

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

**دراسة المناعة الخلوية لدى مرضى التدرن الرئوي**

**Abdul-Razak Shafiq Hasan (Ph.D.Med. Microbiol.) Department of  
Microbiology/ College of Medicine/ Al-Anbar University**

عبد الرزاق شفيق حسن (دكتوراه في الاحياء المجهرية الطبية) فرع الأحياء المجهرية / كلية  
الطب / جامعة الانبار

**Abbas Aboud Al- Duliami (Ph.D. Microbiology), Department of  
Microbiology/ College of Education/ Diyala University**

عباس عبود الدليمي (دكتوراه في الأحياء المجهرية) فرع الأحياء المجهرية / كلية  
التربية / جامعة ديالى

**Hasan Ali Al-Saadi (Ph.D. Microbiol.) Department of Microbiology/  
College of Science/ Diyala University**

حسن علي السعدي (دكتوراه في الأحياء المجهرية) فرع الأحياء المجهرية / كلية العلوم /  
جامعة ديالى

**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 0 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 CD<sub>8</sub> و CD<sub>4</sub> 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 قطرات الجسوء الجلدية كان أكبر بشكل معنوي بين المجموع تحت العلاج وهذه النتائج تناسب طرديا مع معدل انخفاض الخلايا اللمفاوية التائية CD<sub>4</sub> أظهرت النتائج أيضا تدني كفاءة الخلايا الملتزمة المحيطية لدى مرضى التدرن الرئوي مقارنة بمجموعة السيطرة 0 فضلا عن ذلك فان التحسس الجلدي كان منخفض بشكل معنوي لدى مرضى التدرن الرئوي (97.7% مقابل 100%)، وان هذه الاستجابة تناسب طرديا مع معدل تعداد الخلايا اللمفاوية التائية CD<sub>4</sub>

إن انخفاض عدد الخلايا اللمفاوية التائية CD<sub>4</sub> lymphocytopenia وخاصة في مرحلة التدرن الرئوي الفعال يمكن أن تكون السبب في تدني معايير المناعة الخلوية وبالتالي تطور المرض 0

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 20-100/10<sup>5</sup> 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 CD<sub>4</sub> 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 CD<sub>4</sub><sup>+</sup> 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 CD<sub>4</sub><sup>+</sup> lymphocytopenia (often < 300 cell/mm<sup>3</sup>) in PTB patients before treatment, while the reduction in the CD<sub>8</sub><sup>+</sup> T cell count was not so pronounced and the CD4: CD8 ratio remained on regular levels [10,11]. However, renormalization of CD<sub>4</sub><sup>+</sup> 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 CD<sub>4</sub><sup>+</sup> and CD<sub>8</sub><sup>+</sup> 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 <sup>[16]</sup>. 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 <sup>[17]</sup>. 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 <sup>[17]</sup>. 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) <sup>[18]</sup>.

## **Assessment of peripheral phagocytic activity:**

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

## **Estimation of CD<sub>4</sub> and CD<sub>8</sub> T lymphocytes:**

A total CD4/total CD8, an immunoenzymatic assay was used for estimation of total peripheral CD<sub>4</sub> and CD<sub>8</sub> T cells. The assay is based on specific capture of these cells in the blood with paramagnetic microparticles coated with anti-CD<sub>2</sub> 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 CD<sub>4</sub> 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 CD<sub>4</sub>:CD<sub>8</sub> ratio was observed among controls (1.5:1), while the lowest ratio was among defaulters and chronic cases (1.1:1). Generally, the mean CD<sub>4</sub> T cell count in the PTB patients was significantly lower than the control (660.4 vs 841.1 cell/mm<sup>3</sup>).

**Table (4): Mean CD<sub>4</sub> and CD<sub>8</sub> T cell counts (cell/ mm<sup>3</sup>) among study groups.**

Groups	No tested	CD <sub>4</sub> T cell count		CD <sub>8</sub> T cell count		CD <sub>4</sub> :CD <sub>8</sub>
		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 CD<sub>4</sub> T cell count ( $\beta$  regression coefficient – 208.4 and – 136.4 cell/mm<sup>3</sup>) respectively. However, other PTB groups were associated with insignificant change in CD<sub>4</sub> T cell count. Similarly, none of the PTB groups were associated with significant reduction in CD<sub>8</sub> 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<sup>[21,22]</sup>. 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<sup>[8,23,24]</sup>.

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<sup>[25,26,27]</sup>. 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*<sup>[28,29]</sup>. Additionally, our results were extended to showed that PTB patients with  $< 600 \text{ CD}_4 \text{ T cell count/mm}^3$  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  $\text{CD}_4 \text{ T cell}$  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  $\text{CD}_4$  count below  $400 \text{ cell/mm}^3$ <sup>[30,31]</sup>. 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  $\text{CD}_4$  lymphocytopenia accompanied by restoration of DTH to PPD<sup>[12,13,32]</sup>. 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  $\text{CD}_4 \text{ T cell}$  count, being increased in treated groups (mean  $672 \text{ cell/mm}^3$ ) and decreased in untreated group (mean  $606.6 \text{ cell/mm}^3$ ). On the other hand, no such correlation was found concerning the  $\text{CD}_8 \text{ T cell}$  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<sup>[18]</sup>. 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<sup>[15,33]</sup>. Generally, lower DNCB skin sensitization in PTB patients may be attributed to the reversible

impairment of CMI manifested by decreased circulating CD<sub>4</sub> T cells, particularly in the newly diagnosed and defaulters. Additionally, all patients who were non-reactive to TST and DNCD expressed higher reduction in CD<sub>4</sub> 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)<sup>[19]</sup> 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 bellow 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<sup>[20,34]</sup>.

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.

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