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Original Research Article

The induction of Mitsuda type of late response by tuberculin in patients with pulmonary tuberculosis and its significance

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ABSTRACT

Background: Traditionally, the kinetics of tuberculin testing has been studied up to 72 hours. In this study, we have studied the Mitsuda type of late response, usually seen in patients with leprosy after three weeks and also evaluated its significance in patients with pulmonary tuberculosis.
Materials and Methods: Thirty patients of pulmonary tuberculosis were divided into two groups based on sputum Acid-fast bacilli (AFB) examination. Group I patients were sputum AFB positive relapse (n=13). Group II patients were previously treated for pulmonary tuberculosis with negative sputum AFB (n=17). The induration was measured on day 3 and day 21, after tuberculin test with Purified protein derivative (PPD). A punch biopsy was taken from the site of induration on day 21 to study the histo-pathological characteristics.
Results: Four patients (23.5%) of Group 2 had an induration of more than 10 mm on day 21, while none from Group I had more than 10 mm size of induration. Granulomas, typical feature of 'Mitsuda' type of late response were seen in 92% and 100% in Group I and II respectively. The incidence of a granulomatous type of late response is 96.7% in patients with pulmonary tuberculosis.
Conclusions: The Mitsuda type of late response can be induced by the PPD, which we are using for conventional tuberculin testing. The late response appears to offer no protection in preventing relapse in previously treated patients for pulmonary tuberculosis

1. Introduction

The cutaneous response following tuberculin reaction has been studied up to 72 hours. Few studies in the recent and past, demonstrated the late reaction to PPD at the end of three weeks with an incidence of around 40% [1].It is similar to the late reaction (Mitsuda reaction) induced by Integral lepromin antigen in patients with leprosy, which shows well formed epithelioid granulomas [2]. Usually the early reaction in leprosy patients (Fernandez reaction) consists essentially of lymphocytes and monocytes, which is noticed after 24 to48 hours [3].

Intradermal injection of the heat killed whole M. leprae (lepromin) derived from infected armadillos produces both Fernandez and Mitsuda reactions. Fernandez reaction is measured after 48 to 72 hours, considered as positive if erythema is between 10-20mm which indicates delayed type hypersensitivity reaction. Mitsuda reaction is measured after three weeks with induration of more than four mm and presence of granulomas seen in the biopsy of induration site. A positive Mitsuda response denotes the presence of cell mediated immunity against M. leprae and usually seen in tuberculoid leprosy. So, the presence of late Mitsuda response indicates the protection against relapse of the disease. The sonicated M. leprae (Leprosin, a soluble antigen), is known to produce only the Fernandez reaction, not the Mitsuda reaction unless it is liposomised [4].It is generally believed that soluble mycobacterial antigens are not capable of producing the Mitsuda type ofreaction. [5]

But, Ramanathan et al., showed that a group of patients with cutaneous tuberculosis, developed the Mitsuda type of reaction with an induration of more than 10 mm in 43% of

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patients on day 21 biopsy specimen[1].Keeping these facts in mind, the present study was designed to demonstrate the late reaction to the tuberculin test and to evaluate the importance of the late reaction in determining the protective responses in preventing relapses in previously treated cases of pulmonary tuberculosis.

2. Materials and Methods

Sixty nine (69) patients with previous history of pulmonary tuberculosis were screened with sputum AFB examination, X-ray chest and blood routine investigations. The study was approved by the Institutional Ethics committee. They were divided into two groups based on sputum status. A written informed consent was obtained from study subjects. The study was conducted January 2020.

Group I (Study group): comprised patients with sputum positive relapse of pulmonary tuberculosis with no associated co-morbid illnesses.

Group II (Control group) comprised of sputum AFB and CBNAAT negative patients with history adequately treated open pulmonary tuberculosis five or more years back, with no associated co-morbid illnesses.

