

TUBERCULIN REACTION AMONG HEALTHY BCG VACCINATED PRIMARY SCHOOL CHILDREN IN NNEWI, SOUTH EASTERN NIGERIA

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ABSTRACT

Objective: To assess the Mantoux test reaction pattern in healthy BCG vaccinated Primary School Children aged 6 -10 years in Nnewi, South -East Nigeria.

Materials and methods Four primary schools were randomly selected out of 43 government owned primary schools in the town. The entire BCG vaccinated pupils in each school were the sample frame. Mantoux test was administered in 662 pupils that met the inclusion criteria and had their results read 72 hours later.

Results Three groups of children were identified: First group of 470 (75.6%) were negative, 76 (12.2%) had intermediate reaction, while 76 (12.2%) had positive Mantoux test. Increasing age, sex, presence of BCG scar and age at vaccination did not affect the pattern of Mantoux test reaction.

Conclusion: The result indicates that the use of Mantoux test in the diagnosis of Tuberculosis may not be affected by BCG vaccination at birth and therefore, BCG vaccination at birth should be continued.

KEY WORDS: BCG vaccination, Mantoux test.

INTRODUCTION

Tuberculosis is a communicable disease that contributes significantly to global morbidity and mortality.^{1,2} It is estimated that about 2 billion³ people all over the world are infected with tuberculosis. About 8 million new cases occur every year worldwide, resulting in about 3 million deaths.⁴ Of these cases, 86% occurs in the developing world with more than 12% occurring in sub-Saharan Africa.⁴ Tuberculosis is expected to result in the death of about 30 million people in this decade in developing countries.⁵

The emergence of human immuno-deficiency virus (HIV) infection in mid 1980s has led to an increase in the incidence of tuberculosis.^{3,6} HIV sero-prevalence among tuberculosis patients in Nigeria has been reported to have increased from 2.2% in 1991 to 13.2% in 1996.⁷ Also contributing to this recent increase of tuberculosis incidence is multiple drug resistance.^{8,9} In sub-Saharan Africa, worsening cases of civil conflicts have led to worsening economic situation, destruction of health services, displacement, overcrowding and malnutrition contributing significantly to the increasing incidence of tuberculosis.^{10,11} The burden of tuberculosis is even more in children in developing countries, which have little organizational and financial capabilities to fight the scourge.^{2,3,10} It is estimated that only about 15% of the cases are detected, corresponding to a detection rate of 45 per 100,000 as

reported by the National Tuberculosis Control Programme of Nigeria.⁷

The use of BCG as a control strategy for tuberculosis in Nigeria predates the National Tuberculosis Control Programme. Bacille Calmette-Guerin (BCG), a live attenuated vaccine derived from *Mycobacterium bovis* was first introduced in 1921.¹² Despite the conflicting reports on its efficacy in the prevention of tuberculosis, BCG has been generally accepted as protective against the serious forms of tuberculosis infection.¹³ BCG, is mostly given at birth in Nigeria. Though the duration of protection from BCG vaccination has not been fully determined¹⁴, the delayed hypersensitivity reaction following BCG vaccination is initially high (up to 95%) a few months after vaccination.¹⁵ This however wanes progressively but may last up to 10 years.¹³

Tuberculosis infection in children is usually very difficult to diagnose because it often results in paucibacillary infection and is frequently silent. The use of tuberculin skin test (TST, Mantoux and Heaf tests) and chest radiograph¹⁶ in the diagnosis of tuberculosis is usually invaluable in cases with high index of suspicion. Tuberculin skin test is based on delayed hypersensitivity reaction to *M. tuberculosis* antigen. Its value in the diagnosis of tuberculosis is however diminished by a number of factors. One of the most important is that it does not differentiate present or past tuberculosis infection or BCG vaccination. This

may affect the diagnosis of tuberculosis in children in the region with high rate of BCG vaccination at birth.

This study is designed to determine the hypersensitivity reaction pattern to Mantoux test in healthy BCG vaccinated primary school children aged between 6 and 10 years in Nnewi, South Eastern Nigeria.

MATERIALS AND METHODS

Area of Study

This study was done in Nnewi, Nnewi North Local Government Area of Anambra State, in Southeastern Nigeria. It is an urban area as well as a one town Local Government Area. Its projected population for the year 2000 is 121,065.¹⁷

The majority of its inhabitants are of Igbo extraction mostly engaged in trading and subsistence farming, with a few public servants. The town is divided into four quarters with a total of 43 Local Government-run primary schools and a pupil population of 16734 in the year 2002.

