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Estimation of some Immunological Factors in Pulmonary Tuberculosis Patients

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Abstract

This study aimed to assess the status of cell mediated immunity for pulmonary tuberculosis patients through evaluation levels of Interleukins (IFN-γ ,IL-10, IL-12) and estimation vitamin D3 . Whole blood samples collected from 50 pulmonary tuberculosis patients who are admitted to consultant clinic for respiratory diseases in Hilla – Babylon province during the period from February 2016 to February 2017. Out of the pTB patients, there were 27 males and 23 females, the patients age range was between 12-80 years. Cytokines levels and vitamin D3 estimated by using ELISA technique. The mean of serum concentration of IL-10 and IL-12 revealed that there is a significantly increased ($p< 0.05$) in pulmonary tuberculosis patients as compared to controls (4.024 and 1.049)pg/ml (51.563 and 13.514) pg/ml ,respectively. While, the mean of serum concentration of IFN-γ showed no significant increasing ($p>0.05$) in pulmonary tuberculosis patients compared to controls (36.505 and 25.019)pg/ml. The mean of serum concentration of VD3 showed a significant decreasing ($p< 0.05$) in pulmonary tuberculosis patients as compared to controls (18.186 and 29.321) ng/ml.

Conclusions:

The results provide a good correlation between the levels of IL-10 , IL-12, IFN-γ and Vitamin D3 with the cellular immunity against pulmonary tuberculosis.

Key words: Tuberculosis, IL-10, IL-12, IFN-γ, vitamin D3, ELISA Test.

Introduction

Tuberculosis (TB) is a poverty disease that thrives where economic and social determinants of ill health prevail, as well as that mostly affects in young adults in their most productive years living in the developing world [1]. About one-third of the world's population estimated to have been exposed to bacteria of TB and infected potentially [2]. Of those infected, only a little proportion will become sick with TB [3] nevertheless people living with HIV, people with weakened immune systems caused by the prolonged use of medicines such as TNF-α inhibitors or steroids , and diabetes patients, silicosis, and renal insufficiency have a much greater risk of falling ill from TB [4]. Infection with Mycobacterium tuberculosis results in a variety of conditions which ranging from

asymptomatic infection to active tuberculosis with pulmonary or extrapulmonary involvement. In most cases, MT infection has no been fatal. A variety of clinical features of TB results from cell-cell interactions that promoted by cytokines produced by immune cells during response to MT infection [5]. On the way to prevent tissue damage, active tuberculosis is related to decreased Th1 and increased production and action of suppressing cytokines generated by Th2 cells, which act by deactivating macrophages, modulating proinflammatory cytokines, and reducing the function of T cells for antigen presenting [6]. IL-12 is a vital cytokine in the host defense against mycobacterial infections. Additionally, IL-12 favors development of T helper 1 responses by enhancing IFN-γ [7]. IFN-γ is the major cytokine of Th-1 cells, therefore, as proinflammatory immune response, it protects against MTB. IFN-γ activates macrophages for kill intracellular mycobacteria [8]. IL-10 inhibits production of IFN-γ and antigen specific T helper 1 proliferation responses, up-regulates T helper 2 responses and prevents activation of macrophage [7]. Deficiency of vitamin D has implicated as a risk factor for tuberculosis [9]. The important role for vitamin D in modulation of the innate immune via its role is an immunomodulator [10]. 1,25-dihydroxy vitamin D3[1,25(OH)2D3]is the active metabolite form of vitamin D, which modulate immune function mediated macrophages, monocytes, dendritic cells, T and B cells. A recent study demonstrated that vitamin D supplementation restored the impaired immune response with better clinical outcome in TB patients, which reveals that sufficient levels of vitamin D has an important role to control the intracellular infection such as tuberculosis [11].

Materials

Population of the study-included patients with pulmonary tuberculosis (pTB) admitted to consultant clinic for respiratory diseases in Hilla – Babylon province during the period from February 2016 to February 2017. Out of the pTB patients, there were 27 males and 23 females, the patients age range was 12-80 years. Exclusion Criteria: Any patients with DM, malignancy, allergic and pregnant women excluded from this study. In addition to fifty apparently healthy control groups who had no history of pulmonary Tuberculosis.