Among those patients, 10 of them had associated co-morbidities and 5 of them were not willing to participate in the study. So, they were excluded from the study. Tuberculin test was performed in all patients with one TU PPD-RT 23 given intra-dermally in flexor aspect of forearm by using 27 gauge needle. Size of induration was measured on day 3 and day 21by using ball pen method by an independent observer. After informed consent, a punch biopsy of 5mm thickness including epidermis and dermis was taken from the site of induration under local anesthesia by using a disposable biopsy punch forceps. The Specimen was preserved in 10% formalin. Cellular characteristics of the biopsy specimen were examined, after haematoxylin and eosin staining procedures.

The parameters noticed in H and E preparation were location of the granuloma in the dermis, organization of the granuloma, differential cell types as a percentage of total cells, granuloma index, presence or absence of necrosis, presence or absence of edema and presence or absence of congestion.

Six of the patients lost follow-up. Ten patients were not willing for biopsy. Eight biopsy specimens were distorted and too small to be reported by the Pathologist. So, they were excluded from the study. Total numbers of 30 patients were finally available for analysis in the study. Group I (Study group) comprised of 13 patients and Group II (Control group) comprised of 17 patients.

3. Results

There were 13 patients in Group I and 17 patients in Group II with an almost equal mean age distribution of 41.3 years and 41.2 years respectively. There were 9/13 (69%) men and 4/13 (31%) women in Group I and 15/17 (88%) men and 2/17 (12%) women in Group II. As depicted in Table 1, only 5 patients (38%) out of 13 patients in Group 1 had induration greater than 10 mm on day 3. On day 21, none had induration greater than 10 mm in Group I. Mean size of induration was 7.84 mm and 7.05 mm on day 3 and 21 respectively. Out of 17 patients in Group II, only 5 patients (29%) had induration size greater than 10 mm on day 3. After 3 weeks, 4 patients (24%) had induration greater than 10 mm in Group II. Mean size of induration was 4 mm and 6.17 mm on day 3 and 21 respectively (Table 1).

In Group I, none of the patients had increase in induration size from day 3 to 21. But in Group II, 7 patients (41%) had increase in induration size and 4 patients (23%) had same induration size on day 21. In Group II all patients had granulomatous response on biopsy taken on day 21. In Group I, except only one patient (8%), all others (92%) had granulomatous response (Table 2).

Well organized granulomas were noticed in only one case in both groups i.e., 8% in Group I and 6% in Group II. The mean Granuloma Index was 22.30 in Group I and 23.52 in Group II with p value of 0.78, ($p > 0.05$), which was not significant. The differential count of granulomas of both groups was almost identical with predominant lymphocytes, epitheloid cells and macrophages and few Langhans giant cells. Edema was the most striking feature seen in the granulomas of both groups. One patient (8%) in Group I had necrosis in the granuloma (Table 3).

4. Discussion

There were 30 patients with old history of adequately treated pulmonary tuberculosis divided into two groups based on their current sputum AFB status. Group I consisted 13 patients with positive sputum AFB and CBNAAT and Group II with 17 patients with negative sputum AFB and CBNAAT. The mean age of patients in both groups was almost identical with 41.3 and 41.3 years respectively. Both groups had more male patients since tuberculosis is more prevalent among male population and unwillingness of female patients for the biopsy of the induration site.

Induration size was measured on day 3 and 21, after tuberculin skin injection. The mean induration measured after 21 days in Group I was 4 mm, whereas in Group II, it was 6.17 mm with statistical difference of 0.05 ($p < 0.05$), which was found to be significant. On comparing the induration size from day 3 to day 21, about 41% of Group II patients showed increase in the induration size after three weeks, but all Group I patients showed decrease in induration size.