Period of Study

This study was carried out between February and April 2003, a period of three months. Four schools, one from each quarter, were randomly selected for the study. Three weeks were allocated to each school. The first week of study for each school was used for the distribution and collection of questionnaires. The questionnaires were analyzed and the subjects for the study selected. The next two weeks were used for the administration of the tests and reading of results. The tests were administered on Mondays, Tuesdays and Fridays while the readings were done 72 hours later on Thursdays, Fridays and Mondays respectively.

Study Design

Permission for the study was obtained from the Ethical Committee of the Nnamdi Azikiwe University Teaching Hospital, State Primary Education Board and Nnewi North Local Government Education Authority. The aim and methods of the study were explained to the parents and guardians at a Parent Teachers Association Meeting organized by the school heads following a directive from the Local Government Education Authority.

Study Population

This comprised all BCG vaccinated primary school children aged 6 – 10 years.

Inclusion Criteria

Healthy subjects who had BCG vaccination as evidenced by the presence of BCG scar or documented in the immunization card were included for the study.

Exclusion Criteria

Subjects with present or past history of tuberculosis or present history of cough, which has lasted for more than one month, recent history of measles, or receiving steroids or cytotoxic therapy were excluded.

Sample Size

The sample size for the study was calculated using the formula¹⁸:

$$n = \frac{Z^2 P(1-P)}{d^2}$$

Where n = sample size

Z = 1.96 at 95% confidence interval.

P = estimated population proportion. Since this proportion for the community is not known, a value of 50% (0.5) is assigned to obtain maximum value for P.

d = absolute precision required on either side of the proportions = 5%.

$$n = \frac{(1.96)^2 \times 0.5(1-0.5)}{(0.05)^2} = 384$$

Substituting the figures in the formula, the minimum sample size n was calculated as 384; rounded off to 400. A minimum of 600 subjects were targeted for the study on attrition rate of 50% which was guided by the experience of another local study¹⁹ that involved the use of needle puncture in primary school children. There were also uncertainties about the number of subjects that will properly fill their questionnaires or those that will fall within the exclusion criteria.

Pre-Test

A hundred copies of the study questionnaire which also served to convey parental consent was distributed to pupils in a primary school in a pilot study. They were self-completed at home by the parents and guardians. The same questionnaire was equally distributed to parents randomly by one of the authors for self-completion. Fifty-five percent of the completed questionnaire was returned and analyzed. Their responses were similar to those obtained by the authors. Based on this, it was concluded that the questionnaire was understandable and can be completed at home easily by the parents and guardians.

A follow-up on the defaulters showed that their main reasons for not completing the questionnaire were:

- Fear that their children have received too many vaccines and another was about to be given.
- False assumption that their children will be sterilized by the test as it was presumed to be a family planning injection.
- False assumptions that they may be asked to pay for the test.

Based on this experience, the authors attended the meeting of the Parent Teachers Association in each school used for the study to explain the study to the

parents and guardians of the pupils in order to reduce this high default rate.

Sampling Strategy

All the geographical quarters of the study area were involved in the study. The schools in each quarter were listed and one school per quarter was randomly selected by ballot.

In each school selected per quarter, the school register was used to stratify the entire school population per class into the age strata of 6, 7, 8, 9 and 10 years. For the purpose of analysis, each age stratum was further stratified into male and female subgroups.

A total of 1,120 questionnaires were distributed. Of this number, 839 (75%) were duly filled and returned with implicit parental consent. Out of this number, 172 children (20.5%) were not included because their questionnaires were either not properly filled (93) or the subjects were within the exclusion criteria (79). A total of 667 subjects with properly completed questionnaires had physical examination done on them. The subjects were further examined for signs of nephrotic syndrome, heart diseases, and asthma and other conditions that may require steroids in their management. Three subjects had features of sickle cell anaemia and two were asthmatic and on medication including steroids. They were consequently eliminated from the study. A total of 662 subjects were thus enrolled for the study.

Administration of PPD

The PPD used was obtained from Aventis-Pasteur and was composed of purified tuberculin with stabilizing agents at appropriate pH made up of glycine, sodium chloride and dipotassium phosphate. The PPD was in freeze dried forms of 100 i.u. ampoules for dissolution in 1ml of accompanying diluent. It was stored in the refrigerator at a temperature of 2-10 degree centigrade. The refrigerator was powered by public electricity supply with standby generator services during power failure. It was transported in ice-packed immunization bags to the schools. The PPD was reconstituted shortly before use as required for each session. Each reconstituted ampoule was finished before reconstituting another. Each ampoule was used for 10 (ten) subjects and lasted between 30 and 45 minutes.

