



ISSN: 0067-2904

Detection of Serum Levels of IL-17 and CCL-5 in a Sample of Iraqi Pulmonary Tuberculosis Patients

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Received: 1/8/2020

Accepted: 23/6/2021

Abstract

Cytokines and chemokines are small-secreted proteins involved in many aspects of cell development, differentiation, and activation functions. A prominent characteristic of these molecules is their effect on the immune system in relation to the development of cell trafficking and immune tissues and organs. Furthermore, they play an important role in initiating and coordinating the organized and sequential recruitment and activation of cells into *Mycobacterium tuberculosis*-infected lungs. We aimed to evaluate the levels of interleukin -17 (IL-17) and the chemotactic chemokine (C-C motif) ligand 5 (CCL5) in the sera of pulmonary tuberculosis (PTB) patients. About 90 subjects were included, involving 50 patients with pulmonary TB and 40 apparently healthy individuals who were selected as a control group. Sera were obtained for measuring IL-17 and CCL-5 levels by enzyme linked immunosorbent assay (ELISA). The results revealed that serum levels of IL-17 showed no significant differences between each patient's group and control. In contrast, the serum level of CCL-5 was significantly increased in pulmonary tuberculosis patients compared to control ($P \leq 0.01$). The mean \pm SE values of IL-17 level in PTB patients and controls were 43.06 ± 3.64 and 41.009 ± 0.009 pg/ml, respectively. While, the mean \pm SE values of CCL-5 level in PTB patients and controls were 455.40 ± 25.35 and 80.86 ± 5.96 ng/L, respectively. The results of the current study suggest that high levels of CCL-5 in the sera of PTB patients may indicate an important role in the immunopathogenesis of the disease. Therefore, this chemokine could be considered as a useful biomarker for the severity of PTB infections.

Keywords: Pulmonary tuberculosis, IL-17, CCL-5, ELISA.

المستويات المصلية لـ IL-17 أو CCL-5 في عينة من مرضى عراقيين مصابين بالسل الرئوي

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الخلاصة

الحركيات الخلوية والحركيات الكيميائية هي بروتينات صغيرة مفرزة تشارك في العديد من جوانب نمو الخلايا والتميز والتنشيط. ومن السمات البارزة لهذه الجزيئات تأثيرها في الجهاز المناعي فيما يتعلق بتنظيم عمل الخلايا وتطور الأنسجة والأعضاء المناعية، علاوة على ذلك فإنها تلعب دوراً مهماً في عملية بدء وتنسيق تسلسل نشاط الخلايا في حال الإصابة بجرثومة السل الرئوي. لتقييم مستويات الانترلوكين 17 والحركي الكيميائي الجاذب 5 في مصل مرضى السل الرئوي؛ اجريت الدراسة التي شملت حوالي 90 شخصاً

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في هذه الدراسة، منهم ٥٠ مريضاً مصاباً بالسل الرئوي و ٤٠ اصحاء ظاهرياً تم اعتمادهم كمجموعة سيطرة. جرى قياس مستويات الانترلوكين-١٧ والحركي الكيميائي الجاذب باستخدام تقنية الامتزاز المناعي المرتبط بالإنظيم. أظهرت النتائج عدم وجود فروق ذو دلالة إحصائية في مستويات الانترلوكين ١٧ بين مجموعتي المرضى والسيطرة، في المقابل، كانت مستويات الحركي الكيميائي الجاذب مرتفعة بشكل ملحوظ وذات دلالة إحصائية في مصول مرضى السل الرئوي مقارنة بمجموعة السيطرة. كان المتوسط \pm الخطأ المعياري لمستوى الانترلوكين ١٧ في مرضى السل والاصحاء $43,06 \pm 3,64$ و $41,009 \pm 0,009$ بيكوغرام / مل، على التوالي. بينما، كان المتوسط \pm الخطأ المعياري لمستوى الحركي الكيميائي الجاذب-٥ في مصول المرضى والاصحاء $445,40 \pm 25,35$ نانوغرام / لتر و $82,19 \pm 5,84$ نانوغرام / لتر، على التوالي. أن المستويات العالية من الحركي الكيميائي الجاذب-٥ في مصول مرضى السل الرئوي التي اظهرتها نتائج الدراسة الحالية قد تشير إلى دوره المهم في الأمراض المناعية لهذا المرض، لذلك يمكن اعتبارها علامة حيوية مفيدة لشدة الإصابة بالسل الرئوي.