If induration more than 10 mm was considered as positive, none of the Group I patients had a positive response, but four patents (24%) of Group II had a positive response. The study performed by Ramanthan et

Table 1: Distribution of Induration size in Group I and II

Induration size (mm)	Group I		Group II	
	Day 3	Day 21	Day 3	Day 21
0	0	0	0	0
1-5	3 (23)	8 (62)	4 (24)	8 (47)
6-9	5 (38)	5 (38)	8 (47)	5 (29)
>10	5 (38)	0	5 (29)	4 (24)

[Figure in parenthesis denoted percentages]

Table 2: Change in induration size from Day 3 to Day 21

Change in induration size from Day 3 to Day 21	Group I	Group II
Decrease > 8mm	1(8%)	1 (6%)
Decrease by 4 - 8 mm	5 (38%)	3 (18%)
Decrease by 3 mm	7 (54%)	2 (12%)
No change	0 (0%)	4 (23%)
Increase by 3mm	0 (0%)	5 (29%)
Increase > 3mm	0 (0%)	2 (12%)

Table 3: The characteristics of the granuloma

Characters	Group I	Group II
Organization of Granuloma		
0	2 (16%)	3 (18%)
1	10 (76%)	13 (76%)
2	1 (8%)	1 (6%)
Granuloma Index		
0-20	8 (62%)	9 (53%)
21-40	4 (30%)	8 (47%)
41-60	1 (8%)	0 (0%)
Mean	22.30	23.52
Differential count %		
Lymphocytes	52.5	47.64
Macrophages	15.83	15.29
Epitheloid cells	31.25	37.05
Giant cells	0.58	0.88
Eosinophil	0.40	0
Polymorphs	0	0
Features		
Edema	12 (92%)	15 (88%)
Congestion	3 (25%)	3 (18%)
Necrosis	1 (8%)	0 (0%)

al., demonstrated late reaction (induration > 10 mm on day 21) in about 43% of cases in cutaneous tuberculosis. In our study involving patients of pulmonary tuberculosis, late reaction was seen up to 24% in Group II and 13% in total study population. But, the biopsy material Histopathological examination revealed contradictory findings with respect to induration size.

H and E stained preparation of biopsy taken from the test site after three weeks of tuberculin test showed granulomatous response (Figure 1) in all but one specimen (96.7%). This is in discordance with the induration size criteria (>10 mm) for late reaction. The reason may be due to less conspicuous inter and intracellular edema on day 21. Therefore, for studying late reaction, we recommend induration size is not a useful parameter, but biopsy of the induration site is the gold standard for demonstrating late reaction.

With the help of the pathologist, further finer characteristics of granuloma were sorted out. They were scattered in the dermis consisting of epithelioid cells, lymphocytes and a few macrophages along with moderate number of giant cells (Figure 2). The infiltrates were seen around blood vessels and appendages also. These features of the PPD induced granuloma seen on day 21 were similar to the late reaction of Mitsuda evoked by whole M. leprae or liposomised leprosin as reviewed by Narayanan.,[6].Thus, the results of the present study indicate that PPD is capable producing Mitsuda type of reaction in patients with pulmonary tuberculosis.

