

**TUBERCULIN RESPONSE OF
ETHIOPIAN CHILDREN
AFTER BCG VACCINATION AT BIRTH**

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By:

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ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

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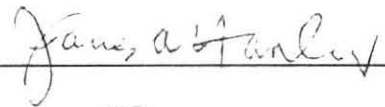
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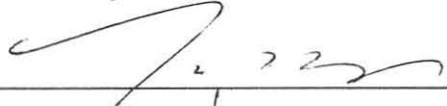
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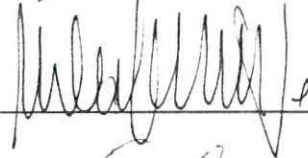
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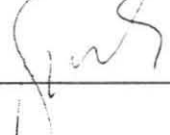
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I wish to dedicate this paper to

My Mother

For her un comprising principle of life

and to

My sister W/o Almaz Getahun

For leading her brother into intellectual
pursuits

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LIST OF CONTENTS

	<u>PAGE</u>
ACKNOWLEDGEMENT	i
LIST OF CONTENTS	ii
LIST OF TABLES	iii
LIST OF FIGURES	iv
LIST OF ABBREVIATION	v
ABSTRACT	vi
INTRODUCTION	1
<hr/>	
LITERATURE REVIEW	5
Quality Control Testing	7
Effect of BCG Vaccine	11
BCG Vaccine in the New Born and Young infants	19
OBJECTIVE	26
METHODOLOGY	27
Study Area	27
Subject Selection	27
Sample Size Determination	30
Data Collection	33
Test Procedure	33
Statistical Analysis	34
RESULTS	37
DISCUSSION	56
CONCLUSION	71
RECOMMENDATION	74
REFERENCES	76
ANNEXES	82
GLOSSARY	85

LIST OF TABLES

<u>PAGE</u>	
1.	Major controlled Trials on Protective Effect of BCG Vaccine Against Tuberculosis 18
2.	Characteristics of study subjects 43
3.	Frequency Distribution of the Size of BCG Scar by Age Group 44
4.	Frequency Distribution of BCG Scar by Sex 45
5.	Frequency Distribution of Tuberculin Reaction by Age Group 46
6.	Frequency of Tuberculin Reaction by Sex 47
7.	Correlation Between the Size of BCG Scar and PPD induration 48
8.	Relationship Between the Absence of BCG Scar and Positive Tuberculin Reaction by Age Group 50
9.	Relationship Between the Presence of BCG Scar and Negative Tuberculin Reaction by Age Group 51
10.	Prevalence of Tuberculin conversion with the Presence of BCG Scar by Age Group 52
11.	Crude Prevalence and odds Ratios of Tuberculin Non Reactors by Selected Characteristics 53
12.	Relationship Between BCG Scar Sizes Before and After the 2 nd BCG Vaccination 54

LIST OF FIGURES

<u>PAGE</u>		
1.	Graphic Representation of Study Design	36
2.	Distribution Pattern of Tuberculin Reaction in Different Age Groups	47b
3.	Correlation Between BCG Scar Size and PPD Induration	49
4.	The Frequency of Tuberculin Reaction in Smear +ve Tuberculosis Patients	55

LIST OF ABBREVIATIONS

BCG	-	Bacillus Calmette Guerin
EPI	-	Expanded Programme on Immunization
LIF	-	Lymphocyte Inhibition Factor
PPD	-	Purified Protein Derivative
U.A.E.	-	United Arab Emerates
WHO	-	World Health Organization

ABSTRACT

In an attempt to evaluate the effectiveness of neonatal BCG vaccination policy, a tuberculin survey was conducted among different age groups of children in Lideta awraja, Addis ababa who had received BCG vaccination shortly after birth. The survey consisted of assessing the immunization records and nutritional status and conducting clinical histories and physical examinations. Thereafter, each study subject's site of BCG vaccination on the right shoulder was assessed and the size of the scar graded. Tuberculin PPD tests were also performed simultaneously, and tuberculin reaction were read within 96-120 hours. A standard data collection form was used to record address, age, sex, body weight, BCG Scar and tuberculin response.

In addition, 60 children were revaccinated at the age of 10 years (within the study period) and tuberculin tests were performed 2 months after the second vaccination.

Furthermore, 70 bacteriologically proven tuberculosis patients were also tuberculin tested at the beginning of the study, in order to check the potency of tuberculin PPD test and to estimate the diagnostic value of the test.

A total of 895 children were studied between October 1992 and February 1993. Of these 563 (63%) had a definite BCG scar while the rest 331 (37%) were without a Scar. The sex distribution in each group was similar. The percentage of tuberculin non reactors in different age groups was 39.5% at 2 months, 34.5% at 18 months, 39.1% at 5-7 years, and 46% at 9-11 years. The number of tuberculin non reactors initially showed a slight decline from age 2 months and then an increase from age 18 months onward; the overall pattern of induration size was

statistically significant different between the age groups ($P < 0.001$). Some 55% of the children without a BCG scar showed no response to the tuberculin test, while 67% of those with a scar showed a positive tuberculin response. A direct correlation was observed between tuberculin reactivity and the size of BCG scar ($P < 0.001$). Meanwhile, a positive tuberculin reaction (> 6 mm) was observed in 11% of children despite the absence of the BCG scar. Children without the BCG scar have 2.53 times the risk of a negative tuberculin reaction compared to those with a BCG scar ($P < 0.001$). All the children revaccinated at the age of 10 years showed a positive tuberculin reaction and elicited a larger size of BCG scar (> 4 mm). The tuberculosis patients showed tuberculin reactions according to an unimodal distribution. Of these 12% showed 10 -15mm induration. A reaction of 10-15 mm were also observed in 3.3% of children without a scar, 11% of children with a large scar size (> 5 mm) and in 10% of revaccinated children without tuberculosis infection. This revealed that the lack of specificity of the test.

Overall, this finding emphasized the poor tuberculin response in infants at the age of 2 months compared to 18 months after vaccination at birth and the waning effect of tuberculin response at the age five years compared to 18 months. It indicates that the BCG induced allergy tended to disappear in this age group and the need for revaccination. Thus we suggest a further study in deciding the age of first vaccination and revaccination at the age of 5 years without tuberculin test.

In estimating coverage of vaccination, the presence of BCG scar used as an indicator may overestimate by almost a factor of two. Hence for accurate estimates we recommend determining the rate of tuberculin reactors in populations who have a BCG scar.

In assessing the diagnostic value of tuberculin PPD test, it was found less specific to determine the problem of tuberculosis in the community at a cut-off point of 10mm. Therefore for a better estimate, a cut-off point of 15mm and above should be considered.

INTRODUCTION

Since the bacillus which causes the disease tuberculosis was discovered by Dr. Robert Koch, a significant proportion of the entire population of the world has been infected by tuberculosis. According to a 1990 WHO publication, it is estimated that more than 20 million people worldwide have been infected and a total of nearly 3 million deaths have resulted from tuberculosis (1).

The global incidence of infectious cases is estimated at about 8 million cases per year. The majority (95%) occur in the developing countries. Indeed, in developing countries tuberculosis is the most important cause of morbidity and mortality with 80% of new cases occurring in the economically productive age group (15 to 54).

According to a recent national tuberculin survey between 1987 and 1990, the annual tuberculosis risk of infection in Ethiopia is estimated at about 1.5%, and the overall incidence of tuberculosis is between 90,000 and 154,000 cases per year while the prevalence is estimated as high twice the incidence(2).

A study in Mendeyo Awraja, Ethiopia indicated the prevalence of tuberculosis infection as 13.4%, with the rate being lower in infants and children under 5 years than in older children. Also the annual risk of infection was 2.7% in non vaccinated children (3).

Moreover, tuberculosis and its complications still remain more important causes of death than all other notifiable infectious diseases combined. In Ethiopia, despite under diagnosis, tuberculosis is the first leading cause for hospital deaths, it accounts for 12.2% in children and 14.3% in all age groups of hospital

deaths(2,4).

Besides this, nowadays, tuberculosis is associated with HIV infection. HIV seropositivity ranges from 10% to 55% in TB patients(5,6,7), which often indicates endogenous reactivation rather than exogenous super infection. Consequently, the incidence of tuberculosis in HIV infected patients can be expected to be high as the HIV/AIDS pandemic is widespread.

Obviously, this may worsen the extent, seriousness and severity of tuberculosis in a population. Additionally, it is a challenge for national tuberculosis control programmes to reduce the morbidity and mortality of tuberculosis with limited resources.

Tuberculosis is not a disease of a single pathogen, rather it has multi-causal factors. Ethiopia is a poor country, where the majority of its citizens have low socioeconomic status, live in over-crowded conditions, have high rates of malnutrition and other related factors, all contributing to the development of the disease by increasing exposure to pathogens.

In general, tuberculosis is one of the major public health problems as mentioned in earlier paragraphs. Based on the magnitude, seriousness and severity of the problem, it requires that it be given priority at all levels to apply more effectively the potent measures available for control at a cost affordable by the country. Considering cost effectiveness, BCG vaccination would be the appropriate measure to prevent tuberculosis.

Since 1921 BCG vaccination has been used to prevent tuberculosis. In 1974 it was integrated into the WHO EPI programme to strengthen childhood preventable diseases in developing countries.

Three vaccination policies are used in the different countries

1. Vaccination after birth without booster or one or two revaccination during childhood.
2. Single dose in a campaign for all eligible subjects.
3. Continuous and sustained revaccination policy.

In 1980, Ethiopia launched the EPI nationwide. A single dose of BCG vaccination has been applied to newborns soon after birth without booster or revaccination during childhood as a matter of policy. Nevertheless, a single low dose of BCG vaccination usually given to newborns can not induce a lasting significant level of protection. Thus revaccination is necessary at school age (8,9).

Beside these, tuberculosis remains still the leading cause of childhood morbidity and mortality in Ethiopia, despite the widespread of neonatal BCG vaccination in the country.

Thus it is important for us to evaluate the effectiveness of neonatal BCG vaccination policy, using a method applicable for the country. Therefore, a tuberculin sample survey in different age groups was conducted, in order to estimate the optimal time for revaccination and to describe the immune response in young infants. Beside this, it is impracticable to measure the health benefit directly. In terms of the number of vaccinated children, it is imperative to measure the output of the vaccination service by estimating the coverage by means of a sample survey of the presence of scar and distribution of the scar size (8). In this regard we assessed the presence of BCG scar, scar size and its relationship with tuberculin reaction among children who had received neonatal BCG

vaccination in order to determine its reliability in measuring vaccination output.

The ultimate goal of BCG vaccination and measurement of vaccination output is to reduce morbidity and mortality resulted from tuberculosis. Hence, the significance of the tuberculin PPD test to measure the tuberculosis problem in the community was also studied.

LITERATURE REVIEW

History of the BCG

Calmette and Guerin at the Pasteur Institute of Lille, France attenuated the Virulent Bovine type mycobacterium tuberculosis isolated by Nocard in 1902 from the tuberculosis mastitis of a heifer. They added ox bile to their culture media in an effort to obtain a well dispersed suspension of the organisms. As an unexpected result they noted that the organisms cultured in this way lost virulence for laboratory animals. After 13 years of sub culturing and testing from 1906 to 1919 they became convinced that they had developed a strain of organism that would remain avirulent and could be used to immunize humans against tuberculosis (10,11,12).

