FIELD RESEARCH

THE EFFICACY OF BCG VACCINATION – A BRIEF REPORT OF THE CHINGLEPUT BCG TRIAL

By

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Though BCG vaccination has been practised for nearly 60 years, its efficacy has always remained a subject of controversy. The efficacy, in terms of the effect of the vaccine in reducing the incidence of tuberculous disease among the vaccinated has been demonstrated conclusively\(^1,2\) in animals. However, among human subjects the protective effect can only be demonstrated through controlled clinical trials. A large number of such studies have been published from various developed countries of the world though all these studies, especially the earlier ones, cannot be classified as statistically valid. Table No. 1 presents the protective effects observed in seven statistically valid BCG trials conducted in different parts of the world.

<table>
<thead>
<tr>
<th>Trial and subjects</th>
<th>Ref No.</th>
<th>Intake period</th>
<th>Duration of Observation (Yr)</th>
<th>Percentage protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. American Indians: (1 to 18 yrs age)</td>
<td>3</td>
<td>1935–38</td>
<td>9–11</td>
<td>80</td>
</tr>
<tr>
<td>Chicago : Infants</td>
<td>5</td>
<td>1937–48</td>
<td>12–23</td>
<td>75</td>
</tr>
<tr>
<td>Georgia : School children (6 to 17 yrs age)</td>
<td>6</td>
<td>1947</td>
<td>20</td>
<td>Nil</td>
</tr>
<tr>
<td>Puerto Rico: general population (below 20 yrs age)</td>
<td>7</td>
<td>1949–51</td>
<td>5½–7½</td>
<td>31</td>
</tr>
<tr>
<td>Georgia and Alabama : general population (age 5 yrs &amp; above)</td>
<td>8</td>
<td>1950</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Great Britain : school children (14 to 15½ yrs age)</td>
<td>4</td>
<td>1950–52</td>
<td>15</td>
<td>78</td>
</tr>
<tr>
<td>Madanapalle, south India: general population (all ages)</td>
<td>9</td>
<td>1950–55</td>
<td>9–14</td>
<td>30</td>
</tr>
</tbody>
</table>

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One of the earliest studies reported was conducted by Aronson and others (1958) among North American Indians. This study demonstrated that BCG provided a high degree of protection, of the order of about 80 percent. Similarly a study conducted by the Medical Research Council, Great Britain (1963) using two vaccines viz., BCG vaccine and a vaccine prepared from cultures of vole bacillus (M. Microti) also demonstrated that both these vaccines gave almost similar degrees of high protection of about 80 percent and further, the same degree of protective effect lasted for about 10 years of observation. Also, a twenty year study among infants in Chicago demonstrated a high degree of protection. On the contrary, the three American studies all started during the late forties demonstrated either a very moderate or no protection by BCG vaccination. Finally, a small study among the rural general population conducted in India by Frimodt-Moller et al., (1973) demonstrated that in the first few years after vaccination there was no evidence of the protective effect which appeared later at about 8 years after vaccination but again virtually disappeared.

An explanation offered for these contradictory results was that infections by mycobacteria other than the M. tuberculosis, causing nonspecific sensitivity to tuberculin and offering a certain degree of protection against tuberculosis, might have masked the protective effect of BCG in the American trials. It was also suggested that the potency of vaccines used in the American trials was low in comparison to that used in the British trial. The vaccines used in these trials were not available for further laboratory and other tests and no further analysis of data collected in these studies provided any other clues on the variability of results between the British and the American trials.

In view of the above and also the fact that BCG was extensively being used in India there appeared a need for undertaking further field trials in India, wherein all the known shortcomings of previous trials could be eliminated. The techniques of freeze-drying, developed in the meanwhile, had made it possible to prepare a stable BCG product and thus maintain the BCG strain as a 'seed-lot' without the risk of further genotypic changes. From the beginning it was envisaged that the protective effect of BCG would be studied in atleast two areas in India viz., an area with a high prevalence of non-specific sensitivity and another without or with a low prevalence of non-specific sensitivity. An extensive search made for an area of the latter type soon showed that in most areas in India the non-specific sensitivity was highly prevalent. Thus even though theoretically a trial in an area with low prevalence of non-specific sensitivity is of considerable interest, its applicability to Indian conditions is small.