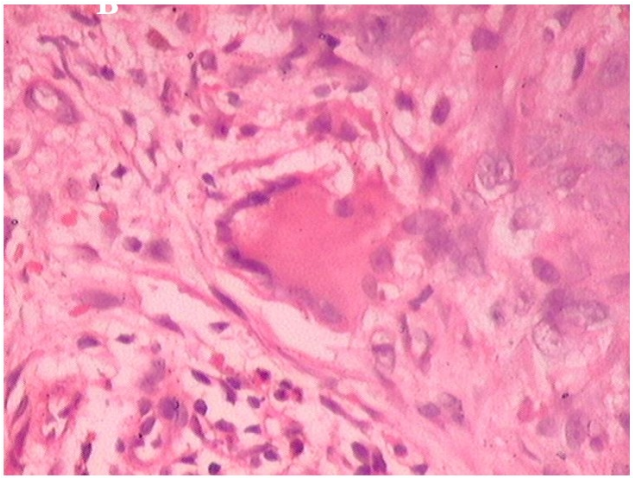

Fig. 1: H and E stained preparation of biopsy showing granulomatous response

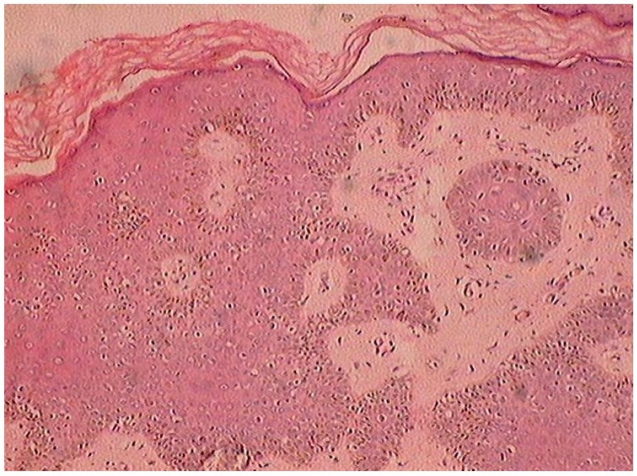

Fig. 2: Negative reaction with absence of the granuloma

The location of the granuloma in the dermis were found to be scattered along all the three layers namely, papillary dermis (Superficial Portion), Mid dermis and Reticular dermis (deeper portion) with the distribution of granulomas were more uniform in Group I than Group II. The pattern of organization was found to be similar between both populations, with more moderately organized granulomas. Two patients, one from each groups (8% and 6%) had a well-organized granuloma.

Granuloma Index (GI) was the most reliable parameter in comparing the granulomas of both groups. It was calculated by observing the percentage of the dermis occupied by the granulomas. Only one patient (8%) of Group I had a GI of 60. All other patients had GI of 40 and less. The mean GI in Group I was 22.30 and Group II was

The Statistical difference was 0.78 ($P>0.05$) which was not statically significant. On correlating the size of induration on day 21 with granuloma index, we could observe a negative correlation (-0.35) in Group I and a positive correlation (+ 0.11) in Group II. But, both parameters were not statistically significant.

The Differential count of cells in the granuloma was expressed as the percentage of individual groups of cells to that of total cells. Lymphocytes were the predominant cell population, followed by epithelioid cells and macrophages. Giant cells were noticed in smaller proportions.[7] Only one patient had eosinophil in the granuloma. There were no polymorphs seen in the picture. The mean differential lymphocyte count was 52.5%in Group I and 47.65% in Group II. The mean differential epithelioid cell count was 31.25% in Group I and 37.05% in Group II. Macrophages were seen in a mean differential count of 15.83% in Group I and 15.29% in Group II. We could infer that the differential count of cell population was almost similar in both groups. This could not differentiate the granulomas of two populations.

The other features like oedema, congestion and necrosis were also studied. The oedema was seen in most of the biopsy specimens, but it was less conspicuous than the day 3 reaction to PPD, as described by Ramanathan, 2004.[1] The inter and intra cellular oedema was abundant in only one patient belonging to Group II. Eight percent of patients in Group I and 12% of patients in Group II had no oedema in the day 21 reaction. This oedema was thought to be the cause for induration measured clinically.

Inflammatory cells which are the primary factors were not represented by the measurement of induration size. So, the induration size did not correlate with the presence of granulomas in the late reaction to PPD. The congestion was seen in a few patients of both groups (Group I - 25% and Group II - 18%). There was no significance with the presence of congestion. Necrosis was seen in only one patient (8%) in Group I. None had necrosis in Group II.

5. Conclusions

The Mitsuda type of late reaction can be induced by the PPD, which we are using for conventional tuberculin testing. The granulomas composed of lymphocytes, epithelioid cells, macrophages and Giant cells are the hallmark of the late reaction, similar to that of Mitsuda reactions seen in the leprosy patients. The incidence of late reaction is 96.7% in patient with pulmonary tuberculosis. The size of induration is not a useful parameter to report the late reaction. The distribution of granulomas and differential count of the cellular population are almost similar in both who are relapsed and not relapsed. The late reaction appears to offers no protection in preventing relapse in previously treated patients for pulmonary tuberculosis.

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8. Conflict of interest

None.

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