

An evaluation study of cellular immunological functions in anergic tuberculous patients

Mohammed A.K.Al-Sa'adi

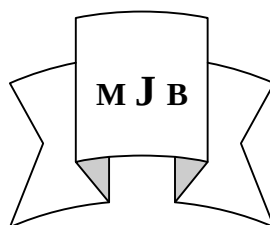
Babylon University, College of Medicine

Ibrahim M.S.Shnawa

Babylon University, College of Science

Salih Al-Mukhtar

TB Center in Babylon



Abstract

The present work aimed to evaluate the cellular immunological functions in TB patients among anergic tuberculous patients (AN), allergic tuberculous patients (AL), and controls (C). This was carried out on 125 tuberculous patients admitted to the TB center in Babylon Province during the period January/2003 to May/2004, and 23 apparently healthy controls. Tuberculin skin test was used to judge the immunoresponsiveness of tuberculous patients. The NBT, E-rosette, and LIF assays were used as parameters to evaluate the cellular immunological functions. The results showed marked decreasing in these functions in AN (4%, 14%, 57.14%) respectively in comparison to that of AL (7%, 21%, 37%) and C (11%, 25%, 88%). Thus these parameters may aid to be used as a diagnostic as well as immunological tools among the tuberculous patients.

الخلاصة

هدفت هذه الدراسة الى تقييم الوظائف المناعية الخلوية لمرضى التدرن الرئوي الخاملين والمتحسسين مناعيا مقارنة مع الاشخاص الاصحاء وتضمنت هذه الدراسة 125 مريضا بالتدرن الرئوي المراجعين لمركز التدرن الرئوي في بابل للفترة من كانون الثاني 2003 الى ايار 2004 وشملت الدراسة كذلك 23 شخصا طبيعيا كسيطرة واستعمل فحص السيلين الجلدي لمعرفة نمط الاستجابة المناعية للمرضى . استخدم فحص اختزال صبغة النايترورباعية الزوليوم الزرقاء وفحص التشكيل الزهري التالي واختبار فاعلية العامل المثبط لهجرة الخلايا البيضاء لتحديد الوظائف المناعية الخلوية وظهرت النتائج وجود انخفاض واضح في مستويات هذه العوامل في مجموعة الخاملين مناعيا مقارنة مع المتحسسين وكذلك السيطرة . لذلك فان هذه العوامل ربما تساعد عند استخدامها كوسائل تشخيصية مناعية في تحديد الحالة المرضية لمرضى التدرن الرئوي .

Introduction

M. tuberculosis had been defined as the causative agent of tuberculosis (TB) in 1882 by Robert Koch (1) . Approximately about one – third of the world's population is infected with *M. tuberculosis* and it is responsible for 8 – 12 million cases of active TB each year and 3 million deaths (

2,3,4,5) . The diagnosis of pulmonary TB is based on clinical symptoms , chest X- ray microscopic examination and sputum smear , cultivation of clinical specimen(mainly sputum)and immunological test (6) . *M. tuberculosis* induces cell – mediated immunity which is expressed by tuberculin

skin test (7) . When the immune system of tuberculosis patients fail to respond to *M. tuberculosis* , those patients are considered as anergic patients (8,9,10) .TB – associated anergy increases the severity of disease , as well as makes several diagnostic and theuraptic problems (11, 12) . This work aimed to point out a test – battery of choice for evaluation of cellular immune functions in anergic tuberculosis patients compared to that of allergic tuberculosis patients and apparently healthy controls.

Materials and Methods

I – samples

A – patients

The samples consisted of 125 (79 males and 46 females) tuberculosis patients of witch 82 were anergic group (AN) (tuberculin skin test was negative), whereas 43 were the allergic group (AL) (tuberculin skin test was positive). The judgment for the anergic status was based on the unresponsiveness to tuberculin skin test (13 , 14) . Those patient were admitted to the Babylon center of TB and chest diseases . Diagnosis of TB was established by the clinical picture X- ray chest examination ,and detection of acid fast bacilli .Fresh blood (5 ml) was drawn from each patient .

B – control

Twenty three apparently healthy subjects (18 males and 5 females) were also involved in this study as controls (C) as confirmed by respiratory clinician specialists . Fresh blood (5 ml) was drawn from each subject .

