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Original Research Article

The Impact of Tuberculosis on Immunomodulatory Markers: A Clinical Study

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Abstract

This study aims to investigate the levels of immunomodulatory markers like interluekin-6 (IL-6), tumor necrotizing factor-α (TNF-α), cell differentiation-4 (CD4) and CD8 levels in those patients with active tuberculosis (TB) disease in comparison with control group.

41 Adults diagnosed with TB were included in comparison to 32 healthy individuals at Babylon health center for pulmonary diseases and TB. Descriptive data for patients and control group were collected by well-trained researcher following a structured questionnaire. In parallel, peripheral blood collected to determineIL-6, TNF-α, CD4 and CD8. Then the assessment for the association between clinical and descriptive data and immunomodulatory markers levels was investigated statistically.

The majority of TB patients were males (56%) and 71% were resident in rural areas;47% of them were living in middle socioeconomic state, moreover, 47% of TB cases had diabetes, furthermore, 51% had chronic obstructive pulmonary diseases, 12% had hypertension and 39% of them had chronic anemia with 47% smokers with no significant difference versus control. Following to that, there was highly increased in IL-6 and TNF-α levels in TB patients versus control (P<0.001), with low CD4 level versus control (P<0.001). While there was no significant change shown in CD8 levels versus control and this might highly be correlated with 30% of abnormal liver function tests among TB patients.

A high proportion of TB patients have low CD4 level mostly associated with active disease. Moreover, the increase of IL-6 and TNF-α levels suggests a inverse impact on CD4 level which closely associated with the outcome of the disease.

Key Words: Tuberculosis, Immunomodulatory markers, IL-6, TNF-α, CD4, CD8.

الخلاصة

هو التحقق من مستويات العلامات المناعة مثل إنترلوكين -٦(IL-6) ، عامل نخر الورم-(TNF- α)α، التمايز الخلوي -٤ (CD4) والتمايز الخلوي-٨(CD8) في المرضى الذين يعانون من السل النشط بالمقارنة مع مجموعة السيطرة.

تم تضمين ٤١ شخصا مصابين بالسل بالمقارنة مع ٣٢ شخصا أصحاء في مركز صحة بابل للأمراض الرئوية والسل. تم جمع البيانات الوصفية للمرضى ومجموعة السيطرة من قبل باحث مدرب بعد استبيان منظم. في موازاة ذلك، تم جمع الدم المحيطي لتحديد مستويات٦-IL، α -TNF، CD4 و CD8. ثم دراسة تقييم الارتباط بين الخصائص السريرية ومستويات علامات المناعية عند خط الأساس باستخدام تحليل الانحدار.

كان معظم المرضى من الذكور (٥٦٪) و ٧١٪ كانوا مقيمين في المناطق الريفية. ٤٧٪ منهم كانوا يعيشون في حالة اجتماعية واقتصادية متوسطة، وعلاوة على ذلك، ٤٧٪ من حالات السل لديهم مرض السكري، و٥١٪ لديهم أمراض انسداد رئوي مزمن، و ١٢٪ يعانون من ارتفاع ضغط الدم و ٣٩٪ منهم يعانون من فقر الدم المزمن اما نسبة للمدخنين فكانت ٤٧% بدون تأثير هام. كان هناك زيادة كبيرة في مستوى TNF-αو6-IL في مرضى السل مقابل مجموعة السيطرة (P <0.001)، مع انخفاض مستوى CD4 مقابل السيطرة (P <0.001). في حين لم يكن هناك تغييرات كبيرة في مستوىCD8 مقابل السيطرة وهذه قد ترتبط بشكل كبير مع ارتفاع ٣٠٪ من المرضى لاختبارات وظائف الكبد.

نسبة عالية من مرضى السل لديهم مستوى منخفض من CD4 مرتبط بالمرض الإيجابي. تشير زيادة مستوى6-IL وTNF-α إلى تأثير قابل للانعكاس من السل النشط على مستوى CD4 والتي ترتبط ارتباطا وثيقا مع نتائج المرض النهاية.