Introduction

Tuberculosis (TB) is a chronic granulomatous bacterial disease caused primarily by *Mycobacterium tuberculosis* (Mtb), which is transmitted predominantly by air droplets comprising bacilli that are inhaled by healthy persons. Most commonly, they affect the lungs, but can damage any tissue, including the brain, lymph nodes, intestines, kidneys and bone [1, 2]. Mtb is a facultative intracellular pathogen that predominantly infects macrophages during the early stages of infection [3]. Host immune cells secrete a number of cytokines that are involved in the defense against Mtb infection [4]. These molecules play active roles in initiating and regulating the immune response at different stages of disease development [5]. Conventionally, the interaction between infected macrophages and T lymphocytes is critical for protective immunity against Mtb and is mediated by a number of inflammatory cytokines produced by several cell types [6, 7].

IL-17 is a proinflammatory cytokine that plays protective roles in host defense against extra- and intracellular pathogens, as well as in chronic inflammatory diseases [8-10]. IL-17 plays a crucial role in the causes of TB [11, 12]. IL-17 was suggested to play a protective role during the first step of mycobacterial infections and granuloma formation [13].

CCL5 is a protein which is encoded in humans by the *ccl5* gene and has a comprehensive clinical importance in an array of human diseases, including HIV, atherosclerosis, asthma, cancer, and autoimmune disorders, such as arthritis, glomerulonephritis, and diabetes [14, 15].

CCL5 plays an important role in the tuberculous granuloma formation [16], and has been shown to play a major role in the anti -mycobacterial immune responses by suppressing intracellular growth of Mtb [17]. This study attempted to evaluate the association between serum levels of interleukin-17A and CCL5 and their possible relationship with pulmonary tuberculosis in a sample of Iraqi patients.

Materials and Methods

Study subjects

A total of 90 individuals were included in this study; 50 patients were diagnosed as having active pulmonary tuberculosis by physicians at the specialized chest and respiratory diseases centers in AL-Sadur city and Wassit city during the period from January 2019 to May 2019. The diagnosis was based on clinical signs and symptoms in addition to chest x-ray analysis and confirmed by positive sputum smear for acid-fast bacilli. Inclusive criteria for patients included new incidence with active TB, while those with history of pulmonary TB disease, diabetic mellitus, autoimmune diseases and human immunodeficiency virus (HIV) infection were excluded from this study.

Patients included 20 males and 30 females, with an age range of 13-85 years. In addition, 40 apparently healthy individuals (20 males and 20 females) with an age range of 16-58 years were included as a control group. Those were blood donors to the blood bank as well as medical laboratory staff from Al-Zahra Teaching Hospital in Wassit city. The criteria of sampling were the lacking of lung lesions on X-ray of chest with no history of TB disease. The study protocol was approved by the ethics committee at the department of Biology/ College of Science/ University of Baghdad, No. BEC/1218/0028.

IL-17 and CCL-5 detection by ELISA procedure

Three ml of whole blood was dispensed in gel tubes and serum was separated by centrifugation at

3000 r.p.m for 10 mins. The yielded serum was divided into several Eppendorf tubes placed in a cool-box under aseptic conditions and stored in the freezer at -20°C till using in further immunological test. ELISA kits ((Mybiosource/ USA) were utilized to detect IL-17A or RANTES/CCL5) based on instructions provided by the manufacturer. Adherent cell supernatants or standards were added to anti-cytokine monoclonal antibody-coated microtitre plates in duplicate. Then, enzyme-conjugated anti-cytokine or anti-chemokine monoclonal antibodies directed against a second epitope of the cytokine or chemokine molecule was added. After incubation and washing to remove unbound antibody, substrate was added then absorbance values at 450 nm were read. Cytokine or chemokine standard curves were prepared and used to quantify unknown cytokine or chemokine levels in experimental samples.

