

# Assessment of Leucocytes Migration Inhibition in Tuberculosis Patients

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## Abstract

Leucocytes Migration Inhibition test in agarose was carried against PPD and *Mycobacterium tuberculosis* antigen . The proportion of different Kinds of migrating leucocytes was calculated and expressed as a function of the migration distance .

Application of this test to clinical problem revealed that with PPD as antigen , a highly significant inhibition was obtained in PPD skin test – positive normal individual , as well as, a high significant inhibition was recorded in individuals recovered from tuberculosis .

These results demonstrated the suitability of this test for routine detection for cell mediated immunity .

## Introduction

When lymphocytes are activated , either by mitogens or antigen , they produce a range of soluble mediators , in addition to antibody , these mediators are known collectively as lymphokines irrespective of the mode of generation or their subsequent action .The various lymphokines are classified according to their mode of action , as :

Lymphotoxin – Kills nucleated cells .Macrophage activating factor – enhances macrophages mobility and phagocytosis.

Mitogenic factor-stimulates mitosis in lymphocyte populations .

Migration-inhibition factor (M I F ) inhibits macrophages migration.

The inhibition of macrophages migration by lymphokines from activated lymphocytes had been studied in detailed and showed an approximate correlation with invivo immune status . [1]

Cell mediated immunity can be detected invitro by several methods. In leucocytes migration inhibition test ( LMIT) , a total population of human leucocytes is incubated in a capillary tube in the presence of Antigen , and the size of their migration out of the capillary is measured .

Several modifications were performed , one of them utilizing agarose gel layer was used to demonstrated the LMIT in presence of Purified Protein Derivative (PPD) in patients with positive PPD skin test [2] . This study demonstrates the phenomenon of leucocytes migration in agarose gel with special attention to the nature of the migrating leucocytes and the dynamic of this type of migration as a function of time .

## Materials and Methods

A total of 40 adults were tested . PPD was carried out by intradermal tests . The reaction was considered as positive when erythema was larger than 4mm in diameter [4] . The patients were divided into four groups :

**Group -1** : Normal individual with positive skin test to tuberculin , and without history of tuberculosis which showed a negative test serologically and bacteriological tests for the presence of *mycobacterium tuberculosis* .

**Group -2** : Normal individual with negative skin test to tuberculin and without history of tuberculosis and showed a negative serological and bacteriological tests for *mycobacterium tuberculosis* .

**Group -3** : Individuals with previous history of tuberculosis ( between 6 – 12 months ) but they were healthy at the time of investigation which gave negative bacteriological and serological tests for *mycobacterium tuberculosis*. All individuals of this group had a positive skin test for PPD.

Group -4: included patients with proven infection with *Mycobacterium tuberculosis* .All showed a positive skin test for PPD.

*Mycobacterium* was purified and isolated from sputum of tuberculosis patients using a procedure that was described by [3],the procedure included a precipitation by ammonium sulphate, filtration on sephadix G200,electrophoresis and ultracentrifugation on sucrose gradient .Antigen was then concentrated by vacuum dialyser in TC medium 199

All experiments were performed under a sterile condition according to [5] . Venous blood was mixed with preservative-free heparin at a concentration of 12 u of heparin /ml of blood. Leucocytes were then separated by adding 7.0 ml of dextran 250(6%).After one hour of sedimentation 37° C , the supernatant was taken , and centrifuged at 300 g for min and washed 3x in TC medium 199.

The Leucocytes were counted and the proportion between lymphocytes , neutrophils and eosinophils . was established ,RBCs – contamination was expressed as percentage of the number of leucocytes. Leucocytes ( $10 \times 10^5$  ) were suspended in 50 ul of each of the following solutions :

- TC 199 and 10% fetal calf serum (FCS ) .
- TC 199; 10% FCS and 25 ug of PPD.
- TC 199; 10% FCS and purified mycobacterium antigen at a final concentration of 1:3

Agaros 300 mg was heated at 100° C in 32 ml of distilled water , then incubated in a water bath at 50° C , and mixed with 4ml fetal calf serum (FCS) , 4 ml of 10x TC 199; 0.5 ml of tissue culture TC

bicarbonate . The final product was a gel with 0.75% agarose and 10% FCS in TC 199 (pH 7.4) . The gel was poured in petri dishes , when solidified , 4 wells were punched .

