

**A study of the cell-mediated immunity
in patients with pulmonary tuberculosis**

دراسة المناعة الخلوية لدى مرضى التدرن الرئوي

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A study of the cell-mediated immunity in patients with pulmonary tuberculosis

Abstract:

Objectives:

Assessment of certain cell mediated parameters during different clinical stages of pulmonary tuberculosis (PTB).

Materials and method:

Two hundred and ten patients with different clinical stages of PTB and 105 healthy individuals were enrolled in this study. Both study groups were submitted to; tuberculin skin test (TST) to assess the delayed type hypersensitivity resection (DTH); skin sensitization to 1-chloro 2,4-dinitrobenzene (DNCB) to determine the non-specific immune response (NIR); assessment of peripheral phagocytic activity (PPA) by nitro-blue tetrazolium (NBT) reduction test. Additionally, correlation of these immune parameters with peripheral total CD_4^+ and CD_8^+ T lymphocyte counts were also sought.

Results:

The TST positivity rate was significantly lower in newly diagnosed (untreated) compared to treated PTB patients (32.3% vs 67.7%), while the mean diameter of tuberculin skin induration was significantly higher among treated group ($P < 0.001$). These observations were positively correlated with the mean reduction in the CD_4^+ T cell count. Furthermore, PTB patients showed a low peripheral phagocytic activity as compared to healthy individuals. The skin sensitization with DNCB was significantly lower in PTB patients and positively correlated with the mean reduction in CD_4^+ T cell count, affirming low non-specific immune response.

Conclusion:

The CD_4^+ T lymphocytopenia particularly accompanied the active PTB may explain the state of hyporesponsiveness that hasten disease progression.

Key words: Cell mediated immunity, Pulmonary tuberculosis, Tuberculin skin test

دراسة المناعة الخلوية لدى مرضى التدرن الرئوي

الخلاصة:

أجريت هذه الدراسة على 210 من مرضى التدرن الرئوي بمختلف مراحله السريرية و 105 من الأشخاص الأصحاء كمجموع سيطرة. هدف الدراسة هو قياس بعض معايير المناعة الخلوية والتي شملت اختبار التوبركلين الجلدي لقياس فرط

التحسس الاجل للمستخلص البروتيني للمتفطرة السلية (Tuberculin skin test) ، اختبار كفاءة الخلايا الملتزمة المحيطية لاختزال مادة NBT و اختبار التحسس الجلدي لمادة DNCB وعلاقة تلك الاختبارات بعدد الخلايا اللمفاوية التائية المحيطية CD₄ و CD₈ . أظهرت النتائج أن إيجابية تفاعل التوبركلين تحت الأدمة منخفضة بشكل معنوي لدى مرضى التدرن المشخصين حديثا (قبل العلاج) مقارنة بمجموع المرضى المعالجين (32.3% مقابل 67.7%) وان معدل قطرالجسوء الجلدية كان أكبر بشكل معنوي بين المجموع تحت العلاج وهذه النتائج تناسبت طرديا مع معدل انخفاض الخلايا اللمفاوية التائية CD₄ . أظهرت النتائج أيضا تدني كفاءة الخلايا الملتزمة المحيطية لدى مرضى التدرن الرئوي مقارنة بمجموعة السيطرة . فضلا عن ذلك فان التحسس الجلدي كان منخفض بشكل معنوي لدى مرضى التدرن الرئوي (97.7% مقابل 100%) ، وان هذه الاستجابة تناسبت طرديا مع معدل تعداد الخلايا اللمفاوية التائية CD₄ .

إن انخفاض عدد الخلايا اللمفاوية التائية CD₄ lymphocytopenia وخاصة في مرحلة التدرن الرئوي الفعال يمكن أن تكون السبب في تدني معايير المناعة الخلوية وبالتالي تطور المرض .
الكلمات المفتاحية: المناعة الخلوية، التدرن الرئوي، اختبار التوبركلين

Introduction:

Tuberculosis (TB), the ancient enemy of mankind, remains one of the most serious infectious disease in the world, accounting or 3 million deaths annually [1]. Among the Eastern Mediterranean countries, Iraq ranks in the intermediate zone of endemicity with an incidence rate 20-100/10⁵ population [2]. However, higher incidence had been reported during the last years [3].

Infection with *Mycobacterium tuberculosis* (MTB) provoke a complex immune response, which can be appreciated as the interplay of two major mechanisms; the cell-mediated immunity and the delayed type hypersensitivity reaction, while the humoral immunity has no definite role in the protection [4,5]. The CD₄ T lymphocytes plays an important central role by expressing and regulating the acquired immunity, which control the primary infection in 95% of the immunocompetent hosts [6,7]. Thus failure of CD₄⁺ T cell response contribute to progressive primary infection and reactivation of endogenous MTB infection, as in HIV/TB coinfection [8,9].

