

**COMPARISON OF ALLERGIC REACTION BETWEEN  
PARATUBERCULIN P.P.D. HEAT KILLED M. PARA-  
TUBERCULOSIS  
AND LEPROMIN IN LEPROSY PATIENTS IN IRAN(\*)**

By

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**INTRODUCTION**

Leprosy is caused in human by *Mycobacterium leprae*, since this organisms can not be cultivated in vitro and there is no suitable laboratory animal for the propagation of lepra bacillus, lepromin, usually prepared from the nodule (leproma) of the patient with clinical form of lepromatosis leprosy, is used for the determination of allergic reactions (FERNANDEZ) and diagnosis of kind of leprosy. Paratuberculosis is caused by another member of the mycobacteria family in the ruminant. *M. leprae* cultivation of this organism is difficult and does not grow on media used for cultivation of *M. Tuberculosis*. The two diseases have long incubation period and do not infect laboratory animals. In infected tissues *M. Leprae* and paratuberculosis exist in great number and the packet of the organism in macrophages are similar and forms the globie. Preliminary studies which were carried out in a leprosarium near Meshad, North East of IRAN showed a good antigenic relationship between the lepromin and killed paratuberculosis organism. In this study further investigations were carried out (On 158 leprous patient and their apparently healthy children located in Baba-Baghi leprosarium near Tabriz.

Two healthy individuals one tuberculin negative and the other tuberculin positive were used as control.

**MATERIAL AND METHOD:**

a) Lepromin (1): Leprom containing large quantity of the organism was taken under sterile precaution from a lepromatosis patient, the material was heated at 110°C for 20 minutes then homogenized at 1% suspension in saline. The homogenate was diluted 20 times in saline and once more heated as above. After addition of phenol at 0.5%, a sample was stained by Ziehl-Neelsen method and 6 to 8 organisms could be seen in each microscopic field.

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b) Paratuberculose Particulate Antigen: M. Paratuberculosis strain 316F, (11) was cultivated in synthetic liquid medium (12) for one month. The culture was homogenized then distributed in 50 ml. bottles and freeze dried, for use 10 ml. of saline was added in one vial and boiled for 30 minutes to kill the organism. The turbidity of the suspension was measured by brown turbidometer and its concentration was adjusted with saline to contain about  $7 \times 10^8$  organisms per ml. The suspension was diluted 100 times, then heated at  $110^\circ\text{C}$  for 20 minutes before use.

c) Paratuberculine P.P.D.(13) Prepared from 3 strains of M. Paratuberculosis 316F, 2e and II, obtained protein was precipitate with 4% trichloroacetic acid at  $+4^\circ\text{C}$ . Then dissolved in special buffer. This solution was titrated on sensitized guinea pigs. Concentration of protein was adjusted at 0.5 mg/ml. and used for the experiment.

d) Injection of Allergens: One hundred fifty eight leprosy patients and their families were selected for the test. These patients had various kind of leprosy, they had been classified and had been under treatment for ten years. All patients are injected I/D with 0.1 ml of lepromin on the right arm and 86 of them received 0.1 ml injection of particulate antigen on the left arm; the rest were injected with 0.1 ml I/D of paratuberculine P.P.D. prepared in this Institute. The results were recorded after 72 hours (Fernandez reaction) and after 21 days for Mitsuda reaction.

Patients tested in this study were as follows: 8,10).

1) Clinical form LL (polar lepromatosis): Eighty four persons of different age from 8 to 75 years. These patients had been under treatment from the date of diagnosis in this leprosarium and 85% were bacteriologically positive.

2) Clinical form BL (Borderline lepromatosis): These patients were from 20-60 years old and 56% of them were bacteriologically positive.

3) Clinical form B.T. (Borderline-Tuberculoide): From nine patients under investigation majority of them were bacteriologically negative.

4) Clinical form BB. (Borderline) which not more than five patients were available and 3 of them were bacteriologically positive.

5) Clinical form T.T.: Polar tuberculoide, from seven patients investigated all were bacteriologically negative.

6) Twenty seven children without any clinical symptom were investigated, these children were from one to fifteen years of age and had kept with their families, in Baba-Baghy leprosarium and had been in close contact with them, but from the beginning they had been under drug prophylaxie (4) with D.D.S.

7) Two healthy individuals were also investigated one tuberculin positive and the other negative who did not have any contacts with leprosy patients and were living in another town.