Following the decision to undertake a BCG trial in India, all the preliminaries, essential for such a trial, were carried out. The objectives of the study were defined, a set of work-instructions for standardised techniques and procedures were drawn up and personnel were meticulously trained for the same. In the selection of an area for the study, one condition appeared to be of paramount importance: that no previous BCG vaccination should have been conducted in that area. Only one or two districts were available in the whole country satisfying this condition and thus a contiguous part of Chingleput District in Tamil Nadu State was selected as the study area.
Prior to start of the study several preliminary investigations were conducted around the study area selected. These investigations, which also formed a part of the training of study personnel, showed that non-specific sensitivity was highly prevalent in the district as also that the prevalence of tuberculous disease as well as leprosy was high. It was assumed that incidence of tuberculous disease would also be high in the area, and that an adequate number of new cases would be available for valid comparisons within a relatively short period of time.

The objectives of the study were to obtain: (1) a precise estimate of the protective effect of BCG vaccination against tuberculosis in the non-infected (i.e. tuberculin negative subjects) (2) the effect of BCG vaccination in persons already infected, (3) protective effect of different strains of BCG, and (4) epidemiological data on tuberculosis in the community.

Choice of BCG strains, vaccine dosages and placebo

It will be recalled that the study was planned on two premises. Firstly, that in previous studies where no protection was observed, the cause was mainly due to the low potency vaccines used; secondly, that the protection, though of a low order, offered by infections with acid-fast bacilli could have masked the protective effect of BCG. It was thus imperative that the vaccine or vaccines selected to be employed in the study would be of a very high quality.

It is well known that all BCG vaccines used in the world originate from the BCG culture first prepared in 1921 by Drs A. Calmette and C. Guerin after repeatedly subculturing *M. bovis* for 13 years. Even so, further subculturing for production of vaccine in different BCG laboratories of the world have changed the characteristics of the BCG bacilli differently in different laboratories. Thus various ‘strains’ of BCG are identified. The quality of BCG vaccine is tested on the basis of three main criteria: animal experiments for protection in animals; in-vitro tests for viability of BCG organisms and finally, allergising ability of the vaccines in human subjects. Several BCG strains were tested as above for selection of BCG strains to be used in the study. Of those strains, three strains, one obtained from Rio de Janeiro, the second from Copenhagen (strain 1331) and the third from Paris (1173 P2) were considered the best among those tested. At the time of the beginning of the study extensive use of the Rio de Janeiro strain in human subjects had not been done. As such, the Copenhagen and the Paris strains were selected to be employed in the study. The Copenhagen strain is also used for BCG vaccine production in India.

As for the dose with which to vaccinate the population in the study, 0.1 mg of moist weight of BCG bacilli has been identified as the appropriate dose (and will be referred to in this paper as standard dose) for vaccination for both the above strains of BCG. This dose is compatible with a high post-vaccination allergy with a very low rate of complications in the vaccinated. However, when BCG is handled badly the viability of the organisms may be sharply reduced and under such conditions, whether BCG would still give protection would be of great interest under programme conditions. Thus two doses were employed i.e. 0.1 mg and 0.01 mg the latter being one tenth the former standard
dose. Samples of vaccines actually used in the study have been preserved as seed-lots at very low temperatures and can at any time be retrieved and used for laboratory, animal or other experiments.

In order to elicit the status of tuberculous infection in the study population, PPD-S was selected. PPD-S, prepared by Florence Seibert in 1941 happens to be still the best tuberculin available, and is also adopted as the international reference preparation of tuberculin by the WHO. For the elicitation of non-specific sensitivity a mycobacterin named PPD-B prepared from Battery bacillus, later classified as *M. intracellulare*, was selected. For eliciting tuberculin sensitivity a dose of tuberculin containing 3 IU (International units) of PPD-S in the freeze-dried form, corresponding to 5IU of PPD-S in liquid form was used. For eliciting the non-specific sensitivity a dose of 10 units of PPD-B was used.

The controls were given an injection with a placebo of Dextran-500. The preparation also freeze dried, closely resembled, freeze-dried BCG vaccine in appearance.