Several studies had reported a peripheral CD₄⁺ lymphocytopenia (often < 300 cell/mm³) in PTB patients before treatment, while the reduction in the CD₈⁺ T cell count was not so pronounced and the CD4: CD8 ratio remained on regular levels [10,11]. However, renormalization of CD₄⁺ T cell depletion after successful anti-TB treatment had been reported [12,13]. Concerning the phagocytic activity, a marked reduction of neutrophil activity had been reported in active PTB as determined by an *in vitro* assay [14]. Additionally, the decreased skin reactivity in PTB patients indicates low non-specific immune response [15]. We sought to explore the defect in CMI among PTB patients through employing different *in vitro* and *in vivo* procedures, and to affirm their correlation with the CD₄⁺ and CD₈⁺ T cell counts.

Subjects, materials and methods:

Subjects:

Two hundred and ten PTB patients were enrolled in this study. The mean age was 31.8 years. Diagnosis of TB was established by radiographic and standard bacteriological criteria at the TB and Chest Disease Institute in Baghdad. 99 patients were newly diagnosed (before treatment), 13 defaulters, 24 treatment failures, 24 relapse and 50 chronic cases. Classification of TB patients was based on WHO guidelines ^[16]. Additionally, 105 healthy individuals with mean age 26.7 years were included as control group.

Determination of delayed type hypersensitivity reaction:

The TST was used to detect the delayed type hypersensitivity reaction (DTH) to purified protein derivative of MTB. TST was performed and interpreted according to the recommendation of the American Thoracic society ^[17]. 0.1 ml of PPD (5 tuberculin units), which was purchased from Sera and Vaccine institute (Baghdad), was injected intradermally on the volar aspect of forearm. Coetaneous reaction was recorded as the greatest diameter induration 48-72 hours post-inoculation following the ballpoint-pen method ^[17]. Induration > 10 mm was considered positive reaction.

Determination of non-specific immune response:

The technique of skin sensitization to 1-chloro 2,4-dinitrobenzene (Hopkin & Williams,Ltd.UK) was applied and the results were scored as described by Catalona et al.(1972) ^[18].

Assessment of peripheral phagocytic activity:

Modified Park method was used for this purpose ^[19]. The test is based on spontaneous reduction of NBT salt (BDH Ltd.UK) to blue black formozan deposit inside the phagocytes (NBT⁺ cells). 100 neutrophils were counted and the percentages of NBT⁺ cells were recorded. The absolute number of NBT positive cells was calculated from the percentage of NBT⁺ cells and absolute number of neutrophils.

Estimation of CD₄ and CD₈ T lymphocytes:

A total CD4/total CD8, an immunoenzymatic assay was used for estimation of total peripheral CD₄ and CD₈ T cells. The assay is based on specific capture of these cells in the blood with paramagnetic microparticles coated with anti-CD₂ antibody. Blood samples were drawn in EDTA anticoagulant tubes and processed within 24 hours according to the manufacturer's instruction (Sanfi diagnostic, France).

All data were statistically analyzed.

Results:

Data presented in table (1) showed that the rate of TST positive reaction was significantly higher among PT patients compared to control group (53% vs 17.1%). Moreover, the mean diameter of skin induration in PT patients was significantly higher than that of controls (10.5 mm vs 5.8 mm). On the other hand, the newly diagnosed (untreated) group showed significantly higher rate of TST negativity rate as compared to treated groups (defaulters, treatment failures,

relapse & chronic) (67.7 vs 32.3). The highest rate of positive skin reaction was recorded among chronic cases (80%).

Table (1): TST positivity rate among different study groups.

Groups	No. tested	TST		Mean diameter skin induration	P Anova
		Negative No.(%)	Positive No.(%)		
Control	105	87 (82.9)	18 (17.1)	5.8	< 0.001
Newly diagnosed	99	67 (67.7)	32 (32.2)	7.4	
Defaulters	13	3 (23.1)	10 (76.9)	11.2	
Treatment	24	14 (58.3)	10 (41.7)	9.0	
failures	24	5 (20.8)	19 (79.2)	12.7	
Relapse Chronic	50	10 (20)	40 (80)	12.3	

Skin sensitization was performed on 94 PTB patients and 52 controls. All controls showed positive skin reaction (22 gave grade 1+, 20 with grade 2+, and 10 with grade 3+), but non of them gave grade 4+ reaction. Whereas, 75 (79.7%) of PTB patients were positive (36 with grade 1+, 20 with grade 2+, 15 with grade 3+, and 4 with grade 4+). The mean DNCB score in PTB patients was insignificantly higher compared to control (2.4 vs 1.8). However, the mean score reaction in treatment failure and relapse groups was significantly higher than control (P< 0.001), table (2).