## RESULT AND DISCUSSION:

- 1) The results of the allergic reactions after 72 hours are shown in Table I.
- a) Allergic reaction with paratuberculin P.P.D. was more severe than the reaction with lepromin and particulate paratuberculosis antigen. In some instances fever and severe local reactions was observed.
  - b) In LL form 45.24% were positive in Fernandez reaction whereas the reaction with particulate antigen were 81.57% positive and paratuberculin P.P.D. 86.95% positive. Therefore, the reaction with paratuberculin quantitatively was nearly twice as severe as the reaction with lepromin. The severity of these reactions is shown in Fig. 1.
  - c) In BL form 26 patients were tested from these 61.54% were positive with lepromin and 88.46% were positive (showing severe reaction) with paratuberculin P.P.D. It should be mentioned that in some individuals skin reaction with paratuberculin was severe and sometimes was associated with fever and shivering.
  - d) BB form, from five patients tested 60% of patients showed positive reaction with both lepromin and particulate antigen.
  - e) B.T. form, from nine patients tested 55.55% were Fernandez positive whereas the positive reaction with paratuberculin P.P.D. was 88.88%.
  - f) T.T. form. The seven patients with this form showed equally positive reaction 71.42% in both Fernandez reaction and particulate antigen.
  - g) Children who were living with their leprosy families were 74.08% Fernandez positive but 96.24% showed positive reaction with paratuberculin P.P.D. and the local and general allergic reaction was more severe than with lepromin (Fig. No. 2.)

These results showed that in all forms of leprosy after 72 hours the allergic reactions with paratuberculous particulate antigen and paratuberculin P.P.D. were more severe than the reaction induced by lepromin.

2) The results of allergic reactions recorded after 21 days are shown in table II.

- a) After 21 days also allergic reaction with paratuberculin P.P.D. and with paratuberculous particulate antigen were more severe both quantitatively and qualitatively than the reactions with lepromin.
- b) In LL. form, 3.15% from the 80 patients tested had positive Mitsuda reaction at the time of this study, but with particulate antigen and paratuberculin P.P.D. the percentage of positive reaction was 83.68% and 69.56% respectively.
- c) In B.L. form, 15.38% were Mitsuda positive from the 26 patients tested, but 50% showed the positive reaction with paratuberculin P.P.D.
- d) In clinical form of B.B., from 5 persons tested 20% were Mitsuda positive but with paratuberculous particulate antigen the rate of positivity was 100%.

- e) In clinical B.T. form, 43.33% from the nine patients tested had positive Mitsuda reaction but with paratuberculin P.P.D. reaction 77.77% showed positive reaction.
- f) In clinical form T.T., from 7 patients 71.42% showed positive Mitsuda reaction and 85.72% had positive reaction with paratuberculose particulate antigen.
- g) In contact children who were apparently healthy and were under drug treatment the results were somewhat different from what was mentioned above. 81% of 27 children tested had positive Mitsuda reaction whereas with paratuberculine P.P.D. 44.44% of children showed positive reaction. Considering the results obtained in children after 72 hours in which 96.3% showed positive reaction with paratuberculin P.P.D. It can be suggested that because of drug treatment *M. Leprae* did not remain in children's body. The results showed that in the patients after 21 days the reaction with paratuberculose, particulate antigen and with paratuberculin P.P.D. was more severe than the reaction with lepromin.

In children however the situation was reverse and the reaction with lepromin was more severe than with paratuberculin and particulate antigen. It is possible to assume that a person who has been in contact with *M. Leprae* and has become infected will show a severe reaction with paratuberculosis particulate antigen. Lepromin can not be used for the detection of leprosy patients, perhaps because it is not pure and enough concentrated but shows the kind of the disease or the reactivity of the body towards lepra bacillus in the case of probable future contact. As human being is not susceptible to paratuberculosis and the percentage of the reactivity of paratuberculin was high in leprosy patients, so paratuberculin is recommended for investigation of allergic reaction of the leprosy patients.

In the two healthy individuals who were tested as control Mantoux test with 10 units (3) of tuberculin (0.2µg tuberculin P.P.D. on the right arm and 0.5µg paratuberculin on the left arm) was performed. One of the control had a positive Mantoux reaction with the size of 26x22 mm. the other control showed negative Mantoux reaction. Both controls had negative paratuberculin reaction. Since majority of leprosy patients, showed positive reaction with paratuberculin it seems that *M. Paratuberculosis* and *M. Leprae* give cross reaction and their reaction is more specific than the reaction with other mycobacterial allergens (2.5). Since there is an antigenic similarity between *M. Leprae*, *M. Tuberculosis* and *M. Paratuberculosis* and since B.C.G. has been used for the prophylaxis and treatment of leprosy (6,7,9) without complete immunity, it is proposed that the use of live *M. paratuberculosis* and mixture of B.C.G. with killed *M. Paratuberculosis* for prophylaxis and treatment of leprosy be investigated.