Collection of Samples

The serum separated for serological studies; 3 ml of the blood sample allowed to clot for about 15 min. at room temperature, then loosed the clot gently from a wall of the tube by means of a wooden stick. After that, the sample centrifuged for 10 min at about 2500 rpm and finally the serum transferred to another tube for storage at - 20°C.

Methods

ELISA (Enzyme linked immunosorbent assay) Test

Measurement of IL-10, IL-12, IFN-γ and vitamin D3. These parameters estimated by ELISA technique according to the instruction of manufacture company (Elabscince-China).

Statistical Analysis: The statistical package for the Social sciences version 18 (SPSS Inc., Chicago, USA) used for statistical analysis. This study used statistical analysis that included calculation of mean values and percentage. The data were analyzed with a chi-square and t-student tests, and the level of significance was set at $p<0.05$ [12].

Results and Discussions

1- Estimation of IL-10 in Serum of Studied Groups

The mean of IL-10 concentration in pulmonary TB (pTB) patients sera (4.024) pg/ml, while in control groups (1.049) pg/ml. The study significantly shows the difference between pTB and controls, ($p<0.05$), Table (1). The study shows IL-10 was significantly higher in pulmonary TB than in controls.

This finding was matched approximately with [13] who mentioned that active pTB patients had higher levels of anti-inflammatory cytokines IL-10 ($P < 0.001$) Compared to healthy subjects. This finding also matched with [14] who found that the levels of IL-10 in pulmonary tuberculosis group were significantly higher than the control group.

Table (1) IL-10 Concentration in serum of studying groups

Study groups	Mean (pg/ml)+ SD	Range	P value
Patients	4.024±1.459	1.28-7.07	0.0002*
Controls	1.049±0.56	0.01-2.37	
***= Significant (P≤ 0.05)**			

[15] who found that significantly raised serum levels of IL-10 in patients with active pTB. The blocking action of IL-10 in vivo during chronic infection stabilized the pulmonary bacterial load and improved the survival. Furthermore, this beneficial outcome was extremely associated with the recruitment of T cells to the lungs and was enhanced T cell IFN-γ production. The results indicated that IL-10 promotes progression of Tuberculosis disease. These findings have important diagnostic and/or the therapeutic implications for prevention of reactivation Tuberculosis in humans [16]. The blocking of IL-10 in *Mycobacterium tuberculosis* (Mtb) infected macrophages, allows phagosome maturation, but the addition of IL-10 to cells infected with killed Mtb successfully inhibits its maturation, resulting in enhanced survival of Mtb via inhibiting phagosome maturation by IL-10 [17]. It predicted that production of low level of IL-10 by activated macrophages was required for an efficient antimicrobial response, control of bacterial growth and for prevent damage of the lung. Consequently, modulation of IL-10 levels through TB therapy might shorten duration of the treatment and accelerate the bacterial clearance [18]. Other studies found that IL-10 has shown to be elevated in the lungs and serum of active PTB patients. The production of IL-10 following Mtb phagocytosis by macrophages may happen as a natural antimicrobial response via the host, otherwise may be induced by the bacteria as mechanism of evasion . IL-10 block maturation of the phagosome, which facilitates survival and outgrowth of Mtb [19]. In human tuberculosis, IL-10 production is higher in anergic patients, suggesting that *M. tuberculosis* induces IL-10 production, suppressing an effective immune response. In populations of some human, an increase in IL-10 expression identified, being possible to

correlate it with an inefficiency in vaccination with BCG (Bacillus Calmette-Guérin). The analysis of IL-10 gene polymorphisms involved in the development of infectious diseases suggests that this polymorphism has a critical role in the immunity and progression of inflammation. The increase production of IL-10 can, in particular, suppress the immune response and promote the disease progression [20]. A study in Gambia investigated that NRAMP1 influenced TB susceptibility by regulation of IL-10. Higher levels of IL-10 could lead to TB development through two different mechanisms:(a) IL-10 control TB infection through up-regulation of macrophage and production compounds of microbicidal. (b) IL-10 regulates development of secondary immune response in tuberculosis. Depended on the reported results, manipulation of the IL-10 pathway may be a novel immunotherapy for treatment of tuberculosis in the future [21].