The study design and study population

The study design can be considered as a factorial design, the factors being the vaccine strain and the vaccine dose. Both these factors combined in a single experiment resulted in the allocation of each eligible individual in the study population, to one of the following 5 'vaccines' (placebo is not a vaccine):

a. Strain 1331, 0.1 mg
b. Strain 1331, 0.01 mg
c. Strain 1173, P2, 0.1 mg
d. Strain 1173 P2, 0.01 mg
e. Placebo

The study population included the total population of about 360,000 persons in 209 contiguous panchayats (a panchayat is a village with its hamlets) and one town in Chingleput district. Individuals were allocated to one of the above 5 'vaccines' according to a randomised double blind scheme.

Methods

The procedures adopted for intake of the study population were briefly as follows: The entire population was first registered on a household basis going from village to village and within the village, from house to house. For each individual, one card was made out at the time of registration, wherein all the identification particulars as well as details of all investigations done at intake were recorded. The eligibility of study individuals for investigations at intake were as follows:

(i) Tests with PPD-S and PPD-B were offered to each individual aged one year and above
(ii) 'Vaccination' according to allocation, was offered irrespective of tuberculin sensitivity to each individual aged 1 month and above.

(iii) X-ray examination (using Mass Miniature Radiography with 70 mm films) was offered to each individual aged 10 years and above.

(iv) 2 Sputum samples were collected from each individual showing any abnormality on X-ray. From the sputum samples collected, direct smear, using fluorescence microscopy, and sputum culture with identification tests for mycobacteria, and drug sensitivity tests on positive cultures were carried out.

The procedures of follow-up for identification of new cases occurring in the study population after intake, were as follows:

(i) Resurvey: Once every 2½ years every individual, in the study population, aged 5 years and above was offered an x-ray examination, with sputum examinations as at intake to those with any x-ray abnormality.

(ii) Selective case-finding: Twice or thrice between any two resurveys i.e. at intervals of 7 or 10 months, individuals showing abnormalities on chest x-rays in any previous x-ray examination as well as those with defined symptoms suggestive of pulmonary tuberculosis were offered follow-up x-ray and sputum examinations.

(iii) Passive follow up: The project established a tuberculosis clinic in the main town situated in the study area. At this clinic, any individual attending with symptoms suggestive of pulmonary tuberculosis was offered x-ray and sputum examinations. In addition, in the area of the study, there were 10 health institutions such as Primary Health Centres, dispensaries, hospitals etc. At each of these institutions x-ray and sputum examination facilities were offered once or twice a month for symptomatics attending voluntarily at these centres. Treatment of cases diagnosed was also carried out through these centres.

It will be appreciated that the intake procedures were designed to identify the tuberculin, chest x-ray and sputum status of individuals at the time of ‘vaccination’ in order to identify accurately persons in whom the protective effect of BCG could be studied. The follow-up procedures were designed to identify all new cases of pulmonary tuberculosis that occurred in the study population after ‘vaccination’.

In addition to the follow-up procedures enumerated above, the status of tuberculin allergy following vaccination (post vaccination allergy) was assessed after three intervals following 'vaccination' viz. at 2 ½ months, at 2½ years and at 4 years.
Post-vaccination allergy soon after vaccination is an index not only of the quality of vaccine used but also the efficiency of vaccination procedures such as dose injected, preservation and use of vaccine etc. The assessment was done in mutually exclusive representative samples of the study population.

Objectivity of procedures and diagnosis

A carefully conducted double-blind study ensures, to a large extent, objectivity of procedures by eliminating bias. Even so, bias in other forms could be introduced. In a BCG trial such as this, bias can be introduced at the time of diagnosis of a new case, because of the presence of a BCG scar, especially when diagnosis is made on clinical basis and not on objective findings such as sputum positivity.

In the present study complete objectivity was ensured. The identity of ‘vaccines’ with which individuals were vaccinated was never available to the project staff. The vaccines were coded in the laboratories and the details of the codes were kept at the Indian Council of Medical Research (ICMR), New Delhi. When finally it was decided to find out the distribution of all new cases as those vaccinated and controls, the decoding was done (on a computer) by the ICMR headquarters. Similarly, establishing the diagnosis was based on sputum examination or x-ray examination results. Further, in order to be sure that the identity of individuals was correct, finger-prints taken at the time of vaccination were compared to a second set of finger-prints taken at the time when an individuals was diagnosed as a case of pulmonary tuberculosis at follow-up.