II – Methods

1- Tuberculin skin test :-

Tuberculin (parified protein derivative (PPD) – 2 unites) skin test was carried out for patient and controls according to (15)

2 – Evaluation of phagocytic activity of neutrophil :-

Nitrobluetetrazolium (NBT) test was used to check the phagocytic activity of neutrophil (16) .

3 – Estimation of T – cell count

E- rosette test was used to estimate the T – cell count (17) .

4 – Measurement of leukocyte migration inhibitory factor (LIF) assays for the factors that inhibit the movement of leukocytes away from their site of release ,are used as tests for the evaluation of cellular immunity (18) . This test was performed in accordance with (19)

III – Statistical analysis

Mean , median and two tailed T- statistics were used as statistical parameters (20)

Results

Age – sex distribution

The results expressed in table (1) revealed the wide range of age in AN patient (0.5-80 years) . The large group 13: 82 (15. 82 %) was fitted with an age range (25 – 29) year, AL patients expressed approximately the same age properties , their age ranging from (11 – 71) year and the large age group8:43 (18:604 %) was included in an age group (25- 29) year .

AN patients consisted of 47 : 82 (57.31 %) males , and 35: 82 (42 . 683 %) females . While AL patient consisted of 32 : 43 (74. 41) % males and 11 : 43 (25. 58) % females (table 1) .

The result of NBT test showed a gradual increasing values from AN,AL, to C (table - 2) .the results expressed in table (3) revealed that the values of T-cells count were higher in C than AL and in AL than AN. The results of LIF assay (table 4)expressed great differences in the LIF values among the three studied groups .regarding the border line (70%)the LIF of AL were significant 37.5%,whereas the lif of AN

were significant to a lesser extent 57.14%, but it was clearly evident that LIF results of C were non-significant 88.2%.

From the results expressed above NBT, T-cell count, and LIF assay were showing great significant differences among studied groups. (table-5)

Discussion

The age distribution of TB patient in this work was in agreement with that have been reported by Kaltenbach *et al.*, (21) who showed that there is no significant difference in the infection with *M. tuberculosis* between young and elderly patients and in the induction of anergic state, likewise, sex – distribution was matched with (22) Who mentioned that males are more susceptible to TB infection than females.

Regarding NBT, Park *et al.* (16) showed that the mean and range of NBT test are 72% and 5 – 11 % respectively in patients with primary TB, these findings were similar to that of AL patients reported in this work. The relatively decreasing value of NBT in AN may be due to: (a) malnutrition (b) immunocompromized effect (c) the deficiency in oxidative metabolism due to genetic defect in the *ets* gene which is responsible for this activity, and (d) drug effect (23 , 25). The decreasing T – cell count in AN greatly contributes to the impaired cell – mediated immunity to mycobacterial infection and in the induction of anergic form (25). Moreover; the immunological anergic state is induced in tuberculous patients when the T – lymphocytes count is sub normal (26).

The variation in the results of LIF could be attributed to the sensitivity of leukocytes, in anergic state, the sensitivity of LIF-producing cells is less than that of AL state (27). The non-significant values of LIF for C was in agreement to that reported by Veselic *et al.* (28). These non-significant values may be due to the absence of long term sensitization

, which is necessary for the expression of inhibitory function.

From the results expressed above NBT, T-cell count, and LIF assay were showing great significant differences among studied group, thus these parameters represent clear probes in the identification and characterization of the immunological state of tuberculosis patients.

Reference

- 1 – Fenton M.J. & Vermeulen M. W (1996). Infection & immunity 64 (3) : 683-690.
- 2 – Phalen , S.W. & McMurray , D.N . 1993 . Infection & immunity 61 (1) : 142 – 145
- 3 – Flynn , J – I . and Chan , J . (2001) . Infection & immunity 69(7) : 4195 – 4201 .
- 4 – Smith , I . (2003) . International paediatrics 18 (1) : 20 : 22
- 5 – Bloomberg , m r 2004 . the facts on tuberculosis
- 6 – Murray , P.R ; baron , E.J ; Jorgenson, J . h (eds) . 2003 . manual of chin . micro . 8th ed vol .1 , pp : 532 – 559 .
- 7 – Kaufmann, S.H. 2003 . Fundamental immunology. 5th ed , pp: 1229 – 1261
- 8 – Parslow , t. G ; Stites , d.p. ; Terr , a.j. ; and imboden , j.b medical immunology , 10 th ed , pp : 386 – 401 .
- 9 – Boussiotis , V. A. 2000 . J. Clinic . invest . 105 (9) : 1317 – 1325
- 10 – Bloom , B. R & McKinney , J . D 1999 , J . national med .
- 11 – Avicene , M . ; Rubio , M . (eds) 2001 . J . immunology . 167 : 2459 – 2468 .
- 12 – Beaman , S . 2003 . MMI 480 b – lecture – 11
- 13 – CDC, 1995 . diagnosis of TB , infection and disease . Atlanta , usa .
- 14 – Martin , P . 2003 . mantoux testing . guide lines for TB in new Zealand .
- 15 – Statens serum institut , 2002 core summary of product characteristics : tuberculin . denmark .