Mantoux test was administered to the subjects using standard techniques.^{20,21} Using an indelible marker, the injection site was demarcated with a circle of about 4cm diameter with the site of the wheal at the center. The subjects were asked not to scratch the area or sponge it during bath. The reaction (induration) was measured 72 hours later at its widest diameter with a transparent millimeter ruler at right angles to the vertical axis of the forearm using the ball point pen method.²²

Grading of the Results

The results of the readings were grouped into three categories and interpreted as recommended^{13, 20} as shown in

Table 1: Grading of Tuberculin Reactions.

Group	Size(mm)	Interpretation
I	0-4	Negative mantoux reaction
II	5-9	Intermediate mantoux reaction
III	≥10	Positive mantoux reaction

Those in Group I (tuberculin reaction of 0 – 4mm) were negative and sent for revaccination.¹³

Those in Group II (tuberculin reaction of 5 – 9mm) showed intermediate hypersensitivity reaction which could be due to BCG effect or previous tuberculosis infection. This, however, does not correspond to adequate immunity.⁴

Those in Group III (tuberculin reaction of ≥10mm) were positive and may have active tuberculosis. They were sent to Nnamdi Azikiwe University Teaching Hospital for further assessment for tuberculosis.

Statistical Analysis

The results were analyzed by simple frequency counts, percentages and proportions using Microsoft Excel package. Chi-square test was employed for the test of significance in each of the characteristics of the study population at $P < 0.05$.

RESULTS

The skin test results were read after 72 hours in 622 subjects, leaving out 40 subjects who were absent from school at the time of reading. This gave a default rate of 6.0%.

The sex distributions of the subjects were 298 males and 324 females, a M/F ratio of 1:1.1. Their age and sex distributions are as shown in

Table 2: Age and sex distribution of the 622 children studied. Age (years)

Sex	6 (n)	7 (n)	8 (n)	9 (n)	10 (n)
Male	56	48	70	50	74
Female	70	44	68	64	78
Total	126	92	138	114	152

Of the 622 subjects studied, 215 had BCG scar while 407 had documented evidence of vaccination in their immunization cards

Table 3: Tuberculin reaction in relation to BCG scar

Reaction (mm)	BCG Scar n (%)	No BCG Scar n (%)	P Value	Significance Level
0-4	147(68.4)	323(79.4)	0.13	S
5-9	32 (14.9)	46 (11.3)	0.23	S
≥10	36 (16.7)	38 (9.3)	0.01	NS
Total	215(100)	407(100)		

The negative and intermediate TST reactions did not show any difference in their reactions, however, the positive TST reaction showed statistically significant difference ($P < 0.02$).

Sex did not influence TST reactions

Table 4 : Sensitivity pattern by sex per age group

Age Yrs	Tuberculin Reactions	Male n (%)	Female n (%)	P value	Significance level (S)
6	0 – 4mm	40 (71.4)	48 (68.6)	0.85	NS
	5 – 9mm	6 (10.1)	16 (22.9)	0.11	NS
	≥ 10mm	10 (17.9)	6 (8.7)	0.15	NS
7	0 – 4mm	36 (75.0)	32 (77.7)	0.90	NS
	5 – 9mm	4 (8.3)	6 (13.6)	0.44	NS
	≥ 10mm	8 (16.7)	6 (13.6)	0.71	NS
8	0 – 4mm	46 (65.8)	60 (88.3)	0.13	NS
	5 – 9mm	12 (17.1)	6 (8.8)	0.18	NS
	≥ 10mm	12 (17.1)	2 (2.9)	0.01	S
9	0 – 4mm	38 (76.0)	52 (81.3)	0.75	NS
	5 – 9mm	8 (16.2)	8 (12.5)	0.62	NS
	≥ 10mm	4 (8.0)	4 (6.3)	0.73	NS
10	0 – 4mm	58 (78.4)	60 (76.9)	0.92	NS
	5 – 9mm	4 (5.4)	6 (7.7)	0.58	NS
	≥ 10mm	12 (16.2)	12 (15.4)	0.90	NS

There is no pattern of reaction to TST among the different sexes per age group. Positive TST for the females seem to have decreased significantly at the age of 8 years but, however, rose to an insignificant level at 10 years. The males did not show this pattern of

response. It was also observed that the subjects who received BCG vaccination after 2 months of birth reacted more positively to TST (19.0%) when compared to those who received BCG vaccination within two months of birth (11.7%). The negative TST reactions are the same in both groups. This however is not statistically significant ($P > 0.05$)

Table 5 : Sensitivity pattern by age at vaccination

Tuberculin Reaction (mm)	Vaccinated within 2months of birth n (%)	Vaccinated after 2months of birth n (%)	P Value	Significance level
0 – 4 mm	438 (75.5)	32 (76.2)	0.96	NS
5 – 9 mm	74 (12.8)	2 (4.8)	0.15	NS
≥ 10mm	68 (11.7)	8 (19.0)	0.19	NS
TOTAL	580 (100)	42 (100)		