## SUMMARY

One hundred fifty eight leprous patients and children apparently healthy from Baba-Baghi leprosarium in Tabriz and two healthy individuals from another city were tested to estimate their response to allergic reaction with lepromin, paratuberculose particulate antigen and paratuberculin P.P.D. From 84 patients with clinical form LL, 45.24% showed Fernandez positive reaction with lepromin 81.57% with paratuberculose particulate antigen and 86.95% with paratuberculin P.P.D. only 3.51% of them had positive Mitsuda reaction but with the above allergens 71.42% should positive reaction. From 27 children apparently healthy tested 74.07% had positive Fernandez reaction but with paratuberculin 96.26% showed positive reaction after 72 hours. In Mitsuda test with lepromin 81.48% and with paratuberculin 44.44% of them showed positive reaction. One of the control healthy individual who was tuberculin positive showed negative reaction with paratuberculin. It seem that there is a great antigenic relationship between M.Leprae and M.Paratuberculosis.

## COMMENT:

It is recommended that gel precipitin test and MIF test be done in the sera of the leprous patients with paratuberculin P.P.D. and paratuberculose extracted antigen comparing with lepromin to find more relation between these two microorganisms.

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**TABLE I**

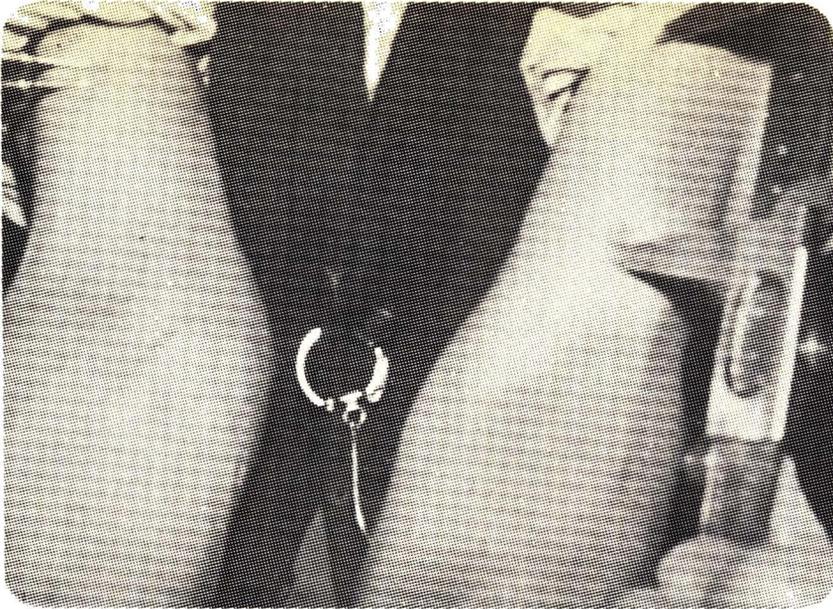
**Shows the comparison of the allergic reactions after 72 hours**

<i>Form of the disease</i>	<i>Lepromin</i>				<i>Particulate Antigen</i>				<i>Paratuberculin P.P.D.</i>				<i>Total</i>
	<i>+</i>	<i>%</i>	<i>-</i>	<i>%</i>	<i>+</i>	<i>%</i>	<i>-</i>	<i>%</i>	<i>+</i>	<i>%</i>	<i>-</i>	<i>%</i>	
LL	38	45.24	46	54.76	31	81.58	7	18.42	40	86.95	6	13.08	84
BL	6	61.54	10	38.46					23	88.56	3	11.54	26
B.B.	3	60	2	40	3	60	2	40					5
B.T.	5	55.55	4	44.4					8	88.8	1	11.11	9
T.T.	5	71.42	2	28.58	5	71.42	2	28.57					7
Children in contact	20	74.08	7	25.92					26	96.3	1	3.7	27

**TABLE II**

**Shows the comparison of the reactions with lepromin and paratuberculin P.P.D. and paratuberculoase particulate antigen after 21 days**

<i>FORM OF THE disease</i>	<i>Lepromin</i>				<i>Particulate Antigen</i>				<i>Paratuberculin P.P.D.</i>				<i>Total</i>
	<i>+</i>	<i>%</i>	<i>-</i>	<i>%</i>	<i>+</i>	<i>%</i>	<i>-</i>	<i>%</i>	<i>+</i>	<i>%</i>	<i>-</i>	<i>%</i>	
LL.	3	3.57	81	96.43	28	73.68	10	26.31	32	69.56	14	30.44	84
B.L.	4	15.38	22	84.62					13	50	13	50	26
B.B.	1	20	4	80	5	100							5
B.T.	3	43.33	6	66.66					7	77.77	2	22.22	9
T.T.	5	71.43	2	28.57	6	85.72	1	14.28					7
Children in contact	22	81.48	15	18.5					12	44.44	15	55.55	27



*Fig. 1*

Comparison of the reaction with lepromin and Paratuberculin P.P.D. After 72 hours in a patient. Left hand shows the reaction with paratuberculin and right hand shows the reaction with lepromin.



*Fig. 2*

Shows the allergic reaction in contact children after 72 hours. Right hand is injected with lepromin and left hand with paratuberculin P.P.D.