glucose analysis were lower than the results of [7, 8]. The results of LDH analysis were higher than the results of [7- 9] and the results of ADA analysis were lower than the results of [11] and higher than the results of [7, 10, 20].

The disagreement between present study results with other results may be due to the differences in the geographic distribution, physiological differences between Iraqi patients and other patients, psychological state of the patients and the differences in the local environment [15, 17, 24]. As a conclusion from the present study, that the glucose level (less than 60 mg/dl) of pleural fluid is not

specific of TPE infection because this level also indicates complicated parapneumonic effusion, tuberculosis (20 %), malignancy (< 10%) and rheumatoid arthritis [16]. As well as the protein level > 5 g/dl is not specific for tuberculous pleuritis infection. The combination of the two parameters protein and sugar may be diagnostic because the tuberculous pleuritis patients have protein level > 5 g/dl and glucose level < 60 mg /dl. Also we found that LDH measurement is a sensitive, but rather non-specific inflammatory marker [19], is in general discretely elevated in pleural effusion tuberculosis and in non-tuberculous pleural effusion patients and the pleural fluids specimens of patients with pleural tuberculosis have the highest levels of ADA activity. So, the higher the level, the greater the chance of the patient having TB while the lower the level the lesser the chance of the patient having TB [17]. Furthermore, ADA measurement is an inexpensive, minimally invasive, rapid, and readily accessible test, ADA determination is a relative sensitive and specific test for the diagnosis of tuberculous pleurisy.

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