In 1921, the first human BCG vaccine was administered live, into the mouth of a young baby whose mother had died of tuberculosis. The baby survived, contracting neither the mother's tuberculosis nor any untoward effect of the BCG. This experience encouraged Calmette to carry out a series of experiments in animals; the result suggested that the vaccine was safe and that it offered a degree of resistance to challenge with virulent tubercle bacilli. Based on this evidence, he began to provide the vaccine free of charge, in particular for use in infants exposed to tuberculosis in the home. Later on the peroral BCG vaccination was continued in France and countries that requested the BCG strain from Pasteur Institute of Paris. In 1928 the League of Nations announced the safety of the BCG strain for use in vaccination of animals and man (10,11).

Despite this, between 1929 and 1930 in Lubeck, Germany, 72 out of 250 perorally vaccinated children died as a consequence, with fulminating tuberculosis.

Confidence in BCG evaporated and Calmette died broken hearted in 1933. But the German health authorities and the law court declared that the BCG vaccine was contaminated with virulent Kiel strain through professional malpractice. In spite of the objections following the Lubeck malpractice, BCG vaccination progressed and in 1948 the first international BCG congress in Paris stated that the BCG vaccination was effective in preventing tuberculosis and that BCG strain stably maintained its residual virulence. By 1948 more than 10 million vaccinations has been carried out(10,11).

To reduce the global problem of tuberculosis after the second world war, BCG vaccination has been applied on an ever increasing scale. The WHO and UNICEF organized BCG mass campaigns to cover the eligible population in a short time in several countries. Since 1974, a BCG vaccination programme has been integrated with the general health services and included in the WHO - EPI programme to strengthen prevention of infectious disease in children. Vaccination is therefore directed to the youngest age groups including the newborn till now (9,12,13).

Effect of BCG on Tuberculosis

Calmette and Guerin demonstrated in successive experiments that BCG vaccine became harmless, kept its antigenic property, viability and an appropriate level of residual virulence to multiply in the vaccinated host to offer premunition, which is the protection as concomitant immunity depending on the presence of the living, metabolizing and persisting BCG in the vaccinated host. Hence, effective BCG vaccine activates the macrophages, the T-cell functions and the molecular communication mechanism of the immune system. Thus, this premunition will be able to inhibit all further invading virulent mycobacteria and offers protection against

tuberculosis(10).

Quality Control of BCG Vaccine

In spite of the precise instructions of Calmette and Guerin on the methods of maintaining the BCG cultures, with modifications to maintenance conditions in different Laboratories, the genotypical and phenotypical variations resulted in various BCG sub strains with decreasing levels of the original residual virulence. The consequence today is that several BCG sub strains are being used in the vaccine manufacturing Laboratories with different viability, residual virulence, immunogenicity and reactogenicity(10,15).

Since 1948, WHO took the responsibility for the large scale international BCG vaccine. In 1974 the twenty-seventh World Health Assembly reaffirmed the importance of quality control of BCG vaccine and recommended that all member countries producing or importing BCG vaccine use the international quality control system set up by WHO "until they have established a competent national control system" (14). All producers of freeze dried vaccine supplied by or through UNICEF were already using this system, which consisted of evaluation in international reference laboratories and centres both in the laboratory and by clinical testing (15).

Quality Control Testing

In-vitro tests:- The In-vitro potency of the vaccine is expressed in viable units. Several in-vitro tests for BCG vaccine have been described. One of these determines the number of culturable particles on solid medium from appropriate dilution level; a laboratory should be able to relate the number of BCG particles determined for a particular vaccine to clinical effects such as scar size,

post vaccination tuberculin hyper sensitivity and incidence of common toxic effects(10,15).

A rapid test for viability is based on measurement of bioluminescence, which is a reliable marker for living cells; the test is useful once the mean content of adenosine triphosphate per culturable particle has been estimated for a given vaccine strain(15).

Vaccine Viability

The viability of a given BCG vaccine, i.e. the proportion of live and dead bacilli, is an important determinant of its characteristics. The final product is filled in containers according to a standard bacterial mass, which is estimated by weight or opacity. The percentage of total bacterial particles that is culturable is then determined. This percentage is subject to further decrease after freeze drying (10,15)).

The extent of the local reaction to BCG vaccination is proportional to the total bacterial mass while the level of tuberculin sensitivity is related to the number of culturable particles (15).

Vaccine Thermal Stability

According to the WHO requirements, the number of culturable particles in a vaccine after incubation for 28 days at 37⁰c must not be less than 20% of that in samples of the same vaccine stored at 4⁰c.

Residual Virulence

This is tested by the relative persistence capacity of the BCG sub strain in the spleen of mice and by the skin reactivity in guinea pigs (Jensen test). The corresponding human field control is the reaction size of the local skin lesion and the frequency of the adverse reaction (10).

Tuberculin Allergy

Purified protein derivative (PPD) of tuberculin is a precipitate found from filtrate of old tuberculin(OT) by Florence Seibert in 1934 and has been used to test cell mediated immunity followed by mycobacterial sensitization in animals and human populations. Its immunological reaction is expressed as PPD, because of cell infiltration and it enhances the activity of regional lymph nodes which leads to release small lymphocytes from bone marrow. T. lymphocytes proliferated to antigenic stimulus and offer specifically sensitized lymphocyte then enter the blood circulation, resulting in tissue induration.

False negative PPD responses in the presence of mycobacterial sensitization have been observed in steroid therapy, malnutrition, INH therapy, children less than 3 months who have recent infection with measles or immunization with live viral vaccine. On the other hand, a booster phenomenon of BCG vaccine enhances tuberculin PPD response (16,17).

Tuberculin tests are still be used as tools for assessing the risk of tuberculosis infection in populations and have an impact on tuberculosis control programmes.

For the purpose of testing the quality of BCG vaccine, the tuberculin reaction size in mm is tested in guinea pig sensitized with defined values of the tested BCG vaccine and the corresponding human field test is the tuberculin conversion rate or the tuberculin reaction size which represents the CMI response(10).

However, it has so far proved impossible to overcome the cross sensitivity by infection with different mycobacteria.

Characteristics of current BCG preparation

A study on the quality control of BCG vaccine by WHO was carried out to determine which preparations of BCG vaccine are currently in use what and their characteristics are. For this purpose information was obtained from 15 manufacturers of BCG vaccine, especially on the three parent strains of BCG, that is Glaxo, Pasteur and Tokyo which account for over 90% of the vaccines currently in use world wide.

Based on the information obtained from manufactures of BCG vaccines in 1989 and reviewing several studies on quality control of BCG vaccine, the WHO study group determined that currently available BCG vaccines have an efficacy of 60 - 90% for preventing disseminated tuberculosis or meningitis in young children, but somewhat lower efficacy for other forms of primary tuberculosis. Moreover, no BCG preparation tested was significantly more efficacious than any other and most of the preparations currently in commercial production have been tested at least once in a careful clinical trial. On the basis of there protective efficacy, there is no evidence at present to substantiate choosing one preparation or manufacturer of BCG vaccine over another (15).

The Effect of BCG Vaccination

The rationale of BCG vaccination is to substitute the natural and potentially harmful primary infection with virulent tubercle bacilli by an artificial and innocuous primary infection with attenuated bacilli that have maintained the immunogenic properties but not the pathogenicity of the virulent bacilli. Among the immunogenic properties are the enhanced resistance to a subsequent exposure to virulent infection, but also immune reaction such as induction of delayed hypersensitivity in a healthy host.

While the protective effect of BCG vaccine against tuberculosis can be demonstrated experimentally through vaccination of suitable animals and subsequent challenge, the effect in man is best obtained by means of controlled clinical trials.

Starting in the 1930s a series of controlled trials was organized in several areas of the world, in order to assess the protective effect of BCG against tuberculosis. The results of these trials have been confusing in their lack of consistency, providing evidence of high level protection in some population, but little or no protection in others (table 1).

A trial in Chicago infants was carried out between February, 1937 and February, 1948 and the incidence of tuberculosis between vaccinated and non vaccinated infants during the following 23 years was compared. The result indicated that among the vaccinated there were a total of 17 cases of tuberculosis (0.43/1000/yr.) and 65 cases in non vaccinated (1.7/1000/yr.), a reduction of 75%. The morbidity was 0.41/1000/yr. in the vaccinated subjects and 1.5/1000/yr. in the controls, a reduction of 74% (18).

A similar result was obtained in a study of a North American indian population; this study in the late 1930's revealed that BCG offered 80% protection against tuberculosis (10). Also a trial among British school leavers, started in 1950, showed 78% protection over almost 15 years of follow up (19).

In contrast to these, in the studies in the United States conducted between 1947 and 1950, the one carried out in a Puerto Rico population showed a modest protection effect (31%) while the two studies in Georgia and Alabama school children failed to show any protection at all after 20 years and 14 years of observation respectively (20,21,22).

On the other hand, a trial in Madanopolle, South India between 1950 and 1955 indicated a moderate protective effect estimated at 30% (23).

So far, the assessments of the protective effect of BCG vaccination in human populations have produced contradictory results. As mentioned earlier the sharpest contrast appears in the large controlled studies conducted by the British Medical Research Council (19) and by the United States Public Health Service (21,22). Acceptance of the U.S Public Health Service results would lead to in view of that BCG vaccination has no value in protection against tuberculosis, while in contrast the British Medical Research result suggests that BCG vaccine offered high protection. Various attempts have been made to reconcile these contradictory results.

Among these, Palmer and Long offered an explanation of the differences in the protection effect of BCG vaccination in Britain and the United States, in terms of differences in the prevalence of atypical mycobacteria infection. They further stated "the crux of the matter is that the anti tuberculosis effect of BCG is not added to that which a population may already have acquired from infection with an atypical mycobacteria; instead the observed effect of BCG is its potential capacity minus the capacity acquired from other mycobacterial infection" (24). In as much as BCG can attain its full anti tuberculosis potential only in a population free of other mycobacterial infections, the observed effect in population with such infections is, therefore, the difference between BCG's potential effect and that acquired from "natural vaccination" with one or more of the atypical mycobacteria. Infections with atypical mycobacteria are widespread, particularly in tropical and subtropical regions of the world; hence, one must not expect the protective effect of BCG to be the same

everywhere. Other studies on the atypical mycobacteria also suggest that such sensitization could be associated with protection against tuberculosis and possibly mask the protective effect of BCG vaccination (24).

Meanwhile other factors were investigated for the observed discrepancy in several controlled trials on the protective effect of BCG vaccination; these include: differences in immunogenicity and virulence among the strains used in some of the trials (25), variation of the dose of BCG vaccine and the presence or absence of tuberculosis infection in the trial subjects; infected subjects have invariably been excluded from vaccination trials. In 1968 a trial satisfying most of these conditions was organized in South India by the Indian Council of Medical Research (ICMR), New Delhi, in cooperation with the World Health Organization and the Centre for Disease Control (U.S.) to meet the objectives

1. To estimate the protective effect of BCG vaccination against tuberculosis in the non infected
2. To assess the effect of BCG vaccination in persons already infected
3. To assess the protective effect of different strains of BCG
4. To assess the influence of dosage of BCG on the protective effect.
5. To gather epidemiological data on tuberculosis in the community. However, the results of the trial revealed that BCG did not confer any protection against the development of pulmonary tuberculosis during the first 7 1/2 years after vaccination. Because of this, the question of examining the significance of the difference in protective effect between vaccine strains or dosage does not arise (26).