2- Estimation of IL-12 in Serum of Studied Groups

The mean of IL-12 concentration in pulmonary TB patients, sera was (51.563)pg/ml, while in control groups was (13.514) pg/ml. The study significantly shows the difference between pTB and controls, ($p< 0.05$); Table (2). The study shows IL-12 was significantly higher in pulmonary TB than in control groups. The result matched approximately with [22] who showed that the samples from patients infected with Mycobacterium tuberculosis was significantly higher levels of IL-12, ($p< 0.05$).

Table (2) IL-12 Concentration in Serum of Patients and Controls

Study groups	Mean (pg/ml) ± SD	Range	P value
Patients	51.563±20.485	12.36- 436.64	0.007*
Controls	13.514±5.051	2.87- 43.74	
***= Significant (P≤ 0.05)**			

Another study found significantly increased level of circulating IL-12 in extensive disease, (241.6±104.6 pg/ml); when compared to minimal (80.7± 21.4 pg/ml) according to the radiographic extent of lung lesions in pTB patients, but found in active pTB patients, serum IL-12 concentrations were lower than in healthy controls [23]. [24] found that serum concentration of IL-12p-70 in active TB patients and MDR-TB elevated than in patients after anti-TB treatment, and mentioned that measuring levels of several cytokines in the serum may useful for evaluating the activity of TB disease and monitoring the clinical effects of anti-tubercular treatment. During Mycobacterium tuberculosis infection, IL-12p70 level was elevated due to activation of macrophages upon a successful interaction of the toll-like receptors (TLRs) present in the phagosome and cell surfaces with the Mtb-associated antigens[25]. While the study of [26] was found that the average level of serum IL-12 in 80 pulmonary TB patients was significantly lower than in the healthy control group. Studying of [27] showed that supplying IL-12 and neutralizing IL-27 enhanced acidification and fusion of mycobacterial-containing phagosomes with lysosomes. In addition, cathepsin D was associated with the bacteria and matured to the active form when IL-12 supplied and IL-

27 neutralized. Lysosomal acidification and cathepsin D activity were associated with control of mycobacteria. Lysosomal acidification, association with mycobacteria, and maturation of cathepsin D required IFN-γ production from macrophage. IL-12 is essential for the generation of a protective immune response to *M. tuberculosis*, with its main functions being induction of expression of IFN-γ and activation of antigen-specific lymphocytes capable of creating a protective granuloma. Patients with defective receptors for IL-12 were highly susceptible to severe mycobacterial infections.

3-Estimation of IFN-γ in Serum of Studied Groups

The mean level of interferon gamma (IFN-γ) concentration in the serum was (36.505) pg/ml for pulmonary TB patients and (25.019) pg/ml in health groups.Table (3) shows interferon gamma IFN-γ concentration no significant difference between pTB patients and controls, ($p > 0.05$). The study found no significant raised serum levels of IFN-γ in patients with pulmonary TB. This finding matched approximately with previous studies [28] who found that the levels of IFN-γ in pulmonary tuberculosis group were no significantly higher than control group (0.123 IU/ml, $p > 0.05$). This means that pTB patients involved in this study have a low level of IFN-γ that inadequate to be capable to activate the mycobactericidal activity of alveolar macrophages and results in the reduction of cell mediate immunity for pulmonary TB patients. Depending on the fact that represent a cytokine regulator of CMI increases dramatically at least 10 fold greater than the concentration in normal state in which there is no an antigenic stimulation [29].