- 16 – Park , B . H ; Fikig , S . M ; & Smithwick , E . M . 1968 . infection & NBT . Lancet 2 : 532 – 534 .
- 17 – Gengozian , N . ; Hall , R .E ; & Whitehurst , C . E . 2002 .J . Exp. Bio .Med . 9 : 771 – 778 .
- 18 – Juttner , S . ; & Bernhagen ,J . 1998 . J . Immuni . 161 (5) : 2383 – 2390 .
- 19 – Soborg , M . 1969 . Acta . med . Scand . 185 : 221 – 225 .
- 20 – Cochran , W . G . 1974 . sampling techniques 2 nd ed .
- 21 – Kaltenbach , G . ; Granenberger , F ; Shlienger , J .I . (eds) . 2001 . press . medicine 30 (29) : 1446 – 1449 .
- 22 – Al – Damluji, S . F 1976 .Tuberculosis for medical students .
- 23 – Moretti , M .I . 1992 . j .am . med . helth . assn . 1 (1) : 1 – 33
- 24 – Anderson , K .I . ; Smith , K . A ; Pio , F . (eds) . 1998 . blood 92 (5) : , 1576 – 1585
- 25 – Dai , J . ; Phalen , S . ; & McMurray , D . (1998) . frontiers in bioscience e 110 – 122 .
- 26 – Layne , s . p . 2003 . tuberculosis , principles & infections disease . ucla school & public helth .
- 27 – Soborg , M . 1968 . acta . medica . scand . 184 : 135 – 139 .
- 28 – Veselic , B . ; Dekaris , D . ; & Hrsak , K . M (1972) . j . immune . 24 : 375 –384

Table (1) Age distribution and male-female ratio in anergic(AN),allergic(AL),and controls(C) .

Age group (Year)	AN	AL	C
0.5-<5	3	-	-
5-9	4	-	-
10-14	8	2	-
15-19	4	1	4
20-24	11	7	10
25-29	13	8	2
30-34	9	8	4
35-39	2	7	3
40-44	6	2	-
45-49	5	3	-
50-54	5	1	-
55-56	1	-	-
60-64	4	2	-
65-69	3	-	-
70-74	2	2	-
75-79	1	-	-
80	1	-	-
Age range (year)	0.5-80	11-71	18-36
Total and male-female ratio	82 (47 male + 35 female) 57.317% male 42.683% female	43 (32 male + 11 female) 74.418% male 25.582% female	23 (18 male + 5 female) 78.260% male 21.740% female

Table (2) Nitroblue tetrazolium in tuberculous and normal subjects.

Patient groups	Reduction values (%)		
	Mean	Median	Range
Anergic	5.4120	4.00	1-14
Allergic	7.1140	7.00	2-14
Controls	11.695	11.0	6-20

Table (3) E-rosette test for tuberculous and control subjects.

Patient groups	E-rosette values (%)		
	Mean	Median	Range
Anergic	14.260	14.0	3-26
Allergic	20.790	21.0	11-27
Controls	25.782	25.0	17-36

Table (4) Leukocyte- Inhibition Factor (LIF) of peripheral blood in tuberculous and control subjects.

Patient groups	LIF values (%)		
	Mean	Median	Range
Anergic	52.270	57.14	4.1-90
Allergic	36.158	37.50	4.1-77
Controls	85.640	88.20	78-98

Table (5) Two tailed t- statistics for the differences in cellular immune functions among the study groups.

Inter-group comparisons	Calculated t_{20+20}	Table t* value	P level	Significance
AL- C NBT	4.8050	3.922	0.001	High significance(HS)
LIF	15.245	3.922	0.001	HS
E- rosette	2.3490	2.101	0.050	Significance(S)
AN- C NBT	6.943	3.922	0.001	HS
LIF	4.300	3.922	0.001	HS
E- rosette	6.810	3.922	0.001	HS