Statistical analysis

All statistical analyses were performed using statistical analysis system (SAS) program version 9.1 for windows (SAS. Inst. Inc., Cary. N.C., USA). The mean and standard error values were calculated for the parametric data. T-test was used to analyse the results and make comparisons between the two groups [18].

Results

The patients were divided into two age groups (< 40 years and ≥ 40 years). The results revealed that the disease was more frequent in < 40 group (54%) than the ≥ 40 group (46%). In addition, tuberculosis disease was more frequent in females (58%) than males (42%) in the patient groups (Table- 1).

Table 1-Distribution of the study groups according to the age and gender.

Percentage	Patients group		Control group	
Age	< 40 years	≥ 40 years	< 40 years	≥ 40 years
	54%	46%	76.32%	23.68%
Sex	Male	Female	Male	Female
	42%	58%	50%	50%

Serum levels of IL-17 and CCL-5

The results of IL-17A serum level showed an insignificant variation ($p > 0.05$) between PTB patients and controls (43.06 ± 3.64 and 41.009 ± 0.009 pg/ml, respectively), as shown in Figure -1. In contrast, there was a significantly ($p \leq 0.01$) increased CCL-5 serum level in PTB patients (455.40 ± 25.35 ng/L) compared to control (82.19 ± 5.84 ng/L), as shown in Figure -2.