In contrast to this, a cohort study was carried out in Norway between 1956 to 1973 to study the incidence of tuberculosis in vaccinated, uninfected (tuberculin negative) and infected (tuberculin positive) subjects. The result showed that the protective effect of BCG vaccination in Norwegian children under 5 years in the first 10 years after vaccination of tuberculin negative subjects is estimated to have been 80%. The effect was higher during the first 5 years after vaccination than during the next 5 years (27).

Parallel to this, in 1983, in England and Wales, the protective efficacy of BCG vaccine at ages 15 - 19 years was estimated to be about 80% and at 20 - 24 years about 75% (28). Similarly a controlled study was carried out in Seoul, Korea between 1984 and 1986 to determine the protective effect of the BCG program in children up to 5 years of age and it showed 70% protective effect for the total observation (29).

After assessing all of these contradictory results in several studies, the WHO study group on BCG vaccination policies attempted to explain the variation in the protective effect of BCG vaccination: It could be that in some trials a vaccine of low potency had been used and in others infection other than the tubercle bacilli had provided natural protection against tuberculosis, thus masking the effect of BCG vaccination. Additionally the poor results in some trials could possibly result from the disease diagnosed being predominantly of exogenous super infection type, whereas BCG vaccination may protect uninfected persons against primary and evolutive tuberculosis as well as endogenous reactivation. While it can not be expected to protect uninfected persons if their eventual diseases were of the exogenous reinfection type; this is because at the time of reinfection the level of immunity would be that

derived from the primary infection, whether BCG had been given or not. Thus controls and vaccinated subjects would have the same risk of disease from exogenous reinfection. So far, the study group recommended that BCG vaccination be applied most often to the newborn and young infants and that this policy was being adopted more and more with the introduction of the EPI. It therefore found that there were no reasons to modify this current policy and it was recommended that the use of BCG as an anti tuberculosis measure be continued, in particular in infants and children (30).

A further recommendation of the study group, confirming that of on ICMR/WHO scientific group, was that research on the effectiveness of BCG vaccination in young children should be undertaken according to methods applicable in developing countries (31).

In view of these facts and recommendations, further case control and other studies were carried out to better define the role of BCG in a tuberculosis control programme.

One such study was a case control and contact study among child contacts of newly discovered sputum smear positive patients with pulmonary tuberculosis in Bangkok. In this study, children who had received BCG vaccination after birth showed clinical or radiological evidence suggestive of tuberculosis only half as often in the unvaccinated children (13). Additionally recent observations in Indian children under 15 years do show some protection against tuberculosis (33). Similar to this, a study was carried out in the Shoa district of Ethiopia, to assess the efficacy of previously administered BCG by comparing the vaccinated and non vaccinated proportion of population. It was found that the rate of acquisition of primary tuberculosis in the unvaccinated would appear to be 3.25/100 children per

year over the years studied and in the vaccination group the rate was only 0.42/100 children /year over a similar period; this would suggest protective efficacy of BCG 87.1% (34).

Another case control study in the metropolitan region of Sao Paulo, Brazil revealed that BCG vaccination was highly effective against tuberculosis meningitis in children below 5 years of age. The estimates of efficacy were the same whether one estimated them from neighbourhood controls (84.5%) or hospital controls (80.2%) (32).

So far, in contrast to the WHO study group explanation concerning BCG protection against exogenous reinfection, one other study suggested that BCG vaccination could affect not only endogenous reactivation but also exogenous tubercle reinfection (36).

Based on these consistency of recent studies and the WHO study group on BCG vaccination policy review ,it is now generally accepted that BCG vaccine imparted some protection against tuberculosis. However,the use of neonatal BCG vaccination raised some controversies. Some important aspects of these controversies are given below.

Table 1. Major Controlled Trials on Protective Effect of BCG Vaccine Against Tuberculosis.

Area (Reference No)	Subjects	Intake Period	Vaccine	Duration of observation	Percentage Protection
1. North American Indians (19)	1 to 18 years	1935 - 38	TICE	9 - 11	80
2. Chicago (18)	Infants	1937 - 48	TICE	12 - 23	75
3. Georgia (22)	School children (6-17yr of age)	1947	TICE	20	Nil
4. Puerto Rico (20)	General population (below 20 years)	1949 - 51	Birkhaug	5 - 7	31
5. Georgia & Alabama (21)	Over 5 years	1950	TICE	14	14
6. Great Britain (37)	14 - 15 yrs	1950 - 52	Danish	15	78
7. Modanapolle, S.India (23)	All ages	1950 - 55	Madras	9 - 14	30
8. Chingleput, S.India (26)	All ages	1968 - 71	Paris Danish	7 1/2	Nil
9. Norway (27)	Under 5 year	1954 - 1973	Paris, Japan	10 years	80

BCG Vaccine in the Newborn and Young Infants

As recommended by the World Health Organization, BCG vaccination should be applied to all babies at birth or as soon as possible thereafter, before being exposed to infection. Thus protection is afforded against the serious forms of childhood tuberculosis-miliary tuberculosis and tuberculosis meningitis - which are still often fatal, even when chemotherapy is available. On the other hand, it is generally considered that a single vaccination at birth does not give protection for life, and that revaccination is necessary at school age (9,38).

Immunization of infants with vaccine requires full participation of cell mediated immune response to specific antigen for adequate immunity induction. However, the effectiveness of BCG vaccination of infants is influenced by preexisting cell mediated immunity to mycobacterial antigen through interference with processing of the BCG organisms. The humoral factor produced by the mother's immune system and reaching the fetus trans placentally and also breast feeding appears to provide the newborn's specific cell mediated immunity. It is obtained by the generation of PPD induced LIF (Targer 1985). The newborn's specific cell mediated is not likely associated with immunological memory, rather

it is akin to passive specific humeral immunity which usually declined in the 3 months after birth. As described earlier, this pre existing passive cell mediated immunity to specific antigen may interfere with the establishment of active immunity by altering the host response to active immunization to that antigen; the resulting post vaccination tuberculin sensitivity in the newborn invariably appears to be lower than in older children given the same dose of BCG. Thus, at least part of the widely varying efficacy of BCG immunization may be related to this passive form of pre existing immunity (9,39,40).

On the other hand, a dose of BCG that is well tolerated in school children often causes suppurative lymphadenitis in a high proportion of the newborn. In order to avoid this high incidence of suppurative lymphadenitis in the lowest age group, the dose of BCG vaccine is usually reduced and this results in a further decrease in the immunological response as evidenced by the post vaccination tuberculin sensitivity (9).

Consistent with the previous observation in other study comparing BCG vaccination at birth and at third month of life, evaluation was made using the results of tests with PPD, by vaccine scar and by complications of the vaccine; it was found that BCG given at the end of third months provided a higher rate of response and fewer

complications than when given during the first few days of life (41).

Furthermore, a study was carried out in children of Asian ethnic origin born in England to assess the protection offered by BCG given during the first year of life against tuberculosis. It found a lower protective efficacy of BCG vaccination in infancy (49%) than in the secondary school age BCG programme (80%). Nevertheless, the protection is substantial and in the U.K, it is considered that BCG vaccination of infants should be continued in order to reduce the incidence of childhood tuberculosis (42).

Moreover, a tuberculin survey in Ethiopian children between 1 month and 5 years was carried out; it has found a trend that as age increases, tuberculin positivity increases. The authors offered two explanations for these findings: older children have well developed immune system and more chance of exposure than newborns (43).

In addition to this, a tuberculin survey among BCG vaccinated children aged 5-14 years in an eastern province of Saudi Arabia found that 71% were tuberculin positive; it was also shown that tuberculin sensitivity declines within a few years of vaccination. In this regard, the authors suggest that the observed decline probably indicates the current policy of BCG vaccination at birth should be reinforced by revaccination at school

leaving age (44). Meanwhile, other investigators observed low rates of natural acquisition of tuberculin positivity in BCG unvaccinated preschool children and they suggest a suitable age for giving BCG would be when the child first goes to school (45). Also, a recent study in India indicated that the prevalence rate of tuberculosis in children aged 5 - 9 years was about 3 times higher than in children aged 1 - 4 years (46).

The negative tuberculin response indicates the elimination of the living BCG bacteria from the vaccinated host and the need to revaccinate in order maintain the activation of the cell mediated immunity (3). Hence the above mentioned studies in Ethiopia and Saudi Arabia suggest that the protective effect of BCG vaccination gradually increases with age until age 5 and it declines at school age, indicating the necessity of revaccination at this age (43,44,45).

In order to assess the optimal age for revaccination, a tuberculin survey was carried out in Sri-Lankan children in different age groups who had received low dose of BCG vaccination in the first month of life; based on their results, the authors suggested that the optimal time for revaccination may be at 5 years rather than at 10 years of age (47).

Furthermore, a long term study of BCG revaccination policy in Hungary between 1959 to 1983 (with a

standardized BCG strain, viability control of the vaccine, a reliable diagnostic and reporting system) revealed that the sustained BCG revaccination system lowered childhood tuberculosis incidence 3 - 4 times more rapidly (23 - 32% per year) than the non vaccinated adult incidence during the first 10 years and(6 - 15% per year) further lowered the incidence during the second 10 years (10).

As mentioned earlier, some studies indicated BCG vaccination at birth provides low protection against childhood tuberculosis. They give as reasons the fact that the effectiveness of BCG vaccination is influenced by pre existing cell mediated immunity to mycobacterial antigen, low dose of BCG vaccine and not well developed immune system in newborns to elicit cell mediated immune response to specific antigen.

Because of these reasons, they suggest that vaccination should be given at the third month of life or thereafter; while others recommend that neonatal BCG vaccination be continued with the same dose and followed by vaccination at the age of 5 year and sustained vaccination of the reconverted tuberculin negative children until young adult age.(9,10,39,40,41,47).

Contrary to this, a WHO study group recommended that a second BCG vaccination should be given at the age of 10 years following neonatal BCG vaccination (38) and also

that a tuberculin test for screening before vaccination should be omitted (48).

Meanwhile in 1980, Ethiopia launched the EPI nationwide, assisted by UNICEF, WHO and UNDP. Since then, BCG vaccination has been applied as primo vaccination after birth, without booster during childhood, as a matter of policy in the country. However, a single vaccination at birth does not give protection for life and a second BCG vaccination should be given at the age of 10 years (6) as recommended by WHO (38).

Moreover, a review of the immunization programme in Ethiopia indicate that although the vaccination coverage of BCG increased from 1980 to 1989 (6% to 44.4%), the incidence of tuberculosis also increased from 1977 to 1988(49). Although several reasons have been mentioned for this increasing incidence, the immunization program also should be evaluated.

In light of all of these contradictory results, the present study attempts to evaluate the effectiveness of neonatal BCG vaccination policy by assessing tuberculin response in different age groups in order to find the optimal time for BCG revaccination and to describe immune response in young infants following low dose BCG vaccination at birth. Additionally to assess the measurement of vaccination output and tuberculosis

problem at present in the community .The aim of this study is to use this information as a basis for reviewing and strengthening the current vaccination policy.

Objectives

General

To evaluate the effectiveness of neonatal BCG vaccination policy.

Specific

1. To estimate the optimal age of BCG revaccination following neonatal BCG vaccination.
2. To describe the immune response in young infants following low dose BCG vaccination at birth
3. To assess the reliability of BCG scar for use as an indicator for coverage of BCG vaccination.
4. To assess the diagnostic value of tuberculin PPD test.