Characteristics of the study population

In all, 366,625 persons were registered of whom 272,455 were vaccinated. The x-ray coverage was of the order of about 82 percent and sputum examination coverage 93 percent. Only 3.3 percent of the total population showed scars in the deltoid region suggestive of previous BCG vaccination. Since no previous BCG programme had been conducted in the area, these persons had probably been vaccinated elsewhere.

On the basis of analysis of the distributions of tuberculin reactions in different age and sex groups as well as among TB cases, in the study population, it was decided that 12 mm would be the demarcation line for classifying the infected (12mm and above) and the uninfected (0-11mm). Based on this criterion, about 50 percent of the total population was classified as infected, the infection prevalence continuously rising as age advanced from about 5 percent in the 1-4 year age group to about 80 percent among those aged 50 years and above. Prevalence was higher among males than among females. These prevalences are higher than those found in similar studies elsewhere in India. Similarly, prevalence of reactors to PPD-B was also very high, being about 34 percent in the 1-4 year age group. By the age of 20, almost all persons were reactors to PPD-B. Annual negative incidence of infection (calculated from those that were, at intake, negative to PPD-S but at the 2½ or 4 year retestings were classified as infected) was also very high in the population.
On the basis of the above observations it can be concluded that the risk of tuberculous infection as well as the risk of infection with non-specific mycobacteria was very high in the study population.

The population had also, a very high prevalence of tuberculous disease. One percent of all persons aged 10 years and above were bacillary cases of tuberculosis proved on culture of sputum samples. The prevalence was 1.7 percent among males and 0.4 percent among females. The annual incidence of new bacillary cases in the community was also high. However, most of the new cases that occurred during the observation period occurred from those who were previously (i.e. at the time of intake) tuberculin positive rather than those who were tuberculin negative. Thus the annual incidence of bacillary disease among the tuberculin positives i.e., those reacting with 12 mm and above to tuberculin at intake was 4.98 per thousand whereas among the tuberculin negatives i.e., those reacting with less than 12 mm to tuberculin at intake, it was only 0.26 per thousand.

In summary, the population can be considered to have had the following main epidemiological characteristics: (a) a high prevalence of tuberculous infection and non-specific sensitivity; (b) a high incidence of tuberculous infection; (c) a high prevalence and incidence of tuberculous disease; (d) prevalence and incidence of tuberculous infection and disease higher in males than in females and, (e) most new cases of tuberculosis at intake and not from among those that were infected after intake - in other words, the risk of manifest disease for those recently infected was relatively small. The other and more detailed epidemiological features of the study population would be reported separately.

The Effect of BCG Vaccination

The proportionate reduction in the incidence of tuberculosis among the vaccinated, initially tuberculin negative individuals as compared to a similar but unvaccinated controls is termed the protective effect of BCG vaccination. In this study as until now, the protective effect is studied over a period of 7½ years of follow-up of the population.

As stated earlier, individuals reacting will 0-11 mm induration to tuberculin PPD-S were regarded as tuberculin negatives. However, it is well known that there is some overlapping at the higher levels of reaction (say 8-11 mm) in the negatives, due to the fact that some actually infected individuals may also show slightly smaller reactions than 12mm. In order to obviate this, individuals reacting with 0-7 mm and those reacting with 8-11 mm. Those reacting with 0-7 mm at intake can be considered as ‘definite’ non-reactors. Indeed new cases occurring in these two groups can be added together to obtain numbers of new cases occurring in the 0-11 mm groups.
TABLE 2

DISTRIBUTION OF NEW CASES DURING THE FIRST 7½ YEARS OF FOLLOW-UP ACCORDING TO VACCINATION STATUS

<table>
<thead>
<tr>
<th>Tuberculin reaction at intake</th>
<th>B.C.G</th>
<th>Placebo</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1 mg</td>
<td>0.01 mg</td>
<td></td>
</tr>
<tr>
<td>Cases with at least two positive cultures</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-7</td>
<td>37</td>
<td>37</td>
<td>28</td>
</tr>
<tr>
<td>8-11</td>
<td>14</td>
<td>17</td>
<td>14</td>
</tr>
<tr>
<td>12-15</td>
<td>40</td>
<td>34</td>
<td>38</td>
</tr>
<tr>
<td>16 or more*</td>
<td>259</td>
<td>257</td>
<td>287</td>
</tr>
</tbody>
</table>

| Cases with one positive culture only |
| 0-7                                | 22     | 28      | 19    | 69   |
| 8-11                               | 4      | 3       | 5     | 2    |
| 12-15                              | 20     | 13      | 8     | 41   |
| 16 or more*                       | 90     | 91      | 82    | 263  |

| X-ray cases according to review** |
| 0-7                                | 19     | 18      | 18    | 55   |