الكلمات المفتاحية : السل، العلامات المناعية، انترلوكين -٦، عامل نخر الورم -α، التمايز الخلوي ٤، التمايز الخلوي ٨.

Introduction

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (MTB) [1]. TB has affected the lungs, but can also affect other organs and tissues in the body. It has a latent infection that means asymptomatic which represent one third of the world's population [2]. The symptoms of active pulmonary TB are a chronic cough with bloody sputum, fever, night sweats, and weight loss [3].

MTB is a high lipid containing pathogen with an outer membrane lipid bilayer [4] and mycolic acid containing cell wall [4], therefore, MTB invade and replicate within pulmonary alveolar macrophages [5,6]. Macrophages, T and B-cells and fibroblasts attempt to eliminate MTB, limit the spreading of them and provide a localization of infection under the action of the immune system [7]. The macrophages and dendritic cells are failing to present antigen to lymphocytes; thus the immune response is suppressed [8]. Therefore, an effective host immune system has crucial role in the eradication of MTB infection even if latent TB infection [9-12]. Inadequate host immunity leads to multiplication of MTB and clinical active disease. Equilibrium between the host and MTB factors can lead to excess, but ineffective host inflammatory responses with tissue destruction. Cytokine like interleukin 6 (IL-6) and tumour necrosis factor-α (TNF-α) may play a role in the clinical manifestations and pathological events of TB infection [13] and both of them have close relation with human cell-mediated immune response against MTB [14]. IL-6 inhibits the production of TNF-α that may accelerate the intracellular killing of MTB and development of granulomata. The role of pro-inflammatory and anti-inflammatory markers with diminishing TNF-α levels [15] is crucial in tailoring TB. MTB-specific antibodies; against MTB antigens increase the protective responses stimulated by T-helper-1 cells (Th1); thus, IL-6 is not as essential as TNF-α for anti-MTB effector mechanisms [16] but it is essential for generating protective Th1 immune responses after vaccination with a subunit vaccine against MTB [17] and has a role as a components of the macrophage-signaling machinery that regulate intracellular survival of MTB [18]. CD4 cells depletion is commonly associated with immunosuppressive status that can cause by opportunistic infection despite of a broad reference range of CD4 level [19]. Low CD4 cell levels are also associated with positive smear of sputum in TB patients with signs of wasting. CD4 cell level and cytokine levels are independent prognostic factors for the severity of immunosuppressive state [20].The increasing of CD8+ cells level can enhance the innate and adaptive immune responses against MTB infection which highly associated with latent TB cases with cytolytic activity against infected cells [21,22].

Regarding the hepatotoxicity induced by anti-TB drugs, the formation of acetyl isoniazid in large amount as rapid acetylators might be associated with the development drug-induced liver toxicity [23]. The of hepatotoxicity induced by drugs is increased also by factors other than anti-TB drugs like old age group, malnutrition, late stage TB disease, alcoholism, chronic viral hepatitis B and C infections and finally infection with HIV [24]. Furthermore, other studies have been shown that the female gender is with high risk for developing drug induced hepatotoxicity during treatment of TB. This hepatotoxicity during the course of MTB infection could have a great effect on the immune responses against MTB infection and leading to worse the disease with dysregulation of cytokines and other modulatory markers [25]. From all above, the aim of this study was to investigate the levels of immunomodulatory markers like IL-6), TNF-α, CD4 and CD8 levels in those patients with active TB disease with comparison to control group.

Materials and Methods

Patients and Samples: This study was carried out at the Babylon general health

center for pulmonary diseases and TB, Babylon, Hilla, Iraq. The study was included 41 patients with TB having smear positive, in addition to 32 control cases.

Inclusion criteria: Eligible patients should not document withHIV, hepatitis B or C virus infections, all TB patients were received anti-TB drugs with duration varied from a two months to one year.

Ethics statement: Both groups provided written informed consent prior to inclusion into the study. No obligations or interventions put to interfere with standard care of patients. The study was approved by the National Ethics Review Committee at University of Babylon which in accordance to the Declaration of Helsinki.