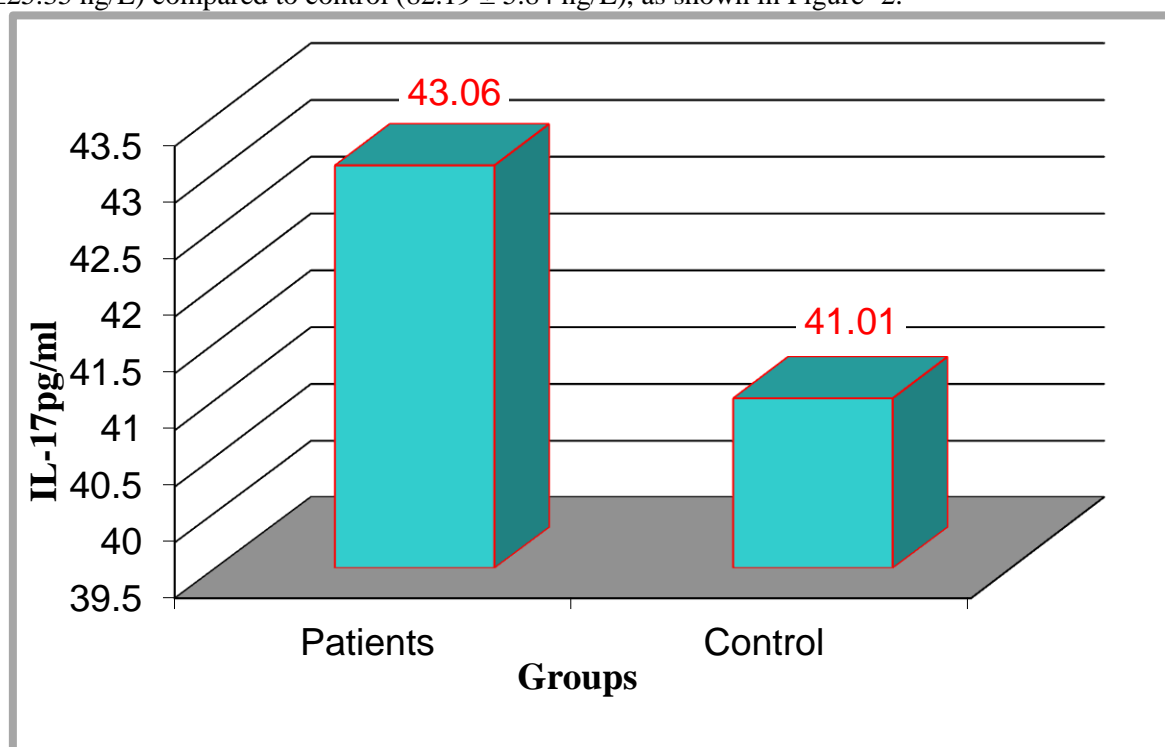


Figure 1-Serum levels of IL-17A in pulmonary tuberculosis patients and controls

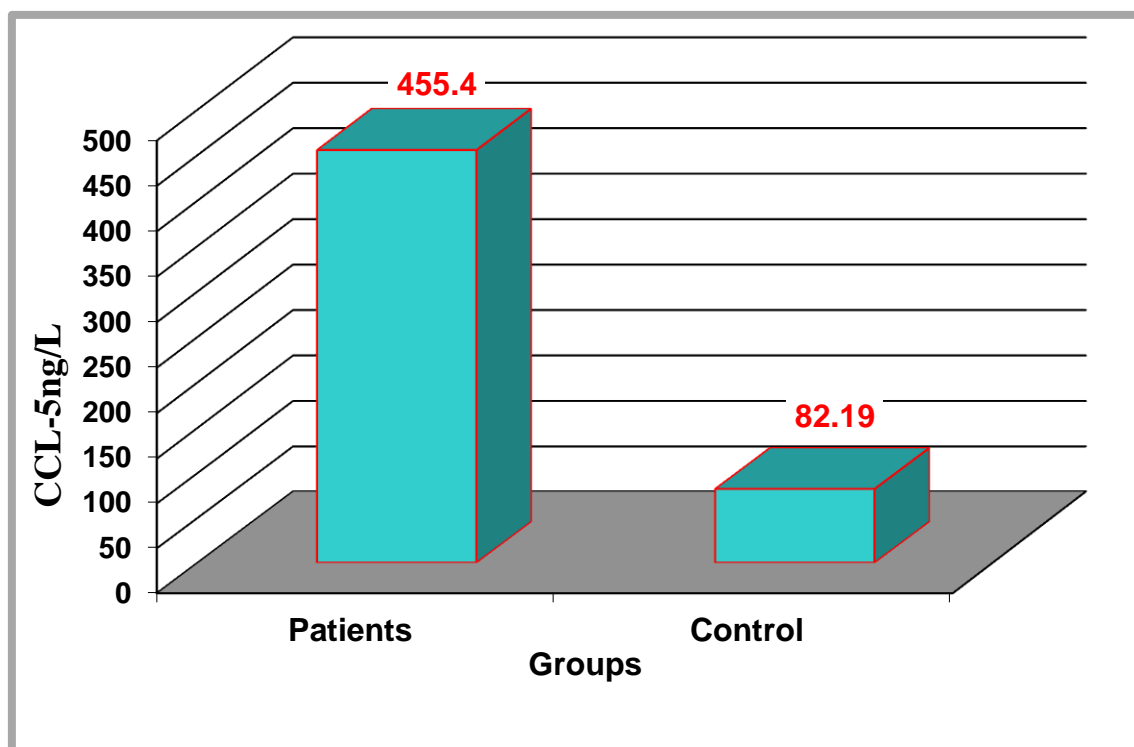


Figure -2: Serum levels of CCL-5 in pulmonary tuberculosis patients and controls.