METHODS

STUDY AREA

The Survey was conducted in Lideta awraja in south Western Addis Ababa, Ethiopia. As of 1992, the awraja population was estimated to be 176,819, of whom it is estimated that 71,911 are children under 15 years of age. Almost all have similar socio-economic status, but a few are in high and low classes. The altitudes of the awraja vary between 2000m and 2500m above sea level.

The survey took place in five randomly selected elementary schools for school age children and in the local health institutes for pre school children.

Ethical Considerations

This study was approved by the research committee of the faculty of medicine, University of Addis Ababa.

Moreover, written informed consent forms were prepared (Annex 1) and individual informed consent was given by the child's parents or guardians. There were some 20 refusals.

Selection Procedure

Of all apparently healthy Ethiopian-born children aged 2 months to 11 years who had received BCG vaccine at birth, four groups of children, aged 2 months, 18 months, 5 - 7 years and 9 - 11 years were selected.

The selection of a child as a study subject comprised the following main activities:

1. Assess immunization record
2. Conduct clinical history and physical examination
3. Assessment of nutritional status

Assessment of Immunization Record

Immunization records were checked to monitor the date of BCG vaccination in order to exclude un-vaccinated children and children vaccinated beyond the first week of life. Additionally, the exact age of a child was obtained.

Clinical History and Physical Examination

The vaccinated children's parents or guardians were interviewed about the health status of their children. Subsequently, general physical examinations were performed by a physician at the testing site. Finally children who had history of past or recent tuberculosis infection, history of contact with an active tuberculosis, INH therapy, symptoms of tuberculosis, history of measles infection, or live viral vaccination in the last 6 months, immune depressant therapy and other immune suppresser or chronic illness were excluded. This was done in order to minimize false negative and false positive Tuberculin tests.

Assessment of Nutritional Status

Measurements of the weight and height of the child were taken by a trained health assistant before commencement of the test procedure. Two indices of nutritional status were constructed: weight for height and height for age. The subjects' measurements were classified according to the published anthropometric reference value for use in the African region namely:

1. Weight for height --- children of preschool age
 - a. Above median - 1SD = normal
 - b. Between 1 and 2SD below median = mild PEM
 - c. Between 2 and 3SD below median = moderate PEM
 - d. Below median - 3SD = severe PEM
2. Height for age --- school age children
 - a. Above median - 1SD = Normal
 - b. Between 1 and 2SD below median = Mild stunting
 - c. Between 2 and 3SD below median = moderate stunting
 - d. Below median - 3SD = severe stunting

Based on this, all children who had the measurement - 1SD below the median were also excluded. The reason for exclusion was also to minimize false negative tuberculin test.

Sample Size Determination

In several studies an apparent lack of Tuberculin sensitivity followed neonatal BCG vaccination was observed ie. 14% of U.A.E infants(50), 80% of Sri-Lankan's children(47) and in 31.3% of Ethiopian children(43) .

In this regard the proportion of Tuberculin anergy found in the previous Ethiopian study was taken for sample size calculation.

The maximum acceptable discrepancy $100p-100P$ between sample and population percentage was taken as $\pm 4\%$ for precision; a 99% certainty was used.

The 99% confidence limits involve a combined total of 1% in the two tails of the normal distribution, so we took

$$Z = +/- 2.58$$

The standard error (SE) of the estimated percentage is 100 times the square root of $P(1-P)$.

The allowable discrepancy of $+/- 4$ percent between sample and population percentage gives us

$$100p - 100P = +/- 4 = Z \times SE$$

Thus, taking the Proportion of Tuberculin anergy as $P = 31.3\%$ or 0.32, as mentioned before, and solving for n, we obtain

$$n = 4160.25 (0.32) (0.68) = 905$$

In order to allow for incomplete data, drop outs etc., the study was therefore planned to recruit 1000 children.

Sample recruitment

During a period of 4 months from November 1992 until February 1993, a total of 980 children (369 pre school age and 526 school age) were recruited for the study. Of these, some 895 (426 male and 469 female) participated, yielding an overall response rate of 91.3%. Home visitors were employed to contact the parents of children who were absent on the day of reading the tuberculin test and to request them to bring their children to the health centre on the same day or the next. Despite this, 8.7% were non participants since parents of children were absent in their residence for various reasons and so could not be contacted.

Of the 895 children, 253 were aged 2 months, 116 aged 18 months, 202 aged 5-7 years and 324 aged 9-11 years. For the purpose of comparison, the proportions of children in each of four age groups was similar to those the study of Sri-Lankan children (4).

Additionally, in order to assess the effect of revaccination on tuberculin reaction and on scar size, 60 children aged 10 years who had received BCG vaccine at birth and showed no response at all to tuberculin PPD

test in the study period were revaccinated during the study period; the revaccination used the same dose of BCG as is used for newborns; these children were then retested two months later for tuberculin response and a scar size also assessed.

Furthermore, to check the potency of the tuberculin PPD solution and to describe the variation of tuberculin reaction in already infected person, 70 bacteriologically proven tuberculosis patients were also tuberculin tested at the beginning of the study.

Training

A one day job-oriented training was held for the employed research assistants. In order to avoid inter-observer variation, one health assistant was assigned to check immunization records and age of the study subjects, one physician (principal investigator) to perform clinical examination, one nurse to measure weight and height, one nurse to screen malnourished children, one health assistant to evaluate BCG scar, one nurse to administer the tuberculin PPD test, one nurse to read the PPD induration, one health assistant to offer BCG vaccine, and one health assistant to administer and complete the questionnaires.

Data Collection

The variables for which data were collected were: age, sex, address, presence of BCG scar, presence of PPD induration and size of PPD induration (annex 2).

The first part of the questionnaire was administered for eligible children on arrival at the study sites and tuberculin PPD test administered. Subsequently, 96 - 120 hours later, the tuberculin PPD induration was read and the questionnaire was completed.

Test Procedure

The administration, reading and recording of tuberculin tests was in accordance with the guidelines of the WHO standard tuberculin test.

A trained nurse injected into the most superficial epidermis of the volar surface of the right forearm of each subject intradermally 0.1 ml of purified protein derivative of tuberculin; this was done as soon as possible after the disposable syringe has been filled under aseptic condition and the needle had not been withdrawn for a few seconds to minimize leakage. The remaining solution was kept refrigerated and stored in the dark.

All results were read within 96 - 120 hours. The maximum transverse diameter induration (not erythema) was measured using the ball point technique.

The test was graded as non reactive (<1mm induration), 2 - 5mm, 6-9mm or >10mm induration.

The site of the BCG vaccination was assessed by a single health assistant before testing; for those who had a scar, the horizontal diameter of the scar was measured with a transparent plastic ruler. Then the site of BCG vaccination was graded as no scar, 1 - 2mm bud, 3-4mm bud, 4-5mm bud or more than 5mm bud.

For those 60 children who had been selected for revaccination, 0.05ml of BCG vaccine was given intradermally on the right deltoid muscle by a trained nurse. Of these 60, some 52 children returned 2 months later for the 2nd tuberculin PPD test.

Statistical Analysis

The coded questionnaire responses were entered into the EPI-INFO computer program in order to analyze tuberculin responses in different age groups and to correlate tuberculin response, BCG scar and other variables.

Chi-square tests were performed to compare the prevalence of tuberculin anergy according to different categories of age, sex and BCG scar; these were performed without and with stratification. The stratified analysis were performed to correct the comparison for any imbalances in the composition of the sub-groups being

compared with respect to the other variables. eg. the age comparison was stratified on sex and BCG scar etc. The chi-square test for linear trend was used to evaluate the trend of tuberculin anergy across age after adjustment for BCG scar status.

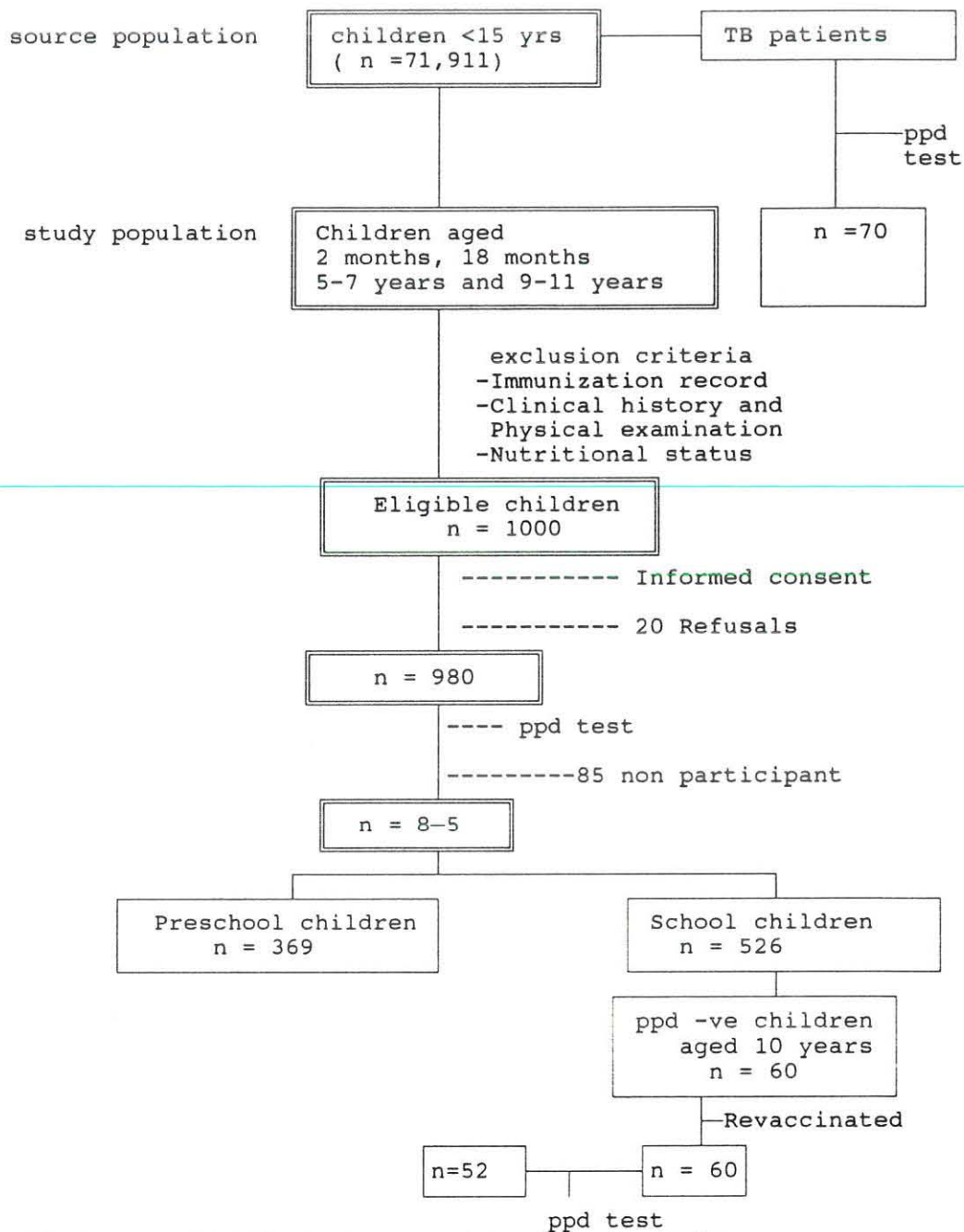


Fig 1 Graphic representation of study design

RESULTS

A total of 980 children who had received BCG vaccination at birth in Addis Ababa between October 1992 and February 1993 were tuberculin PPD tested, but 895 (91.3%) returned for the test to be read.