Demographic and clinical variables

Descriptive data for patients and control were collected by trained researcher following a structured questionnaire. Age, gender, residency, economic and social states and body mass index (BMI) were added as questionnaire to all subjects (patients and control). Moreover, the searching for history of some chronic diseases like diabetes mellitus (DM), hypertension, respiratory diseases and anemia was done in addition to history of smoking.

Liver enzymes: The performing a series of tests as support of patients' health state like baseline liver function tests (aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphate (ALP) and gamma glutamate transferase (γ-GT)) by Biochemistry autoanalyzer-COBAS to measure the hepatotoxicity, in addition to bleeding tendency test (INH).

Immunological Markers:

IL-6, TNF-α, CD4 and CD8 levels

Inflammatory cytokines and cell differentiation indices were measured by ELISA technique (elabscience company, USA). IL-6 is by E-EL-H0192 kit, TNF-α by E-EL-H2305 kit, CD4 by E-EL-H0752kit and CD8 by E-EL-H2359 kit. According to the manufacturer's protocol; each assay was run with known standards (provided with the kit) that were used to determine the quantity of cytokines in each sample in pg/ml and cell differentiation in ng/ml.

Statistical Analysis

Descriptive measures and T-test analysis were used to examine associations and differences in Demographic and clinical variables, biochemical, and immune-modulatory markers. The significance level for all analyses was set at a probability (*P*) of less than or equal to 0.05. All analyses were performed by GraphPad Prism 5.3 for Windows (GraphPad Software, San Diego, CA, USA).

Results

Demographic and clinical variables

The demographic characteristics of all patients included in the study are summarized in Table 1. The age range was from 17 to 87 years with mean for cases and controls were 38.3 and 32.4 years, respectively. The majority of the patients were males (56%) and 71% among all study sample were resident in rural areas while only 29% of them were in urban areas ($P<0.01$). Regarding the socio-economic state, 47% of them were living in middle socioeconomic state, 37% were in low socioeconomic state and only 16% were living in good state. Most of study sample are married (78%).

The majority of cases have TB disease for more than one year (61%), 32% of them have disease between 6-12 months and only 7% have disease for less than 6 months (data not shown). Regarding the body weight, about 90% of study sample have body mass index (BMI) lower than 25 (relatively low BMI) (Table 1).

Table 1: Demographic variables of tuberculosis (TB) patients and control group.

Variables		**TB patients (n=41, %)**	**Control (n=32, %)**	***P value***
Age (yrs)	20-40	22 (54%)	15 (47%)	-
	40-60	13 (32%)	10 (31%)	-
	>60	6 (14%)	7 (22%)	-
Sex	Male	24 (59%)	17 (53%)	-
	Female	17 (41%)	15 (47%)	-
Residence	Urban	7 (17%)	14 (44%)	*P*<0.01
	Rural	34 (83%)	18 (56%)	-
Economic State	Good	2 (5%)	10 (31%)	*P*<0.01
	Middle	21(51%)	13 (41%)	-
	Poor	18 (44%)	9 (28%)	-
Social State	Married	36 (88%)	21 (66%)	-
	Non-Married	5 (12%)	11(34%)	*P*<0.05
BMI	16-20	12(29%)	11(33%)	-
	21-25	24(59%)	12(39%)	-
	26-35	5(12%)	9(28%)	*P*<0.05

BMI: body mass index

Moreover, there were 47% of TB cases had diabetes and it was statistically significance versus control (P<0.001) (32% had NIDDM and 15% had IDDM), furthermore, 51% had chronic obstructive pulmonary diseases (COPD) and 12% had hypertension (P<0.001 versus control for each). In addition to the history taking from patients, 39% of them had chronic anemia with statistically significance versus control (P<0.001) and 46% of them were smokers without significant difference versus control (Table 2).