Table (2): DNCB score among different study groups.

Groups	No. tested	Positive DNCB skin Reaction (Mean score)	P Anova
Control	52	52 (1.8)	< 0.001
Newly diagnosed	65	53 (1.9)	
Defaulters	2	2 (2.0)	
Treatment failures	13	9 (2.7)	
Relapse	4	4 (3.3)	
Chronic	10	7 (2.4)	

The mean percentage of NBT positive cells was significantly higher in all PTB groups (p< 0.001), with the exception of chronic cases, table (3).

Table (3): Mean percentage of NBT positive cells for different groups

Groups	NBT positive cells No. (%)	P Anova
Controls	28 (2.6)	< 0.001
Newly diagnosed	34 (7.8)	
Defaulters	5 (5.8)	
Treatment failures	19 (5.9)	
Relapse	7 (5.9)	
Chronic	25 (3.5)	

Results in table (4) revealed that the mean CD₄ T cell count in the newly diagnosed and defaulters group was significantly lower than controls ($p < 0.001$), while it was insignificant in the remaining PTB groups. The results also showed that the highest CD₄:CD₈ ratio was observed among controls (1.5:1), while the lowest ratio was among defaulters and chronic cases (1.1:1). Generally, the mean CD₄ T cell count in the PTB patients was significantly lower than the control (660.4 vs 841.1 cell/mm³).

Table (4): Mean CD₄ and CD₈ T cell counts (cell/ mm³) among study groups.

Groups	No tested	CD ₄ T cell count		CD ₈ T cell count		CD ₄ :CD ₈
		Mean	P Anova	Mean	P Anova	
Control	10	841.1	< 0.001	0		1.5:1
Newly diagnosed	14	606.6		.5 [NS]		1.2:1
Defaulters	3	508.3		508.1		1.1:1
Treatment failures	3	803.7		472.3		1.3:1
Relapse	4	743.0		618.0		1.3:1
Chronic	4	632.8		574.8		1.1:1
				555.8		

A multiple regression analyses revealed that the defaulters and newly diagnosed PTB cases were associated with significant reduction in the CD₄ T cell count (β regression coefficient – 208.4 and – 136.4 cell/mm³) respectively. However, other PTB groups were associated with insignificant change in CD₄ T cell count. Similarly, none of the PTB groups were associated with significant reduction in CD₈ T cell count.

Discussion:

In the immune competent host, the protective immunity against MTB infection is chiefly relay on effective interaction between CMI and DYH [7,20]. The CMI involves recognition of MTB antigens, stimulating of specific lymphocytes, and elaboration of cytokines, IL-2, INF- and TNF-, which in turn

activate alveolar macrophages (AMs) to kill MTB and promote granuloma formation, while the DTH functions to kill intracellular MTB. However, an overt clinical disease may develop most likely due to the ability of MTB to survive and replicate within the AMs^[21,22]. It has been reported that under certain circumstances, suppression of CMI may increase the host susceptibility for primary infection and reactivation of latent MTB and enhanced disease progression^[8,23,24].

In the present study, high rate of negative TST reaction was observed in PTB patients particularly among the newly diagnosed (untreated) group. Since negative TST imply the incapacity of patients to mount DTH to PPD of MTB, therefore, our results were consistent with other studies reported that 20-30% of newly diagnosed untreated PTB patients dose not respond to TST^[25,26,27]. Furthermore, other studies had reported a T cell dysfunction among newly diagnosed PTB patients, which was manifested by suppressed T cell proliferative response to PPD *in vitro* and decreased production of cytokines *in vivo*^[28,29]. Additionally, our results were extended to showed that PTB patients with < 600 CD₄ T cell count/mm³ were generally associated with coetaneous anergy to PPD. While patients with higher counts restored their ability to evoke a DTH reaction, indicating a close positive correlation between the CD₄ T cell counts and the induction of DTH reaction to PPD. Of note, most previous studies in this regard were performed on HIV/TB coinfectd patients. However, they agree that skin anergy to PPD were more common in HIV positive individuals at CD₄ count below 400 cell/mm³^[30,31]. Similarly in treatment failures, high TST negativity rate was obtained compared to other PTB treated groups. Again this result indirectly affirm that successful anti-TB treatment may revert the CD₄ lymphocytopenia accompanied by restoration of DTH to PPD^[12,13,32]. The results also showed that the mean diameter of tuberculin skin induration was significantly higher in PT treated groups compared to controls. Whereas, the mean diameter in the newly diagnosed untreated group does not differ significantly. Extension of these findings revealed that the mean diameter of skin induration was positively correlated with the CD₄ T cell count, being increased in treated groups (mean 672 cell/mm³) and decreased in untreated group (mean 606.6 cell/mm³). On the other hand, no such correlation was found concerning the CD8 T cell in different PTB groups.