Characteristics of Study Subjects

The study subjects characterized by age and sex are summarized in table 2. It is observed that there are fewer children in the age group 18 months (13.2%) than in the age group 2 months (28.4%). This may be due to less frequent attendance at the health centre during the study period. There was no significant difference regarding sex in different age groups i.e. there was a close to 50:50 sex distribution in each age group.

Distribution of BCG Scar by Age Group

Table 3 shows the distribution of the size of BCG scar by age groups. Of the total, some 564 (63%) had a definite BCG scar while the rest 331 (37%) had no visible post vaccination BCG scar. The percentage without a BCG scar was almost similar between different age groups. However the large size of BCG scar was more frequently observed in school children than in preschool children i.e. a scar 4 - 5mm nodule in 14.6% and a scar >5mm nodule in 13.3% of school children compared to in only 9.2% and 3.5% of pre school children respectively (P<0.001).

As indicated in table 4, the distribution of the presence and absence of BCG scar was similar in both sexes.

BCG Induced Tuberculin Sensitivity

Development of BCG induced tuberculin sensitivity was studied in different age groups of children (table 5). Overall, 368(41.1%) showed no response at all to the tuberculin PPD test; the percentage of non reactors in different age groups was 39.5% at 2 months, 34.5% at 18 months, 39.1% at 5-7 years and 46% at 9-11 years. Among tuberculin reactors in all age groups a reaction size was observed as 2-5mm in 39.6%, 6-9mm in 14.9% and 10-15 mm in 4.3% of children ($P < 0.001$). Figure 1 illustrates the general pattern distribution of the percentage of tuberculin non reactors: initially there is a slight decline from age 2 months and then an increase from age 18 months onwards; the same pattern is seen for those reactors showing a 2 - 5mm induration.

Although there was no statistically significant difference, tuberculin anergy was observed more frequently in females (43.5%) than in males (38.5%) (table 6).

The Size of BCG Scar Versus PPD Induration

In order to determine the correlation between the size of BCG scar and tuberculin induration, a correlation analysis was made. It shows a direct correlation between

tuberculin reactivity and the size of BCG scar. As presented in table 7 and Figure 2, the percentage of tuberculin anergy tends to decline as the size of BCG scar increased.

The percentage of low grade tuberculin reactors (2-5mm induration) also showed a tendency to decline as the size of the BCG scar increases while the percentage of reactors (6-9mm induration) increased substantially with the size of BCG scar ($P < 0.001$).

Moreover, of those children without BCG scar, 55.2% had no tuberculin reaction while 67% of children with scar showed positive tuberculin reaction invariably: 43% had induration of 2-5mm, 19% 6-9mm and 7% 10-15 mm induration ($P < 0.001$). Of those children who had the large BCG scar (i.e. > 4 mm), 25% were Non reactors whereas 75% were Tuberculin reactors.

A positive tuberculin reaction without a BCG scar

Of 331 children without a scar, 148(44.7) were tuberculin reactors invariably. The percentage distribution of reactors was higher in the 18 month age group compared to others. They have 1.63 times the risk of a positive reaction compared to the infants at 2 months. However, the trend is not statistically significant (table 8).

A negative tuberculin reaction with a BCG scar

Among children with a scar, 185 (32.8%) showed no tuberculin response. There was no significant difference observed between age groups (table 9). Also it was not found statistically significant ($p=0.48$).

Tuberculin conversion with a BCG scar

Table 10 shows the crude prevalence of a positive reaction in those children who had BCG scar by age groups and the age-specific adjusted odds ratio (Mantel Haenszel) comparing reactivity between those with and without a BCG scar. It revealed that past BCG vaccination contributes to current tuberculin sensitivity. In each age-group, there was a greater likelihood of having a positive tuberculin reaction when a scar was present than when it was absent: 2.65 higher in the age group 2 months and 2.83 in the age group 9-11 years, while in the age group 18 months and 5-7 years, it was 1.92 and 2.21 respectively. The result was found statistically significant in each age group except in the age group 18 months. In general there was a higher probability of having a positive tuberculin reaction with a scar than without one in infants and older children than younger children i.e. 18 months and 5-7 years.

Table 11 presents prevalence of tuberculin non

reactors according to different predictor variables. It shows that the risk of having a negative tuberculin reaction after neonatal BCG vaccination was lower in the age group of 18 months than 2 months while it was higher in the group 9-11 years. However, it is not statistically significant.

A minimal difference regarding sex was also observed, but it was also insignificant statistically.

Moreover the table indicates that children without a BCG scar have 2.53 times (95% CI 1.9, 3.38) the risk of a negative reaction compared to those with a scar ($p < 0.001$).

Tuberculin response after the 2nd BCG vaccination

In order to estimate the rate of tuberculin conversion after the second vaccination followed neonatal BCG vaccination, a group of 60 children aged 10 years who had no response to the first tuberculin test were revaccinated with a single dose of BCG vaccine. When 52 of them were retested with tuberculin 2 months later a reaction was observed as a size 2-5 mm in 29%, 6-9 mm in 61.5% and 10-15 mm in 9.5% of children. Also, the scar site was assessed before and after 2nd vaccination and it was observed that those children without a scar before the 2nd vaccination developed a large scar size as 4-5 mm in 19% and >5 mm in 81% of children (table 11).

Tuberculin response in TB patients

As illustrated in fig.3 the distribution of tuberculin reactions in TB patients showed a unimodal pattern. It ranges in reaction size between 2 mm and 30 mm. The mean tuberculin reaction diameter was 16.5 mm and 8% had a reaction of <10 mm.

Table 2 Characteristics of study subjects by age and sex(n=895) in Lideta awraja from oct 1992 to feb 1993

Character Age	Male		Female		Total	
	No	%	No	%	No	%
2 months	123	48.6	130	51.4	254	28.4
18 months	64	55.2	52	44.8	118	13.2
5-7 years	95	47.1	107	52.9	202	22.6
9-11 years	144	44.4	180	55.6	321	35.9
Total	426	47.6	469	52.4	895	100

Table 3 Frequency Distribution of the size of BCG Scars by age group in Lideta awraja from oct 1992 to feb 1993.
(numbers in parentheses are percentages)

AGE	BCG Scar size					Total
	Negative	1-2 mm	2-3 mm	4-5 mm	> 5 mm	
2 months	79(31.2)	64(25.3)	76(30)	25(9.9)	9(3.6)	253(100)
15 months	46(39.7)	24(20.7)	33(28.4)	9(7.8)	4(3.4)	116(100)
5-7 years	61(30.2)	25(12.4)	47(23.3)	40(19.8)	29(14.3)	202(100)
9-11 years	145(44.8)	33(10.2)	68(21.0)	37(11.4)	41(12.7)	324(100)
Total	331(37.0)	146(16.3)	224(25.0)	111(12.4)	83(9.3)	895(100)

$$\chi^2 = 74.26(12df)$$

$$P < 0.001$$

Table 4. Frequency Distribution of BCG Scar by Sex in Lideta awraja from oct 1992 to feb 1993.

Sex	Scar +ve		Scar -ve	
	No	Percent	No	Percent
Male	272	48.2	154	46.5
Female	292	51.3	177	53.5
Total	564	100	331	100

Table 5. Frequency Distribution of Tuberculin Reaction by age group in Lideta awraja from Oct 1992 to Feb 1993.

AGE	PPD Induration				Total
	0 -1mm	2-5 mm	6-9 mm	10-15 mm	
2 months	100(39.5)	119(47)	28(11.1)	6(2.4)	253(100)
18 months	40(34.5)	56(48.3)	14(12.1)	6(5.1)	116(100)
5 - 7 Years	79(39.1)	74(36.6)	42(20.8)	7(3.5)	202(100)
9 - 11 Years	149(46)	106(32.6)	49(15.1)	20(6.2)	324(100)
Total	368(41.1)	355(39.6)	133(14.9)	39(4.3)	895(100)

$\chi^2 = 26.65$

$P < 0.001$

Table 6 Frequency of Tuberculin Reaction by Sex in Lideta awraja from oct 1992 to feb 1993.

PPD Induration	Male		Female	
	No	%	No	%
0-1 mm	164	38.5	204	43.5
2-5 mm	177	41.5	178	38.0
6-9 mm	63	14.5	70	14.9
> 10 mm	22	5.2	17	3.6
Total	426	100	469	100

$$X^2 = 3.3$$

$$P = 0.3473$$

DISTRIBUTION PATTERN OF TUBERCULIN REACTION IN DIFFERENT AGE GROUP

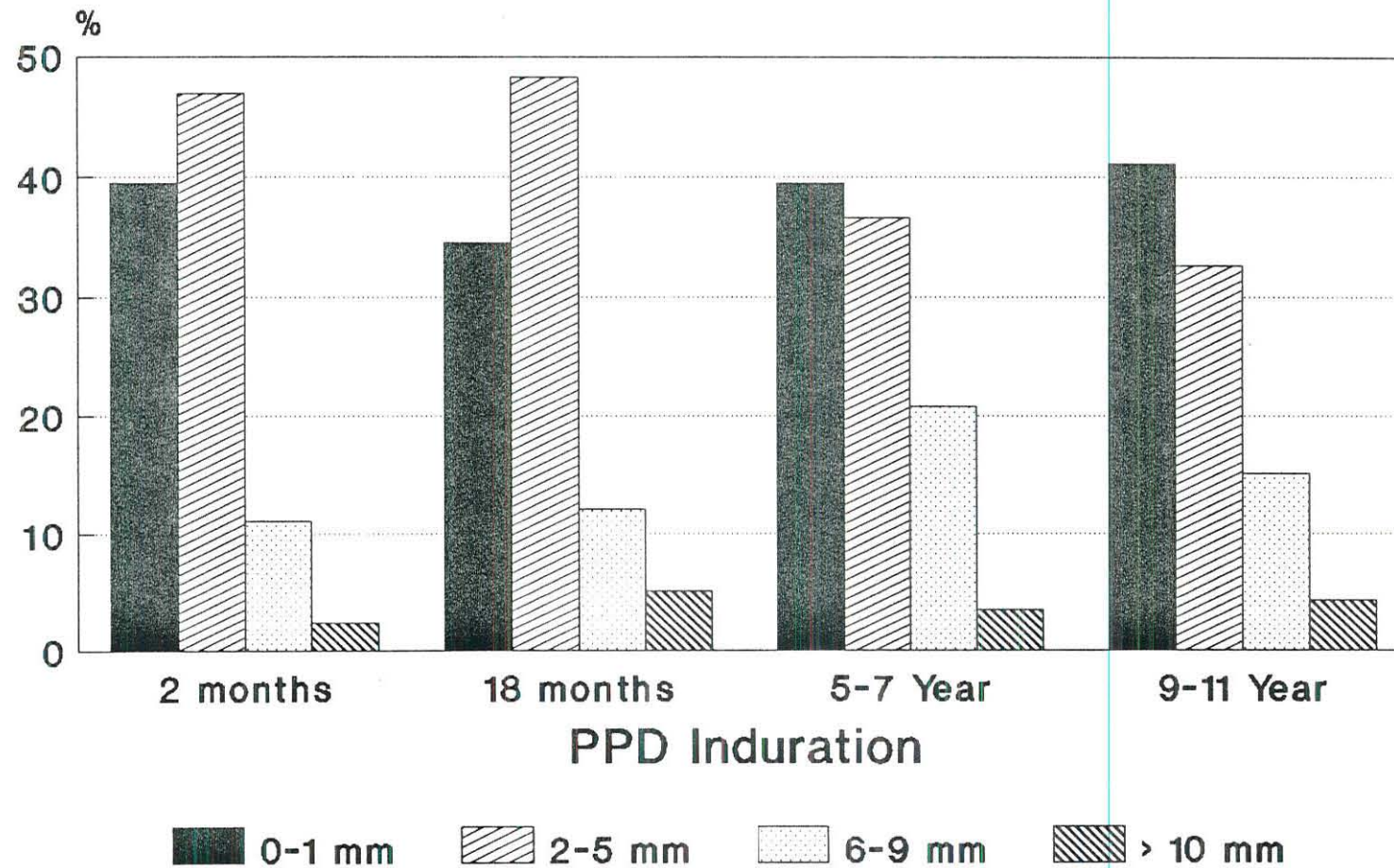


FIG 2

Table 7 Correlation between the size of BCG Scar and PPD Induration (n=895) in Lideta awraja from oct 1992 to feb 1993.