Table 2: Clinical variables (associated chronic diseases & risk factors) in TB patients and control

Disease	TB patients (n=41, %)	Control (n=32, %)	*P value*
IDDM	6 (15%)	0 (0%)	P<0.001
NIDDM	13 (32%)	4 (13%)	P<0.001
Hypertension	5 (12%)	7 (22%)	-
COPD	21 (51%)	3 (9%)	P<0.001
Anemia	16 (39%)	0 (0%)	P<0.001
Smoking	19 (46%)	11 (34%)	-

IDDM: insulin dependent diabetes mellitus, NIDDM: non- insulin dependent diabetes mellitus, COPD: chronic obstructive pulmonary diseases

Liver function tests

The prevalence of bleeding tendency among TB patients in this study was 29% with statistically significant difference versus control (P<0.001). Moreover, more than 30% of TB cases had abnormal liver function tests and most of them after 6 months of therapy (↑ AST and ALT (P<0.05), ↑ ALP (P<0.01), and ↑ γ-GT (P<0.01) versus control) (Table 3).

Table 3: The assessment of liver function of tuberculosis (TB) patients and control group.

Disease		TB patients (n=41, %)	Control (n=32, %)	*P value*
INR > 2.0		12 (29%)	0 (0%)	P<0.001
Abnormal LFT	↑ AST&ALT	6 (15%)	3 (9%)	P<0.05
	↑ ALP	5 (12%)	0 (0%)	P<0.01
	↑ γ-GT	9 (22%)	3 (9%)	P<0.01

INR: International normalized ratio; LFT: Liver function test; AST: Aspartate transaminase; ALT: Alanine transaminase; ALP: Alkaline phosphatase; γ-GT: Gamma glutamine transferase.

Immunomodulatory Markers

There was highly increased in IL-6 and TNF-α levels in TB patient in comparison with control (P<0.001) (Figure 1 and Figure 2 respectively), moreover, CD4 level was significantly decreased versus control (P<0.001) (Figure 3), while there were no significant difference shown in the level of CD8 in comparison to control (Figure 4).

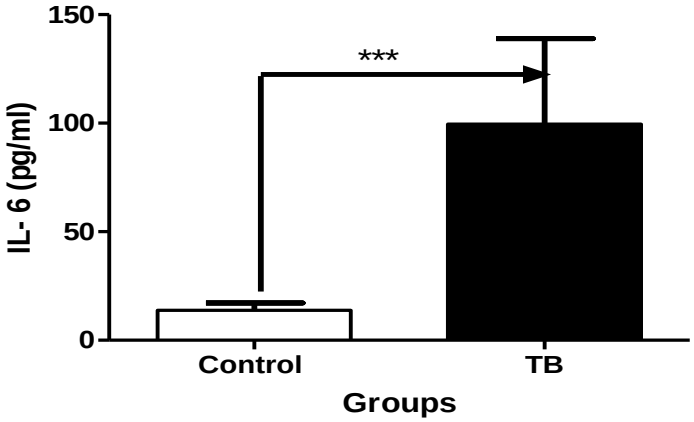

Figure 1: Variation in IL-6 concentration (pg/ml) among patients with tuberculosis (TB) and control groups. The number of asterisks (***) correspond to the level of the statistical significance (P < 0.001).

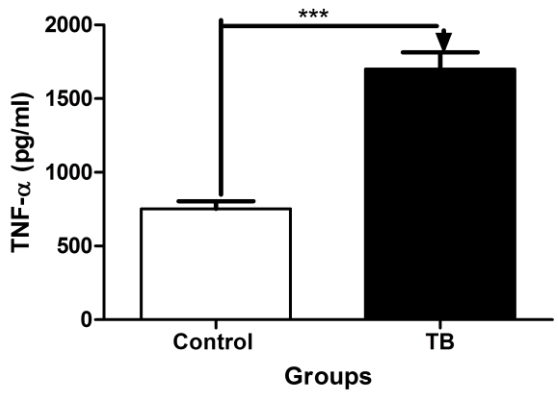

Figure 2: Variation in TNF-α concentration (pg/ml) among patients with tuberculosis (TB) and control groups. The number of asterisks (***) correspond to the level of the statistical significance ($P < 0.001$).