* Only a part of this group was eligible for follow-up at 2nd and 3rd resurveys

** Follow-up period: 5 years.

Table 2, presents the number of new cases occurring in different reaction ranges and given different vaccine-strengths or placebo irrespective of strains of BCG. It will be observed that 102 new cases of pulmonary tuberculosis, in whom at least 2 sputum samples were position on culture at the time of diagnosis were diagnosed among those reacting with 0-7 mm to PPD-S at intake. Of these 102 cases, 37 occurred among those vaccinated with the standard dose of BCG, 37 among those vaccinated with the weaker dos and 28 among those given placebo. It is seen at a glance that BCG did not offer any protection against the development of pulmonary tuberculosis over a period of 7½
years after vaccination. In effect, in none of the reaction ranges, BCG offered any protection.

The protective effect was also analysed according to whether BCG gives protection in different age groups, e.g. in children below 15 years of age. No such effect was apparent. The material was further analysed in relation to time of development of new cases. During the first and the second 2½ year periods after vaccination, the tendency was to obtain a larger number of cases among the vaccinated but during the third 2½ years this tendency was reversed, though it cannot be said that protective effect did become apparent during the third 2½ years.

Discussion

The study was planned to obtain a precise estimate of the protective effect of BCG vaccination. In previous studies where no protection was observed the main reasons attributed were the presence of infections with atypical mycobacteria offering low degree of protection and thus masking the protection offered by BCG and, use of vaccines of low potency especially the latter. In this study, every care was taken to control the latter while the former was not controllable. Even so, BCG showed no evidence of any protective effect. Under the circumstances, it is perhaps appropriate to discuss the probable reasons for the complete lack of protective effect in this population.

One cause that obviously suggests itself is the influence of non-specific mycobacterial infections. Carefully conducted animal studies do not support this hypothesis. In those studies, non-specific mycobacteria of the type most prevalent in the area of the Chingleput study gave only a low degree of protection. Thus, even if these mycobacteria did offer some protection, it could not have completely masked the protective effect due to BCG.

Another reason one must consider is that whether some methodological errors had crept in giving fallacious results. A painstaking study of the procedures adopted, was made by more than one group of experts—both national and international. It was decided that no methodological errors could have crept in.

Finally, it appears that the differences between protective effect observed in this study and that observed in the equally carefully conducted British study (Medical Research Council, Great Britain, 1963) where a high protection was observed, can probably be attributed to the differences in the epidemiological situation between the study populations.

Briefly, it is as follows: The British study population consisted of only adolescents aged about 15 years, whereas in this study the total population formed the study population. There are several such differences because of which the studies are not strictly comparable. Even so, in the British study, over half of all new cases occurred among the initial tuberculin negatives. In the present study, less than 5 percent of all new cases occurred among the initially tuberculin negative population. A more detailed epidemiological reasoning suggests that the evolution of pulmonary tuberculosis, from infection to manifest disease, may be vastly different in India than in the British population.
This hypothesis indeed needs confirmation through further studies.

Thus, it is seen that in this country, BCG vaccine did not protect against pulmonary, especially bacillary tuberculosis. This is possibly true of many other developing countries. The study does not answer the question whether BCG vaccination offers protection against 'childhood forms of tuberculosis' such as tuberculous meningitis, tuberculous lymphadenitis, miliary tuberculosis, tuberculosis of the spine, joints etc. Though no valid data are available on the size and extent of the problem of these kinds of tuberculous disease, especially in the rural areas, it is common knowledge that the management of many of these forms of tuberculosis is difficult and the sequelae are crippling, if not death altogether. The evolution of these forms of tuberculosis is also essentially different from pulmonary bacillary tuberculosis and is more akin to the tuberculosis observed in animal experiments where BCG has repeatedly been shown to offer protection. It is likely therefore that BCG may offer protection against childhood forms of tuberculosis. It is prudent in view of the seriousness of tuberculosis in childhood that the practice of vaccination of infants may not be discontinued.

* Detailed report of the study is published as follows:


REFERENCES


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