PPD Induration	SIZE OF BCG SCAR					total
	Neg (%)	1mm (%)	2-3mm (%)	4-5mm (%)	> 5 mm (%)	
0-1 mm (non reactive)	183(55.2)	56(38.7)	80(35.7)	28(25.2)	21(25.3)	368
2-5 mm	112(33.8)	73(50)	101(45.1)	44(39.6)	25(36.1)	355
6-9 mm	25 (7.6)	14(9.6)	33(14.7)	33(29.7)	28(33.7)	133
> 10 mm	11(3.3)	3(2.1)	10(4.5)	6 (5.4)	9(10.8)	39
Total	331(100)	146(100)	224(100)	111(100)	83(100)	895

$$\chi^2 = 101.15$$

$$P < 0.001$$

CORRELATION BETWEEN BCG SCAR SIZE AND PPD INDURATION

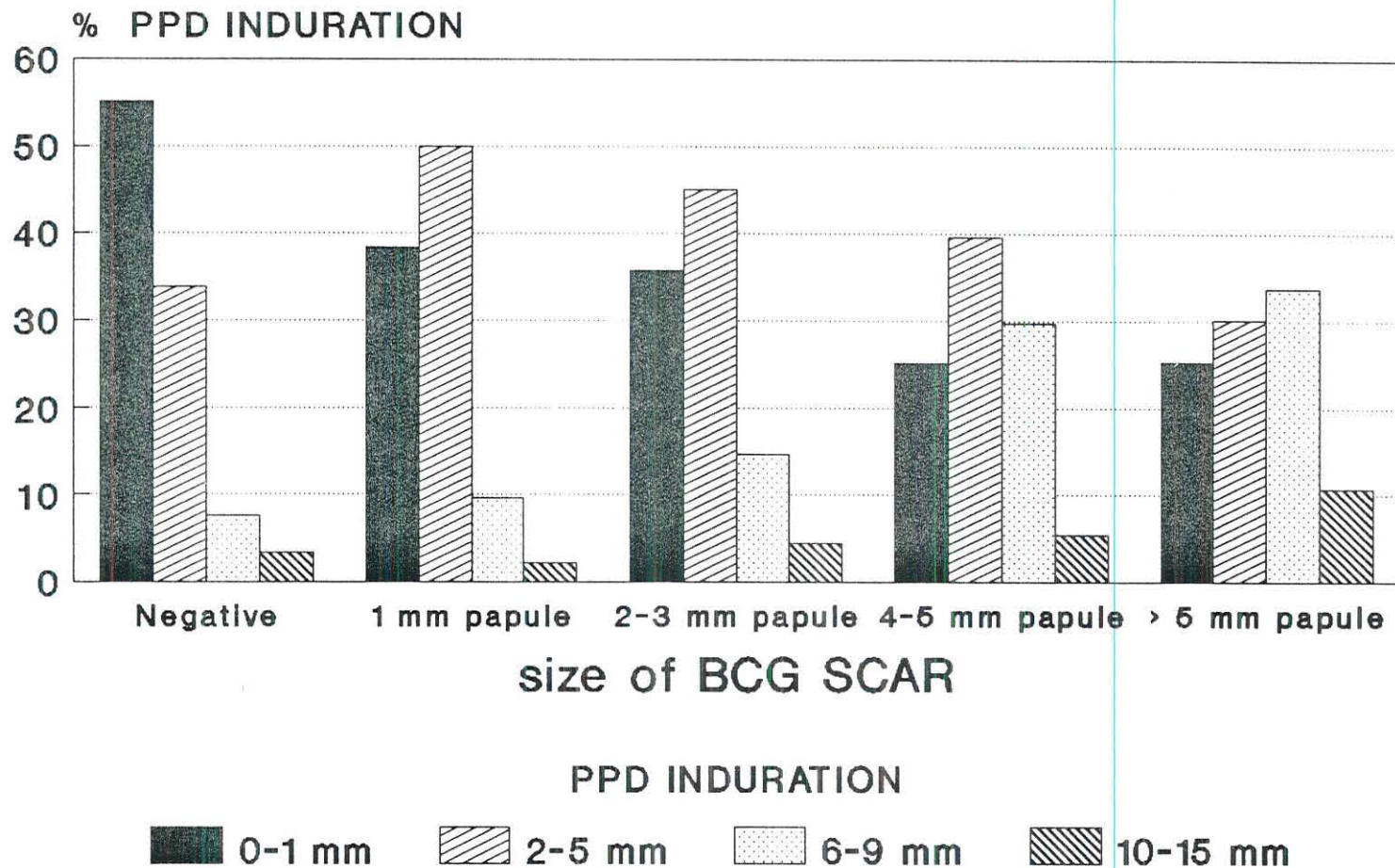


FIG 3

Table 8. Relationship Between the Absence of BCG Scar and Positive Tuberculin Reaction by Age Groups.
In lideta awraja from oct 1992 to feb 1993.

Age groups	+ve tuberculin reaction without BCG scar		
	No	Percent	OR
2 months	35	44.3	1.00
18 months	26	56.5	1.63
5 - 9 yrs	29	47.5	1.14
9 - 11 yrs	58	40.0	0.84

$$x^2 = 1.81(p = 0.17)$$

The presence or absence of confounding should never be assessed by using a statistical test of significance.

Table 9. Relationship Between the Presence of BCG Scar and Negative Tuberculin Reaction by Age Group. in Lideta awraja from oct 1992 to feb 1993.

Age Groups	-ve Tuberculin Reaction with BCG Scar		
	No	Percent	OR
2 months	56	32.2	1.00
18 months	20	28.6	0.84
5 - 7 yrs	47	33.3	1.05
9 - 11 yrs	62	34.6	1.12

$\chi^2 = 0.49 (p = 0.48)$

Table 10. Prevalence of Tuberculin Conversion in those with a BCG Scar by age group in Lideta awraja from oct 1992 to feb 1993.

Age Group	No	%	* MH	CI	OR	p -value
2 months	118	67.8	2.65	1.48, 4.78		< 0.001
18 months	50	71.4	1.92	0.82, 4.52		0.1
5-7 Years	94	66.7	2.21	1.14, 4.27		< 0.01
9-11 Years	117	65.3	2.83	1.76, 4.57		< 0.001

* For example, those 2 month old children with a positive BCG scar were 2.65 times more likely to have a positive tuberculin reaction than similar aged children who did not have a BCG scar.

Table 11. Crude Prevalence and Adjusted Odds Ratios of Tuberculin Non Reactors by selected characteristics in Lideta awraja from oct 1992 to feb 1993.

Variable	Category	Non reactive		MH * OR	95% CI	P-value
		No	%			
AGE	2 months	100	39.5	1.00		
	18 months	40	34.5	0.81	0.50, 1.31	0.35
	5-7 Years	79	39.1	0.98	0.66, 1.46	0.92
	9-11 Years	149	46.0	1.30	0.92, 1.84	0.12
Sex	Male	164	38.5	1.00		
	Female	204	43.5	1.23	0.93, 1.62	0.1
BCG Scar	Yes	185	32.5	1.00		
	No	183	55.3	2.53	1.9, 3.38	<0.001

* Adjusted for other variables in table

The p-value should be considered not as a hard or fast rule for establishing the role of chance but as a guide that chance is an alternative explanation.

Table 12 Relationship between BCG Scar sizes before and after the 2nd BCG vaccination in Lideta awraja from oct 1992 to feb 1993.

Before 2nd vacc. BCG Scar size	After 2nd Vacc. BCG Scar size		Total
	4-5 mm	> 5mm	
Negative	5 (19.2)	21 (80.8)	26 (100)
1-2 mm	1 (16.7)	5 (83.3)	6 (100)
2-3 mm	-	9 (100)	9 (100)
4-5 mm	-	4 (100)	4 (100)
> 5 mm	-	7 (100)	7 (100)
Total	6 (11.5)	46 (88.5)	52 (100)