Table (3) IFN-γ Concentration in serum of studying groups

Study groups	Mean (pg/mL) ± SD	Range	P value
Patients	36.505±16.509	16.93- 49.52	0.132*
Controls	25.019±11.339	11.41-59.93	
*= No significant (P > 0.05)			

This may because most of clinically diagnosed TB patients on antituberculosis treatment and, therefore, the healing effect on granuloma could reduce the number of local and circulating IFN-γ-producing activated T-cells. Investigators were reported increased levels of IFN-γ in the sputum or pleural effusion and observed a correlation between IFN-γ and disease activity in some studies. IFN-γ produced by a variety of cells involved in the immune response against Mtb. Th1 cells that produce gamma interferon IFN-γ confer resistance to infection with mycobacteria. At the site of infection with Mtb, it is the major cytokine of type 1 T helper (Th1) cells, therefore, as it protects against Mtb [24]. [30] who mentioned that serum IFN-gamma level was not found statistically significant ($p= 0.4$) in the differential diagnosis of active and inactive pulmonary tuberculosis. While study of [31] who found that the levels of IFN-γ in pulmonary tuberculosis group were significantly higher than control group ($P<0.01$ or $P<0.05$). In study of [32],who mentioned that count of T cell was decreased significantly for TB patients in study carried out in Babylon province. TGF-β

mediated regulatory mechanisms that down modulated responses of Th1during active TB in human, CD4+ CD25+ T cells reported to be present at elevated level in TB as well as to be able to depress T-cell-mediated IFN-γ production in patients with TB. The study of [28] indicated the persistence of measles infection as one inhibitory mechanism for cell mediated immunity against TB infection. Therefore, infection with measles virus leads to immune suppression resulting in decreased CMI, and at the same time, increased susceptibility to TB infection for the duration of immune suppression. [33] who mentioned that most TB patients in Babylon province/Iraq were affected by immune anergy. Numerous predisposing factors increased frequency of immune anergy including viral infections in addition to metabolic disorders especially renal failure and diabetes mellitus. Results of no increasing Th1 cytokine profile in TB patients were indicated presence of reducing cell mediated of TB patients in spite of mycobacterial antigens presence. This unresponsiveness reflect the inhibitory signals for cell- mediated immunity induced by CMV which was considered as on viruses with potential activity for inducing immune anergy. The study of [34] were found that the level of IFN-γ significantly (P = 0.001) changed and the sputum smear conversion was significantly earlier in the zinc and vitamin A supplemented group. There is in vitro facts that TB patients with progressive disease fail to generate IFN-γ in response to stimulation with mycobacterial antigens.

4- Estimation of VD3 in serum of studied groups

The mean of VD3 concentration in pulmonary TB patients sera was (18.186) ng/ml, while in control groups was (29.321) ng/ml. The study significantly shows the difference between pTB and controls, ($p< 0.05$), Table (4). The study shows VD3 was significantly lower in pulmonary TB than controls. This finding matched approximately with [9]who mentioned that patients with pulmonary tuberculosis was significantly deficient of Vitamin D when found the mean ±SD serum level of 25-hydroxyvitamin D3 in pTB and control groups were 27.1±9.7 and 36.8±8.1 respectively (p=0.0001). This finding also matched with [10] who showed that the concentrations of VD3 in pTB patients lower than the healthy adults.

Table (4) Vitamin D3 Concentration in serum of Patients and Controls

Study groups	Mean (ng/ml) ± SD	Range	P value
Patients	18.186±5.019	2.60-25.90	0.0007*
Controls	29.321±7.700	16.81-89.4	
*= Significant (P≤ 0.05)			

The reason for low level of VD3 in the serum in pTB could be reflect increased utilization of VD or reflect lower exposure to the sun [35] or possibilities that result from tuberculosis itself [9] . Low levels of vitamin D in the serum may also be associated with poor nutritional status in patients with TB [36]. In a cohort follow-up study from Pakistan, low levels of vitamin D were associated with progression to active TB disease in healthy