Leucocytes ( $2 \times 10^5$ ) were incubated for 18 hours at  $37^{\circ}\text{C}$  in humid atmosphere containing 2%  $\text{CO}_2$  in order to keep pH constant . The area of migration was measured after 8 , 18 and 24 hours of incubation . The migration index (MI) was calculated as :

$$\text{MI} = \frac{(\text{Area of migration with Antigen})}{(\text{Area of migration with Antigen})} \times 100 \quad [4]$$

## Results

The average number of migrating cells was  $108 \times 10^2$  (standard deviation  $51 \times 10^2$ ) . The value represented about 4-8% of leucocytes introduced into the well.

Table (1) showed the proportion of the different kinds of leucocytes introduced into well ; into zone of dense migration and in zone of non – dense migration . Figure (1) shows that the proportion of the cells within the zone of dense migration varied greatly according to the distance from the wells . This distance was expressed as the percentage of the longest distance of migration . Eosinophils could not be seen further than 25% of the maximum distance , Lymphocyte-monocyte could be seen as far as 75% of the maximum distance but their proportion was very low when counted so far from the well.

The average of the control migration (without Ag) was  $155 \text{ mm}^2$ , this corresponds to a surface of  $10 \text{ mm}^2$ . A correlation could be demonstrated between the proportion of polymorph nuclear cells (PMNs) and size of area of migration ( $r=0.47$  ,  $p=0.001$ ) . The contamination by RBCs was on the average 18.3% of total number of leucocytes and this contamination did not influence the size of migration area ( $r=0.04$ ) .

Table (2) shows the results of migration inhibition in individuals with negative and positive purified protein derivative (PPD) skin test . The results showed that the negative PPD skin test group characterized by a migration index greater than 80. In contrast , all (PPD) – positive healthy individuals had a migration inhibition less than 80 .

Figure (2) the average migration and the average migration inhibition as a function of inoculation time in individuals with positive PPD at 18 and 24 hours intervals . Both curves showed that the migration was negligible between 18<sup>th</sup> and 24<sup>th</sup> hour .

Table (3) showed that migration inhibition was higher in control group in comparison with patients group . This difference was significant ( $p<0.05$ ) .

### Discussion:

Leucocytes migration inhibition test is regarded as a rapid economical and simple with reproducible results and used as a tool to reflect the immune response [5]. A good correlation could be obtained between the PPD skin test and the inhibition of leucocytes migration by

Antigen in the control group and lesser degree in the patients group. This suggests that this test represents a state of delayed type hypersensitivity . Such correlation could not be

obtained during the course of tuberculosis due to depression of lymphocytes activity by the bacteria concerned [6] .

The depression might prevent migration inhibition by PMN but insufficient to prevent positivity of PPD skin test [3] .

It was demonstrated that leucocytes migration inhibition test (LMIT) was happened , after 6 hours of incubation . The area of migration in the presence of Antigen remained the same . While the migration in control specimens continued . This might suggest that this phenomenon reflect cell-mediated immunity rather than the effect of cytophlic Antibody [7]

When **Mycobacterium** antigen was used as an antigen a highly significant inhibition was observed from tuberculosis [7] .

The inhibition was independent of the presence of antibody , which suggests that the LMIT detect cell – mediated immunity rather than the effect of cell-bound Ab[5] .

In the majority of cases , it can be concluded that lymphocytes inoculate in blood in the presence of *mycobacterium* antigen which may saturate their binding capacity for an antigen , so they are unable to react ( a few hours later ) with weaker concentration of Antigen . While for PPD , are due to action of bacteria or liberation of inhibitors of lymphokines . [8]

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**Table (1) Morphological separation of leucocytes expressed as percentage (± SD)**

Cell	Suspension before migration	None of migration	
		Dense	Non-dense
<b>Neutrophils</b>	45±7.0	89 ±2.0	100
<b>Eosinophils</b>	7 ±2.2	2± 0.6	0
<b>Lymphomonocytes</b>	28± 3.5	9± 1.0	0

The results are the mean of 15 experiments

**Table (2) Leucocytes migration inhibition test in presence of PPD**

Group	1	2	3
<b>Group (a+b): Controls</b>	154	158	103
<b>Group b</b>	134	90	68
<b>Group c ( convalescence )</b>	179	137	73
<b>Group d (patients)</b>	170	153	93

1-migration in TC199

2-migration in TC199+Myc. Ag

3-migration index

**Table(3) Leucocytes migration in presence of mycobaterium AG**

Group	1	2	3
<b>Controls Group (a+b)</b>	130	124	105
<b>Group c</b>	199	126	71
<b>Group q</b>	168	178	103