DISCUSSION

The high negative Mantoux test noted in this study (75.6%) compares with the findings of many other studies.²³⁻²⁹ One notable exception³⁰ reported a negativity rate of 23%. This can be attributed to the fact that the study involved only subjects treated of confirmed tuberculosis. The high negative TST in this study may signify that delayed hypersensitivity to BCG wanes rapidly after vaccination and probably before the age of 6 years as no further significant decline in hypersensitivity rate was observed from 6 – 10 years. This rapid decline in hypersensitivity following BCG vaccination has been documented by earlier researchers.¹⁵

This study shows an intermediate reaction to tuberculin in 12.2% of the subjects. This is much higher than 0.7% and 1.7% from Middle East studies^{28, 29} and lower than 19.4% reported in Lagos³¹. The low intermediate reaction in the Jordanian study²⁸ could be accounted for by the presence of anergy since the study was carried out among sick children, while Nottidge et al²⁹ studied unvaccinated children, in whom a negative reaction would ordinarily be expected. The intermediate TST of 12.2% noted in this study could be the effect of BCG vaccination or hypersensitivity reaction to natural TB infection.

The positive reaction of 12.2% observed in this study is comparable to the findings of 7% in BCG vaccinated children in Botswana²³ and 9.3% reported in adolescent school boys in Lagos.³¹ It is higher than the rate of 0.4% and 1.7% reported by workers in Jordan and Saudi Arabia^{28,29} respectively, but much lower than 49% and 68% reported in Turkey³² and Qatar³⁰ respectively. The fairly high positive TST of 12.2%

observed in this study at ages 6 – 10 years may be because of the epidemiological factors associated with tuberculosis infection rather than the effect of BCG vaccination as increasing age did not lower the positive reactions which would have been expected if it were due to BCG effect. Although not statistically significant, for unclear reasons, males seem to have more positive TST reactions than females. Among the factors that influence the outcome of tuberculin skin test survey³³, are the potency of the PPD, the expertise of the administrator, the sample size, the study population and the epidemiology of tuberculosis in the study area. This may account for the differences in the observed results in various studies²³⁻²⁹.

Some explanation may be offered for the findings of much higher positivity rates in some studies. The study in Turkey²⁶ was carried out in children less than 5 years at which age the effect of BCG on tuberculin reaction is presumably still high. Also, the study included children with positive contacts of tuberculosis in the group studied, another factor that would enhance tuberculin conversion. The Qatar study³⁰ was carried out in children that were treated of tuberculosis. It is therefore, not unexpected that the tuberculin positivity rate in that study would far exceed the observation in this work done on healthy children. Of the BCG vaccinated subjects studied, 215 had BCG scar against 407 without BCG scar. Generally, there was no statistically significant difference in their reactions to Mantoux test ($P=0.062$), but on testing for significance along the reaction patterns, positivity rate ($\geq 10\text{mm}$) showed some significant difference. Those with BCG scar showed more positive TST than those without BCG scar ($P < 0.01$). This finding contrast the finding of workers in Turkey.²⁴ They found that those without BCG scar had more positive TST reactions than those with BCG scar. The finding in this work is more in keeping with the expected as BCG scar is an indication that the subject has responded adequately to BCG vaccination, and therefore, is expected to have more hypersensitivity reactions on tuberculin stimulation later. This pattern of response was also observed in Iran.²⁷

Sex differences did not affect the pattern of response to tuberculin at each age group (Table 4). This result is comparable to the findings of other workers who studied non-vaccinated primary school children under 5 years²⁸ and post tuberculosis treated subjects³⁰ respectively. In this study, a significant difference in the positive reaction between the sexes was observed at 8 years. This trend was not observed in any other age strata and did not affect the overall pattern of the reaction. The reason for this difference is unclear.

This study also revealed that the subjects who were vaccinated within 2 months of birth did not show any difference in their pattern of reaction to tuberculin (Table 5). This finding, however, is unexpected as it is theoretically assumed that BCG vaccination at an older

age will elicit more conversion which ought to give stronger hypersensitivity reaction than when vaccinated at a younger age when immune maturity is less.³⁴ This therefore implies that the age at which BCG vaccination is given does not affect the pattern of hypersensitivity reactions.

The general TST reaction shows very high negative Mantoux test in the study population, and since increasing age and sex did not influence the pattern of TST reactions, Mantoux test is still useful as an integral aid in the diagnosis of tuberculosis in this age group and BCG immunization should continue.

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