household contacts. The findings suggested as well the higher susceptibility of women to the infection, due to their poor nutrition, low socioeconomic status, traditional/cultural traits, and little exposure to sunlight [37]. Vitamin D3 is important for the host resistance to TB; pulmonary TB patients with deficiency of VD3 was not able to mount adequate control of primary Mtb infection in the lungs. Additional, VD3 has implicated in the immunological control of TB in humans involving the orchestration of IFN-γ, IL-15 and IL-32 signaling. A recent evaluation of therapeutic potential of VD3 in combination with PBA has shown an improved effects of anti-mycobacterial and faster the conversion to AFB-negative sputum by participating TB patients. In vitro the evaluation of Mtb-infected macrophages treated with VD3 lead to upregulation of a collage of anti-inflammatory (IL-10, ARG1) and genes of pro-inflammatory (IL1B, TNF) over a 72-hour exposure period. In endemic countries, the therapeutic potential of VD3 in patients with MDR-TB therefore warrant further clinical evaluation in larger cohorts of TB patients [38]. [39] who mentioned that high doses of vitamin D were widely used to treat tuberculosis patients in the preantibiotic era. This approach was successful: vitamin D could suppress growth of intracellular of M tuberculosis in vitro. Vitamin D as well induced expression of cathelicidin, which was involved in the first line defense in patients with TB. Single nucleotide polymorphisms in vitamin D receptor (VDR) gene have been associated with susceptibility to tuberculosis [40]. The genetic alterations of the VDR gene may lead to defects in gene activation or to changes in protein structure of the VDR, both of which could affect the cellular functions of 1,25-dihydroxyvitamin D3. Various VDR polymorphisms could also be linked each other or to unidentified genes that were important determinants of disease risk [41]. Results of other study suggest that 1,25(OH)2D3 down regulates the production cytokines of pro-inflammatory in addition to may control the exacerbated inflammatory response that may protect the host from excessive damage of tissue at infection site [42]. Epidemiological and experimental researches highlight that low level of the serum vitamin D was associated with impaired pulmonary function and it was potentially involved in a number of lung diseases. Moreover, a study of case-control in Ethiopia was determined the role of VDD as predisposing factor for pneumonia in children with age under 5 years [43]. There are others studies on others vitamins in pulmonary TB patients such as a study of [44] was found that The serum concentrations of vitamins A, D, and E were significantly lower in patients with tuberculosis than in control subjects . The net effect of 1,25(OH)2D3 to polarize T-helper responses toward a more regulatory Th2 phenotype, which was considered a key component of its capacity to suppress Th1-driven autoimmune responses. vitamin D deficiency was impairs the ability of macrophages to mature, to producing specific surface antigens via down-regulating the membrane expression of major histocompatibility complex class II (MHC-II) molecules to generate the lysosomal enzyme acid phosphatase and to secrete H_2O_2, essential tools for their antimicrobial function. In contrast, the addition of VD3 increases expression of macrophage-specific surface antigens and of lysosomal enzyme acid phosphatase [40].The utilization of vitamin D for treatment of tuberculosis was common in the pre-antibiotic era [43].

CONFLICT OF INTERESTS.
There are non-conflicts of interest.

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الخلاصة

هدفت هذه الدراسة إلى تقييم بعض معايير المناعة الخلوية لمرضى التدرن الرئوي من خلال تقدير مستويات الانترلوكينات (IFN-γو IL-10و IL-12) وتقدير فيتامينD3 . تم خلال الدراسة جمع عينات الدم من 50 مريضا بالتدرن الرئوي من المراجعات الى العيادة الاستشارية للأمراض الصدرية في الحلة –محافظة بابل خلال الفترة مابين شهر شباط 2016 إلى شباط 2017. ومن بين مرضى التدرن الرئوي، كان هناك 27 ذكور و23 إناث، وعمر المرضى يتراوح بين 12–80 عاما. تم قياس السايتوكينات وفيتامين D3باستخدام تقنية الاليزا. أظهر متوسط تركيز مصل الدم IL-10 و IL-12أن هناك زيادة معنوية ($p< 0.05$) في مرضى التدرن الرئوي بالمقارنة مع مجموعة السيطرة (4.024 و1.049) بغ / مل , (51.563 و13.514) بغ / مل على التوالي. في حين لم يظهر متوسط تركيز مصل IFN-γزيادة معنوية ($p>0.05$) في مرضى التدرن الرئوي مقارنة بمجموعة السيطرة (36.505 و25.019) بغ / مل. أظهر متوسط تركيز مصل الدم ل VD3 انخفاضا معنويا ($p < 0.05$) في مرضى التدرن الرئوي مقارنة بمجموعة السيطرة (18.186 و29.321) نغ/ ل.

الكلمات الدالة: التدرن ، انترلوكين(10،12)، انترفيرون كاما، فيتامين دي3، فحص الاليزا