$$X^2 = 4.27 \quad (p = 0.37)$$

THE FREQUENCY OF TUBERCULIN REACTION IN SMEAR + VE TUBERCULOSIS PATIENTS

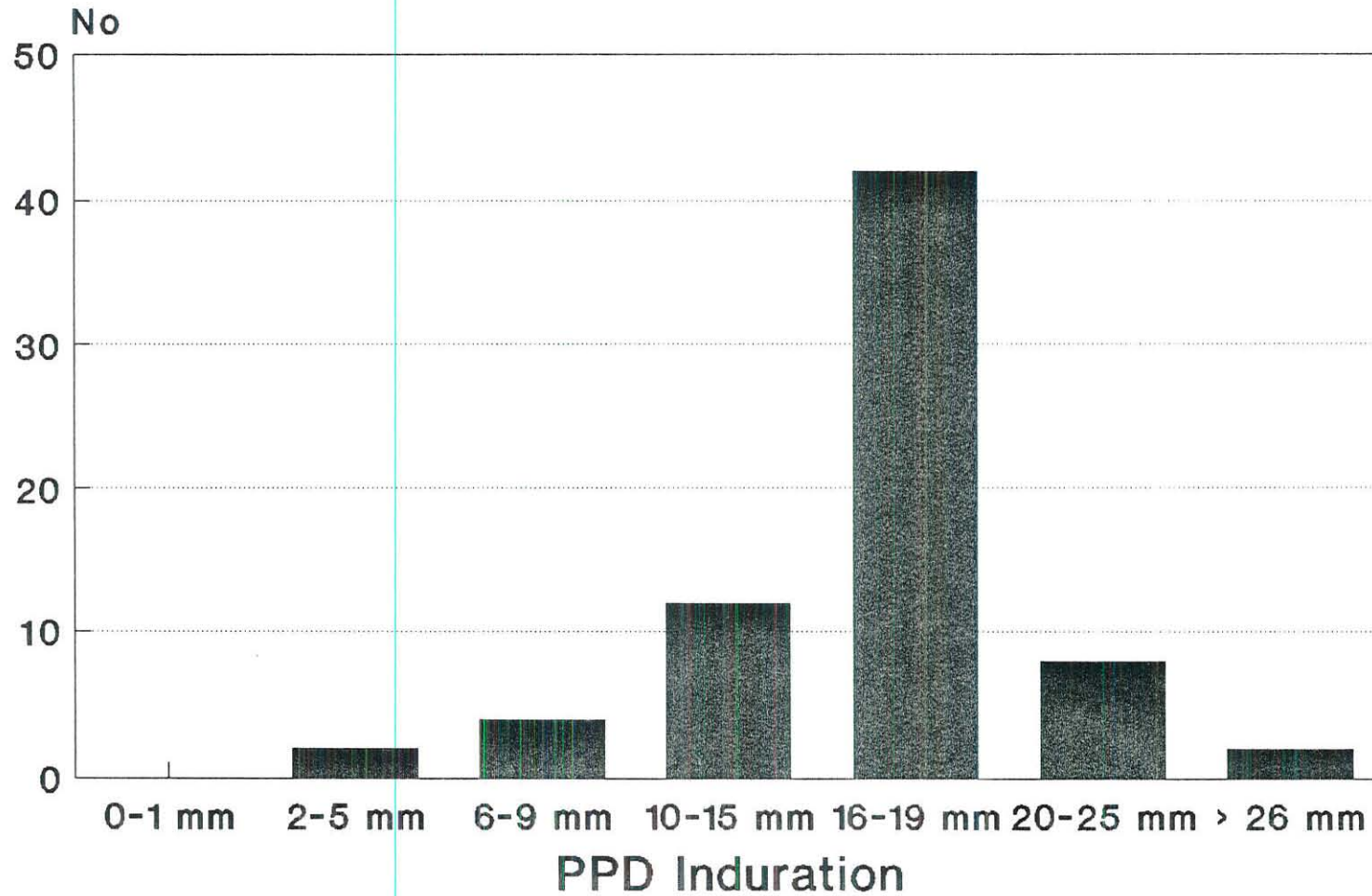


FIG 4

DISCUSSION

In many developing countries like Ethiopia, tuberculosis is still a major public health problem. BCG is the only vaccine for tuberculosis known to be effective in at least in some places. Unfortunately it tends to be less successful in just those areas of the developing world where a vaccine is most needed. The effectiveness of BCG vaccination depends on the quality of vaccine, its transportation and on the technique of the vaccination (59). However currently there is no evidence that when administered to newborns, different preparations of BCG vaccine exhibit different efficacies (15).

The policy of the Ministry of Health in Ethiopia is to offer a single BCG vaccination to all babies within the first few days after birth. Nevertheless, the efficacy of neonatal BCG vaccination in older children and adults has shown some controversy (9,38). BCG vaccination at birth does not give protection for life and thus revaccination is necessary at school age. This stimulated us to evaluate the effectiveness of neonatal BCG vaccination in different age groups of children by testing tuberculin conversion and the occurrence of a scar after vaccination. Additionally, the relationship between the size of the scar and the response was also studied.

Tuberculin response in the absence of BCG scar

The presence of BCG scar is used as an indicator to assess the coverage of the vaccination (48,50). The heat-killed BCG vaccine, with poor sensitizing capacity, can produce a large vaccination (9,47), while the absence of a BCG scar after BCG vaccination has been found between 3 and 30% in different studies (47, 50, 51, 52, 53, 54). In a study among Sri-lankan children (47), 3% of those neonatally vaccinated showed no visible BCG scar, whereas in the Swedish study (54), 30% had no scar, while the figure was 37% in our study. Of these the proportion of children who had a tuberculin reaction (>6mm induration) was 11% and the proportion with a negative tuberculin reaction (0-1mm) was 55%. The corresponding figures were 27% and 46% in the U.A.E. study (50). In an earlier investigation of Ethiopian children, consistent with the present study, 57% of children without a scar also showed no response to tuberculin test.

BCG scar is a local skin lesion resulting from the residual virulence of the BCG sub strains used for vaccine production and it indicates a good take of vaccination and quality of a vaccine (9,10,47). Since it is influenced by the technique of administration of a vaccine, in our study the absence of BCG scar in 37% of tested children could be due to poor administrative

technique of vaccination and poor maintenance of the cold chain system.

The absence of post-vaccination tuberculin sensitivity represents elimination of BCG-induced immunity (10). The negative tuberculin response in 55% of those children without a scar revealed that they were un-protected with BCG vaccination. At the same time, 11% of children showed a strong response (>6mm induration) despite the absence of BCG scar. This might be due to sensitization by atypical mycobacteria. Because, when we analyze the tuberculin response in different age groups after adjusting with BCG scar, it revealed that the likelihood to have positive tuberculin response, despite the absence of BCG scar, was higher in children aged 18 months and 5-7 years compared to infants at 2 months. However, the trend is not statistically significant on chi squared trend analysis(table 8); these groups of children had more chance to be exposed to environmental mycobacteria. Sensitization by atypical mycobacteria may induce some protection against tubercle bacilli but such protection is weaker than that induced by a potent BCG vaccine (8). Moreover these group of children have a well developed immune system compared to infants at the age of 2 months.

Besides this suggested reason, unreliable information about the contact history with tuberculosis patients from the mother should be considered as a possible explanation.

Tuberculin Response in the Presence of BCG Scar

In our study, among group of children showed tuberculin anergy 50% of them had a definite BCG scar. It was comparable with other studies, 50% in karallidde study (47) and also 50% in Indian children (52).

Thus, this consistent observed result revealed that to use BCG scar as indicator to vaccination coverage may over estimate the real situation.

The percentage distribution of tuberculin anergy of those who had BCG scar was found similar between different age groups (table 9). But, it tends to decline as a size of the BCG scar increases: 15% when there was a 1mm nodule and 5.7% when a >5 mm nodule, compared to 35% and 5.4% in U.A.E study (50). The trend was not statistically significant (table 7).

The extent of the local reaction (scar) to BCG vaccination is proportional to the total bacterial mass or residual virulence (15). Hence the large scar size is a good indicator for coverage evaluation, while taking only the large scar size also under-estimates the coverage of BCG vaccination because some of small BCG

scar had BCG induced allergy partially in different age groups.

Consistently, those children who had the second BCG vaccination at the age of 10 years elicited a maximal BCG scar size as 4-5 mm and >5 mm nodule in 19% and 81% respectively. Also, in all of them, a positive tuberculin response was observed. This revealed that the currently available vaccine is potent to induce tuberculin allergy and elicit a large BCG scar, if it has been given in a good administrative technique to those children who have a well developed immune system.

Correlation between BCG scar and tuberculin reaction

This study also demonstrates the direct correlation between BCG scar size and tuberculin reaction ie. as the size of BCG scar increases, the tuberculin reaction induration also increases and vice versa (fig. 3). The correlation was statistically significant ($P < 0.001$). This result also agrees with a study of UAE (50) which has shown a positive correlation.

Moreover children without a BCG scar have 2.53 times (95% confidence interval 1.9, 3.38) the risk of a negative tuberculin reaction compared to those with a scar. The result was found statistically significant ($p < 0.001$) (table 11).

Additionally a higher percentage of a large scar size (>4mm) was observed in school children than preschool children. It indicates that a good take and quality of vaccine in that given calendar year. A scar size could also increase as a body mass increased. Overall, these results emphasize that there is a significant correlation between the size of BCG scar and tuberculin induced induration and the likelihood of having a positive tuberculin reaction of those children with a scar is higher compared to those without. However, in this study 50% of them showed tuberculin anergy despite the presence of BCG scar. Thus the use of a BCG scar as a measure of coverage of vaccination in the communities is likely to be very imprecise; probably it may overestimate the coverage of vaccination. WHO recommends post vaccination tuberculin testing for accurate estimates of the coverage of vaccination(48).

Tuberculin Response Between Different Age Groups

In this study, the distinction between tuberculin reaction representing BCG vaccination and atypical mycobacteria is not precise. The reason is that in tropical countries like Ethiopia post-vaccination sensitivity can not be entirely attributed to BCG in the presence of naturally acquired low grade sensitivity due to environmental mycobacteria. Also, recent studies

display wide variation in tuberculin sensitivity; indurations in excess of 10 mm were documented in children vaccinated at birth. Although this may pose a problem in the determination of BCG induced hypersensitivity, the rate of tuberculin anergy (negative reaction) has been used to assess the effectiveness of our neonatal BCG vaccination policy.

The negative tuberculin reaction indicates the elimination of the living BCG bacteria from the vaccinated host (10). The negative tuberculin response after BCG vaccination has been found in 80-% of Sri-Lankan children (47), 50% of those in the study of Grindulis (51) and 38% in United Arab Emirates infants (50).

In our study, 41% of children had no tuberculin response after neonatal BCG vaccination, excluding 40% nonspecific tuberculin sensitivity (2-5mm) (table 5). Several factors can be responsible for this high rate of tuberculin anergy: factors operating at the time of BCG vaccination; poor maintenance of the cold chain system; and the method of intradermal injection of PPD. However, much has been done in the country, particularly in urban settings, to improve the technique of administering vaccine, monitoring the cold chain system and in supplying the necessary equipment which would be used for EPI.

Apart from these, a reduced dose of BCG vaccine to the newborns causes further decreases immunological responses to a specific mycobacterial antigen (47). Nevertheless, post vaccination tuberculin sensitivity appears to be lower in the newborn than in older children given the same dose of BCG (9). In this study infants at the age of 2 months showed lower rates of tuberculin sensitivity compared to 18 months following low dose of neonatal BCG vaccination and those children who had been revaccinated at the age of 10 years with a low dose of BCG vaccine (0.05ml), all of them showed BCG induced tuberculin allergy in our study. Similarly, 83% of revaccinated children with a low dose of BCG vaccine showed tuberculin conversion in Karalliedde's study (47).

Hence a low dose of BCG vaccine probably may not be a factor for the higher rate of tuberculin anergy after vaccination at birth.

The poorly developed immune system in newborns may decrease the development of immunity in the infants after immunization (9,47). This could be the reason for the higher percentage of tuberculin anergy in our infants compared to 18 month old children. Since the response in the two age groups may not necessarily be the same, even the immediate response to the clinical types of tuberculosis in the two age groups are different(9).

Moreover others offered an explanation for the high rate of tuberculin anergy followed neonatal BCG vaccination: passively transferred maternal immunity, specifically to mycobacterial antigen, interferes with the establishment of active immunity by altering the host response active immunization. But it is passive specific humeral immunity rather than cellular immunity and it usually declines in the 3 months after birth (39,40).

Additionally, a recent study revealed that BCG, given at the end of the 3rd month, provides a higher rate of post vaccination hyper sensitivity than when given during the neonatal period (30). In this regard in Ethiopia the prevalence of tuberculosis is very high: it is estimated to range between 180,000 to 308,000 cases(2), so the proportion of newborns whose mother contracted tuberculosis previously surely would be higher. Besides this, the current policy in the country is that BCG vaccine be given soon after birth. Thus, this also could be the reason for the higher rate of tuberculin anergy in our study subjects.

Because of the above mentioned factors and other related problems, some countries discourage neonatal BCG vaccination.