1-migration in TC199

2-migration in TC199+Myc. Agenl. 1500x

3-migration index

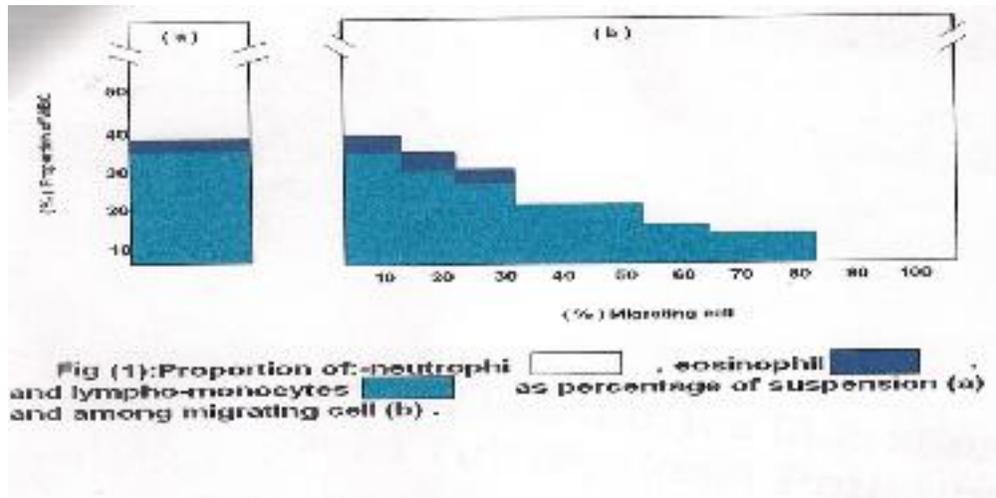


Fig (1): Proportion of neutrophils, eosinophils and lympho-monocytes as percentage of suspension (a) and among migrating cell (b).

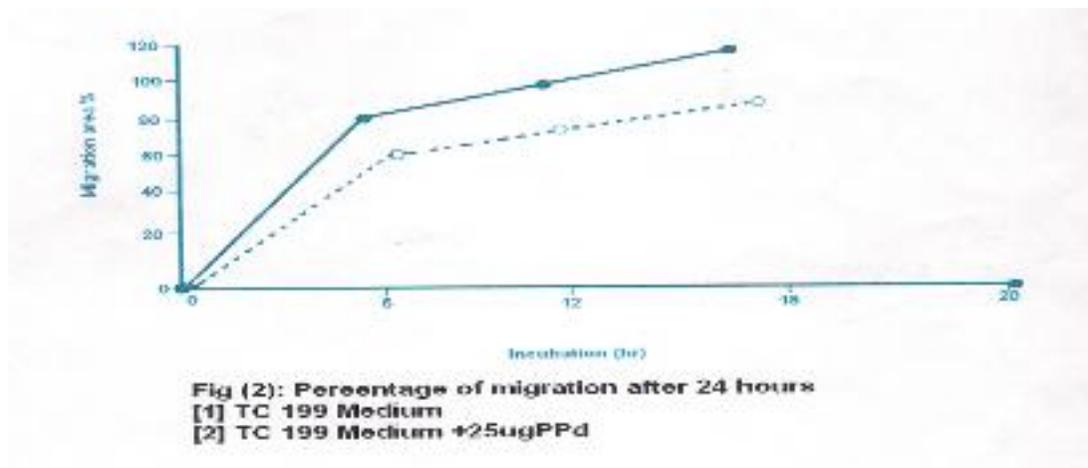


Fig (2): Percentage of migration after 24 hours  
 [1] TC 199 Medium  
 [2] TC 199 Medium +25ugPPd

مجلة ابن الهيثم للعلوم الصرفة والتطبيقية  
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تقييم كبح هجرة خلايا الدم البيض لمرضى السل

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### الخلاصة

نفذ اختيار منع هجرة كريات الدم البيض في الاكاروز تجاه مستضد السل *PPD, Mycobacterium tuberculosis*.

حسب نسبة اختلاف وتنوع هجرة خلايا الدم البيض بوصفها وظيفة لفعل مسافة الهجرة . وضح تطبيق هذا الاختبار في المشاكل السريرية باستخدام PPD مستضداً عالي، يثبط بشكل معنوي عالياً في الافراد الطبيعيين ذوي فحص PPD الجلدي الموجب وكذلك تبين ان التثبط عالياً معنوياً في الافراد المتعافين من السل . وضحت نتائج هذا الاختبار ملائمة للكشف الروتيني عن المناعة تتوسطها الخلايا .