Skin sensitization with DNCB is mediated by circulating lymphocytes in the regional lymph nodes, which induce an allergic response upon contact with epidermal protein bound DNCB. Moreover, circulating antibodies does not develop with contact sensitization, making this test highly specific for CMI^[18]. In our study, the NIR was significantly lower in PTB patients compared to control (79.7% vs 100%), although the mean score of DNCB skin reaction was insignificantly different among treated, untreated and control groups. These results were in full agreement with the previous studies^[15,33]. Generally, lower DNCB skin sensitization in PTB patients may be attributed to the reversible

impairment of CMI manifested by decreased circulating CD₄ T cells, particularly in the newly diagnosed and defaulters. Additionally, all patients who were non-reactive to TST and DNCD expressed higher reduction in CD₄ T cell counts, while those who were DNCD positive showed mild reduction, so they respond to stronger stimulus.

The NBT response is most reliably expressed as a percentage of neutrophils containing formozan. Very few studies had utilized this technique to assess the peripheral phagocytic activity in PTB patients. Park et al. (1968)^[19] reported that PTB patients had consistently negative NBT response based on 10% as a cut-off value. In the current study, the mean percentage of NBT positive cells was 5.7 %, which is below the cut-off value, indicating negative NBT response. The low peripheral phagocytic activity in PTB patients may be attributed to the influx of neutrophils to the site of bacterial invasion (neutrophilic alveolitis), which is an early event in the local immunopathogenesis of PTB^[20,34].

Evidences emerged through this study clearly implies that CD4+ T lymphocytopenia, particularly accompanied active PTB may explain the state of immune hyporesponsiveness, that at least partly, hasten disease progression.

References:

- 1.Borgdoff, M.W. Annual risk of tuberculosis infection. Time for an update?. Bull.WHO, 80(6): 501-2.
- 2.Seita A. Surveillance for tuberculosis in the Eastern Mediterranean region. East.Medit.Pub.Health J. 1996; 2(1):129-34.
- 3.Mohan A. Guidelines for national tuberculosis control program. Ministry of Health,Iraq,1998.
- 4.Flynn, J.L. Immunology of tuberculosis and implication in vaccine development. Tuberculosis,2004; 84: 93-101.
- 5.Ellner JJ. The immune response in human tuberculosis; Implication for tuberculosis control. J.Infect.Dis. 1997; 176: 1351-59.
- 6.Horsburgh CR. Tuberculosis without tubercle. Tuberc.Lung Dis. 1996; 77: 197-98.
- 7.Al-Faraj, S. The role of cell-mediated immunity (CMI) in intracellular infections. Saudi Med.J. 1996; 17(3): 281-5.
- 8.Munsiff S, Alpert P, Chung C & Kline R. A prospective study of tuberculosis and HIV disease progression. J.Acquir.Immune Defic.Syindr.1998; 19(4): 361-66.
- 9.Grange JM. CD₄⁺ T lymphocytopenia in pulmonary tuberculosis. Int.J.Tuberc.Lung Dis. 1998; 2(3): 261-62.
- 10.Villarino,M.; Geiter, L. and Hopewell, F. Parameters of cell-mediated immunity in TB patients with HIV positive and without HIV infection. Am.Rev.Respir.Dis. 1993; 147(4): 216-20.