For this reason we suggest that probably to give BCG vaccine at a dose of 0.1ml at the age of 1 year (the dosage recommended by WHO for 1 year olds and older) may

overcome most of these objections. However, deciding the age of the first vaccination requires a considerable amount of data on the age specific prevalence of tuberculosis infection, which merely reflects the accumulated epidemiological history, particularly in infants under one year. Additionally a cohort analysis of the development (incidence) of infection among groups of children vaccinated at birth and at the age of 1 year is necessary. A system of continuous or periodic surveillance of the incidence of tuberculous meningitis in children is also important(48).

Optimal Time for Revaccination

As discussed in the above paragraph, infants at the age of 2 months showed high rates of negative tuberculin reaction compared to children at 18 months of age. After an initial decline in the rate of tuberculin energy, it tends to increase from age 18 months onwards (fig 1). As indicated in table 2, the rates observed were 34.5%, 39.1% and 46% in the age groups of 18 months, 5 - 7 years and 9 - 11 years respectively. In spite of the increased chance of exposure to environmental mycobacterial as the age increases, the risk of children to have negative tuberculin response is higher in older children compared with the young.

This result indicates the waning effect of tuberculin response was at the age of 5 years compared to 18 months following neonatal BCG vaccination, meaning that post-vaccination hypersensitivity disappeared in the age group 5-7 years.

Meanwhile the observed low grade tuberculin response might be due to sensitization with environmental mycobacteria.

Based on this, we suggest that the optimal time for revaccination should be at the age of 5 years and that it be administered without prior tuberculin test. There is evidence that even vaccination of infected person has no harm or benefit (12) and also WHO recommends the omission of tuberculin test for screening before vaccination (48). In addition, this prior tuberculin test would reduce the coverage of vaccination and also in our country where cost is of major importance, it would more than double the cost .

Additionally, this study demonstrates that when children who had no tuberculin response in the first tuberculin test were revaccinated with low dose of BCG at the age of 10 years, 71% showed >6 mm and 29% 2 - 5mm induration to the second tuberculin test. Also this group of children without BCG scar before the 2nd vaccination produced BCG scar with a diameter of 4 - 5mm (19%) and >5mm (81%) after the 2nd BCG vaccination.

In general, all children who underwent a second BCG vaccination in our study showed a positive tuberculin reaction and exhibited a maximal BCG scar size (>4mm nodule). This reflects the necessity of revaccination in order not only to prevent but also to interrupt the chain of transmission of tuberculosis, since there is no transmission without infected hosts in the community.

The results observed here regarding the necessity of revaccination and its optimal time were also consistent with and supported by other studies.

The study of Karalliedde (47) had a similar design, namely a tuberculin survey in different age groups of children after BCG vaccination at birth. It was observed that there was a waning of the tuberculin response at 5 years and a strong tuberculin response after a second BCG vaccination. With this, they suggested the optimal time for revaccination is at 5 years.

A tuberculin survey in an eastern province of Saudi Arabia also showed that tuberculin sensitivity declines within a few years of vaccination and the authors recommend revaccination at school leaving age following BCG vaccine at birth (44); others indicate the prevalence rate of tuberculosis in children 5 - 9 years was about three times higher than in children aged 1 - 4 years after vaccination at birth (46).

Furthermore, in Hungary between 1959 - 1983 the authorities applied a sustained BCG re vaccination policy. With this policy the incidence of childhood tuberculosis has been reduced by a factor of 3 - 4 times than the vaccinated adults during the first 10 years (10).

Additionally, a recent study demonstrate that the longer the time elapsed since BCG was last administered, the lower is the risk of a positive reaction, since the effect of BCG vaccination on tuberculin sensitivity wanes with time (55).

Moreover, the WHO expert committee (8) notes that the reduced dose of BCG given to the newborn will never induce a lasting significant level of protection. Hence, vaccination at school age should be undertaken irrespective of vaccination at birth.

Specificity of Tuberculin PPD Test

The diagnostic value of the tuberculin PPD test has been questionable for assessing the risk of infection in populations like Ethiopia where BCG vaccination at birth is widespread.

In our study the degree and persistence of tuberculin response is variable. Infants at the age of 2 months in this study who had neither tuberculosis infection nor exposure to environmental mycobacteria

invariably responded to tuberculin test after neonatal BCG vaccination.

It was observed that there was 2 - 5mm induration in 47% and >10 mm induration in 2.4% of infants, while in a study of United Arab Emirate infants (50) it was found that there was 2-5 mm induration in 22% and >10 mm in 39% of infants.

Even though the general pattern in tuberculosis patients displays a unimodal reaction size, the mean reaction size was 16.5mm and 12% showed 10 -15mm induration comparable to the study of Karalliedde (47) where it was 10%.

On the other hand, in this study 3.3% of children without a scar, 11% of children with a large scar size(>5mm) and 10% of revaccinated children also showed a reaction size 10 -15mm without any evidence of tuberculosis infection. BCG vaccinated children may also have enhanced ability to recognize environmental mycobacteria (57); this might have a boosting effect on tuberculin response. Similarly, in Finnish children vaccinated at birth, even tuberculin induration of >15 mm was observed (53). In accordance with this, a potent vaccine is known to induce tuberculin sensitivity as strong as that induced by natural infection (58). Finally Sneidder's recent review (55) concluded that

there is no sure way to distinguish a reaction due to BCG or mycobacterium tuberculosis infection.

Therefore in our country, with a high prevalence of atypical mycobacteria and wide spread of BCG vaccination at birth, the diagnostic value of tuberculin test in assessing risk of infection at a cut-off point 10mm may be less specific.

CONCLUSION

That this study demonstrated the absence of BCG Scar in 37% of children followed neonatal BCG vaccination may reflect poor administrative technique of vaccination and poor maintenance of the cold chain system. Also, to some extent, vaccination of newborns with poorly developed immunity systems might be the reason for high prevalence of the absence of BCG Scar. Since tuberculosis is highly prevalent in our country, the passively transferred maternal immunity to mycobacterial antigen may also reduce the protection of neonatal BCG vaccination.

The positive tuberculin reaction (> 6 mm Induration) was observed in 11% of children without BCG Scar. This indicated that they developed natural protection against tuberculosis resulting from infection with atypical Mycobacteria. This can be expected in a tropical country such as Ethiopia.

The prevalence of tuberculin anergy in 50% of children with a BCG Scar revealed that there is no protection with a given BCG Vaccination. So the presence of BCG Scar, used as an indicator for BCG vaccination, may over-estimate the coverage by a factor of two.

Considering the size of BCG Scar, a lower rate of tuberculin anergy was observed in children who had the large scar size (>5mm) than the small scar size (<3 mm).

Thus the large scar size (>5mm) is a better estimate of the degree of protection offered by BCG vaccination. But to take only this proportion of BCG Scar (>5mm) as an indicator, it also under-estimates the coverage. So for accurate estimate of BCG vaccination coverage in the community, it would be preferable to take the percentage of tuberculin reactors in children who had BCG scar.

However, there was no observed difference in the percentage of tuberculin anergy between different age groups despite the presence of BCG Scar.

All children who had the second BCG vaccination at the age of 10 years elicited a maximal BCG Scar size (>4mm) and positive tuberculin reaction without any complication. This shows that the currently available vaccine is safe and potent but the problem lies on the technique of administering the vaccine and maintenance of the cold chain system. There is also the question of whether it would be administered to those infants with poorly developed immune system or to young children. So this requires further studies.

Tuberculin response in different age groups was also studied and it revealed a relatively higher percentage of tuberculin anergy in infants (39.5%) than in children at the age of 18 months (34.5%). This might be due to poorly developed immune system in infants to induce allergy for a given BCG vaccine than children at the age

of 18 months. Moreover the higher rate of tuberculin Allergy in the age group 5-7 years (39%) compared to 18 months (34.5%) indicates the waning of BCG induced Allergy. It was also observed in 46% of 9-11 years.

Even the low rate of tuberculin allergy observed in age groups 5-7 years and 9-11 years could be due to sensitization by a typical Mycobacteria rather than BCG induced allergy, because these age groups had more chance to be exposed to environmental mycobacteria bacteria.

Therefore since the waning of BCG induced allergy begun at the age of 5 years, we suggested that the optimal time for revaccination should be at the age of 5 years without prior tuberculin testing.

The diagnostic value of tuberculin test was assessed in this study and the tuberculin response of 10 -15mm induration was observed in 4.3% of children vaccinated at birth and in 10% of revaccinated children without any evidence of tuberculosis infection.

The reason, as has been discussed, may be that post vaccination contact with a typical mycobacteria may enhance the tuberculin response; also BCG by itself also can induce allergy > 10 mm induration, therefore the diagnostic value of the test becomes less specific and highly sensitive at the cut off point of 10 mm to assess the risk of tuberculosis infection in the population, since it over estimates the incidence of tuberculosis.

RECOMMENDATION

Based on our finding and reviewed studies we recommend as follows.

1. The presence of BCG Scar, used as an indicator for BCG vaccination, may over-estimate coverage of vaccination by almost a factor of two as it has been observed in this study. Therefore we recommend ~~for accurate estimate of coverage, to determine the rate tuberculin reactors in population who had BCG scar rather than to take only BCG scar.~~
2. The prevalence of Post vaccination hypersensitivity following low dose of neonatal BCG vaccination is lower in infants compared to children revaccinated at the age of 10 years. Hence we recommend further study in deciding the age of first vaccination.
3. A single BCG vaccination at birth could not be expected to provide life long immunity and waning of BCG induced allergy was observed at the age of 5 years. Therefore we strongly recommend the second BCG vaccination at the age of 5 years without prior tuberculin testing.
4. The diagnostic value of tuberculin PPD test in assessing risk of tuberculosis infection at a cut off point 10 mm induration over-estimates the incidence of tuberculosis; thus we suggest for a

better estimate that a cut off point of 15 mm and above should be considered.

5. The currently available BCG vaccine offered is potent and safe but it requires skilled health personnel for a good administration of a vaccine as well as the necessary equipment such as maintenance of cold chain system, sharp needles to properly administer the vaccine intradermally and regular monitoring of the vaccination programme.
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ANNEX 1

Written informed consent was obtained using a written document which stated that

1. The purpose of study was to evaluate the efficacy of BCG vaccination at birth could protect older children against tuberculosis and description of the procedure.
2. Non of the procedures, that is BCG vaccination, PPD test, and others pose a risk or complication to study subjects.
3. Based on the finding of the study, the expected benefit could be revaccination of school children in order to reduce childhood morbidity and mortality caused by tuberculosis.
4. Parents will be informed that they will be returned once or twice to the health institution depending on the results in the study subjects.
5. They also be informed that participation was voluntary and refusal to participate will involve no penalty or loss of benefits.

Before beginning of the study procedure the informed consent format was given to parents for their agreement; in the case of illiterate parent the format was read by a research assistant Consent would obtained with signed form of parents.

Date _____

Signature _____

Glossary

Effectiveness = the actual outcome or benefits of the vaccination in a given population under real life conditions.

Efficacy = protection effect of vaccination as the ratio of the percentage difference between the incidence in unvaccinated and vaccinated children to the incidence in the unvaccinated under ideal conditions i.e the maximum potential.

Tuberculin anergy = loss of tuberculin hypersensitivity or absence of reaction of cell mediated immunity.

Tuberculin non reactor = a person or animal that elicits no tissue response (a reaction size $>1\text{mm}$) for a given tuberculin test.

Tuberculin reactor = a person or animal capable of eliciting a tissue response (a reaction size $< 2\text{mm}$) for a given tuberculin test.