Discussion

Infection with mycobacteria promotes the secretion of a number of cytokine and chemokine signals by host immune cells, which play active roles in host defense against Mtb infection [19, 20]. IL-17 A is a potent pro-inflammatory cytokine that is mainly produced by Th17 lymphocytes. It is capable of inducing chemokine expression, neutrophils migration, and Th1 cells recruitment and trafficking to parenchymal tissues during TB infection. Along with the tumor necrosis factor (TNF), IL-17 A plays an important role in the first steps of TB and granuloma formation [21, 22].

There are many cytokines that induce the production of IL-17 from naive CD4+ T cells, such as IL-23 and IL-6. However, TGF- β and IL-10 are anti-inflammatory cytokines produced by regulatory T cells and can reduce the expression of IL-17 and IFN- γ , especially in Mtb infection response [23].

Some studies suggested that patients with active tuberculosis exhibit high level of IL-17 production [24, 25]. In addition, Oejo-vingals *et al.* [26] reported a significant difference in serum level of IL-17A in PTB patients as compared to controls.

Other studies identified increased IL-17 release in latently TB infected (LTBI) individuals [27] and reduced IL-17 release in patients with active TB [28].

Alimari *et al.* [29] found that high serum level of IL-17 may serve as a protective factor in healthy subjects, enabling them to overcome PTB infection, when compared with the significantly low IL-17 serum level in PTB. During active tuberculosis, Th17 cells are decreased in number but, however, the association with inhibitory immune regulation is unclear [28]. This may explain the low serum level of IL-17A in this study.

It has also been documented that there was an insignificant difference in serum level of IL-17A between latent and active TB subjects and healthy control [30-32], which agrees with the results of this study.

Recently, evidence has been presented pointing to the role of IL-17 in the optimum induction of immune responses of the Th1-type and Th2-type, although the mechanism has not yet been explained [33].

We speculate that lower IL-17 serum levels can reflect a weaker Th1 immune response to mycobacterial infections, as IL-17 is an important cytokine in inducing the optimal protective Th1 response against MTB infection, via preserving granuloma integrity by limiting the death of neutrophil [34, 35].

As a major chemokine, CCL5, also known as Regulated on Activation, Normal T-cell Expressed and Secreted (RANTES), plays a major role in co-stimulation of T cell proliferation and activation in anti-mycobacterial immunity [36].

Moreover, CCL5 has a protective role in MTB infection by forming granuloma, limiting pathogen growth, and preventing lung tissue damage [37]. CCL5 (β -chemokine) is not only a T-cell and macrophage chemo-attractant that activates and extends T cell populations [36], but also a macrophage coactivator, thus inducing a Th1 response [38]. This study is in agreement with that of Pokkali and Das [39], who documented that the plasma level of CCL5 in PTB patients was significantly increased in comparison to healthy subjects.

In contrast, this study is not in agreement with that of Almeida *et al.* [40], who found that plasma level of CCL-5 was not significantly different between patients with active pulmonary TB and BCG-vaccinated healthy controls.

Elevated Th1 response could promote cell-mediated immunity to fight against intracellular pathogens [41]. The concentration of CCL5 increased in patients with PTB and decreased during recovery time, which suggests that it plays a key role in the immune response to TB infection. CCL5 works at an appropriate concentration, and the production of CCL5 above or below this concentration means a dysfunction in this chemokine [42]. The observed discrepancies in the serum levels of IL-17 and CCL-5 in TB patients and control group between the present study and previous studies may be due to differences in several factors. These include differences in ethnicity and clinical form of TB disease, analysis of cells or serum samples, stimulation antigen, the method of analysis used, and/or the underlying host immunity. These observations should be addressed in future investigation.

Conclusions

Significantly up-regulated CCL-5 level in the sera of PTB patients reflect its crucial role in early immunopathogenesis of PTB, which may support its consideration as a useful biomarker for the severity of active tuberculosis.

Acknowledgements

The acknowledgment is presented to all the staff of the primary health care center/AL-Sadur city sector, the consultant clinicians of the chest and respiratory diseases at AL-Rusafa health directorate, and the chest and respiratory disease specialized physicians at Wassit- center, for their support in blood sampling.

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