- 11.Singhal, M. and Bananalikar, J.N. Peripheral blood T-lymphocyte subpopulations in patients with tuberculosis and the effects of chemotherapy. *Tubercle*, 1989; 70:171-8.
- 12.Turett GS & Tezalk EE. Normalization of CD4+ T lymphocyte in patients without HIV infection treated for tuberculosis. *Chest*, 1994; 105(5): 1335-37.
- 13.Dieli F, Friscia G, Disan O, Lavany I, Singh M, Spallek R, Titonel L& Salerno A. Sequestration of T lymphocytes to body fluids in tuberculosis; Reversal of anergy following chemotherapy. *J. Infect.Dis.* 1999; 180: 225-28.
- 14.Matula G & Paterson PY. Spontaneous *in vitro* reduction of nitroblue tetrazolium by neutrophils of adult patients with bacteria infection. *N.Engl.J.Med.* 1971; 285(6): 311-17.
- 15.Malaviya AN, Sehgal KL, Kumer R & Dingley HB. Factors of delayed-type hypersensitivity in pulmonary tuberculosis. *Am.Rev.Respir.Dis.* 1975; 112; 49-52.
- 16.World Health Organization. Tuberculosis. *Bull. WHO*, 1998; 76(Suppl.2): 141-43.
- 17.Al-Jahdali, H.; Memish, Z.A. and Menzies, D. The utility and interpretation of tuberculin skin tests in the Middle East. *Am.J.Infect. Control*, 2005;33(3): 151-6.
- 18.Catalona, WJ, Tylor PT, Rabso PS & Chretien PB. A method for di-nitro chlorobenzene contact sensitization. *N.Engl.J.Med.* 1972; 286(8): 399-402.
- 19.Park B, Hfikring S & Simthwick E. Infection and nitroblue tetrazolium reduction by neutrophils. *Lancet*, 1968; 2:532-34.
- 20.Schluger, N.W.; and Rom, W.N. The host immune response to tuberculosis. *Am.J.Respir. Crit. Care Med.*1998; 157: 679-91.
- 21.Kurashima, K.; Mukaida, N.; Fujimura, M.; Yasui, M.; Nakazumi, Y.; Matsuda, T. and Matsushima, K. Elevated chemokine levels in bronchoalveolar lavage fluid of tuberculosis patients. *Am.J.Respir.Crit. Care Med.* 1997; 155: 1474-77.
- 22.Bloom,B.R, and Small, P.M.The evolving relation between human and Mycobacterium tuberculosis.*N.Engl.J.Med.*1998; 338(10): 677-8.
23. Hirsch CS, Toossi Z, Vanha G, Johnson JL & EllnerJJ. Apoptosis and T cell hyporesponsiveness in pulmonary tuberculosis. 1999; 179: 945-53.
24. Read C. *Mycobacterium tuberculosis*; A stow away in the cell. *Lancet*, 1996; 348: 50.
- 25.Converse PJ, Jones SC, Astemborsici J, Vlahov D& Graham MH. Comparison of a tuberculin interferon assay with the tuberculin skin test in high risk adults; Effect of human immunodeficiency virus infection. *J.Infect.Dis.* 1997; 197:144-50.

26. Andersen P, Munk M, Pollock JM & Doherty TM. Specific immune-based diagnosis of tuberculosis. *Lancet*, 2000; 356: 1099-1104.
27. Moreno S, Baraia J, Miralls P, Beneguer J, Cosin J & Bernaldo J. Tuberculin reaction size in patients infected with HIV. *AIDS*, 1995; 11: 1289-95.
28. Zhang M, Ln Y, Lyerd V, Gong J, Abrams JS & Barnes PE. T cell cytokine response in human infection with *Mycobacterium tuberculosis*. *Infect.Immun.* 1995; 63:3231.
29. Ting CM, Kim AC, Cattamanchi A & Ernts JD. *Mycobacterium tuberculosis* inhabits of INF-B transcriptional response without inhibiting activation of stat-1. *J. Immunol.* 1999; 163: 3898-3906.
30. Mukedi Y, Perrines H, Louis M, Brown C, Prignot J & Willam J. Spectrum of immunodeficiency in HIV-1 infected patients wit pulmonary tuberculosis in Zaire. *Lancet*, 342: 143-46.
31. Ackah NA, Coulibaly D, Digben H, Diallo K, Yetter KM, Greenburg AE & Decock KM. Response to treatment, mortality and CD₄ lymphocyte count in HIV infected persons with tuberculosis in Abidjian, Cote d'e Ivoire. *Lancet*, 1995; 345: 607-10.
32. Martin DJ, Sim JG, Sole GJ, Rymer I, Shalekoff S & van Niekerk AB. CD4+ lymphocyte count in African patients coinfectd with HIV and tuberculosis. *J.Acquir.Immune Defic.Syindr.* 1995; 8 (4): 386-91.
33. Al-tawil NG. Study of the immunological states of patients with pulmonary tuberculosis. M.Sc. thesis, College of Medicine, Baghdad University, 1975.
34. Schwander SK, Sada E, Torrer M, Esocobedo D, Sierra JC & Rich EA. T lymphocytic and immature macrophage alveolitis in active pulmonary tuberculosis. *J.Infect.Dis.* 1996; 173:1267-72.