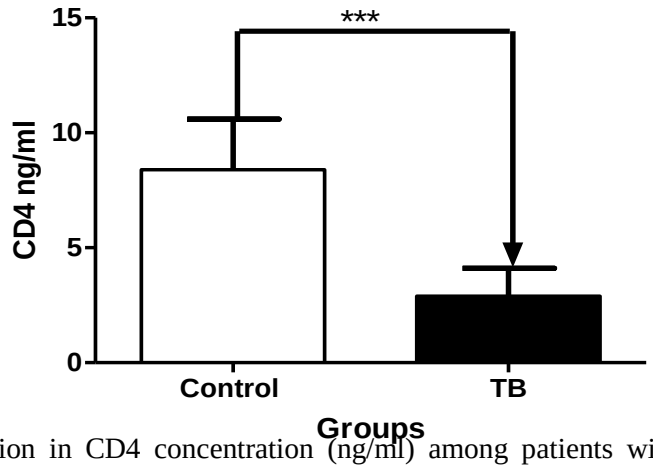

Figure 3: Variation in CD4 concentration (ng/ml) among patients with tuberculosis (TB) and control groups. The number of asterisks (***) correspond to the level of the statistical significance ($P < 0.001$).

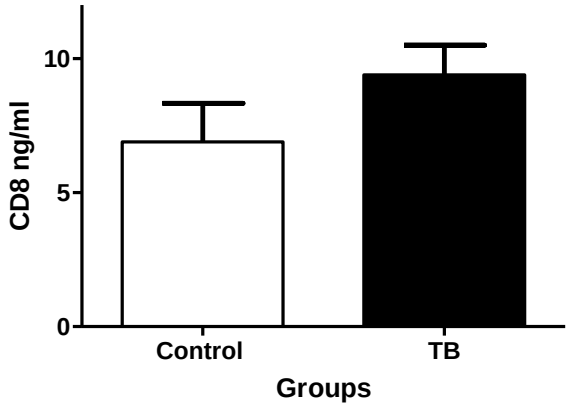

Figure 4: Variation in CD8 concentration (ng/ml) among patients with tuberculosis (TB) and control groups.

Discussion

Our experiments reveal a striking role of IL-6 in TB that MTB is an intracellular bacterium which survives in resting macrophages [26]. The macrophage is activated by cytokines to eradicate MTB [27]. Accordingly, even in immunocompetent individuals, MTB persists to grow and disease is developed after reactivation as a consequence of impaired immunity [28]. The previous studies suggested that different T-cell populations are required for protection against MTB. This includes CD4 and CD8 T-lymphocytes [29]. Th2 immune response has crucial role in progression of TB infection while Th1 cells play a minor role in protection against tuberculosis [30]. Further, a role of TNF-α in protection against tuberculosis has been demonstrated with cytokines as activated by macrophages that increase the resistance against TB [31]. The essential role of IL-6 in a protection against MTB as early immune response is to induce the inhibition of intracellular MTB growth with other cytokines like IL-1 and TNF-α, which are represented as potent pro-inflammatory cytokines [32]. In another hand, IL-6 has been shown to promote differentiation of T cells especially to Th2 responses that stimulate cytotoxic functions with impaired Th1 cell development [33]. Similarly, the deficiency in CD8 T cells, and/ or TNF-α receptor are more obvious in latent MTB infection [34, 35]; the low CD4 levels in TB patients have been reported that closely associated with clinical severity and more advanced of disease and the continuous increase of CD4 level during treatment for TB is strongly associated with the improvement [36]. The CD4 level depletion is also related with wasting and weight loss with high proportion of BMI of TB patients and CD4 level that CD4 level might also correlate with the degree of malnutrition leading to severe immune suppression state with peripheral blood lymphocytopenia [37].

The depletion in CD4 level and lymphocytes in peripheral blood could be due to pooling of T-cells to the site of infection, or due to hypermetabolism and malnutrition secondary to TB infection [38,39].

In conclusion, our study has allowed correlating between the clinical variables with IL-6, TNF-α, CD4 and CD8 levels with some limitations in TB diagnosis that was based only on sputum smear positive and we could not exclude the existence of other factors that might have an impact on the immunity state. Moreover, the study might help to define the cytokines are critical marker to protect against TB and facilitate the development of new immunologic means for prevention and therapy of TB.

Conflict of Interest

The author declares that the article was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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