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## **Evaluation of the inner thigh as site for mantoux test among children in Owerri South Eastern Nigeria**

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**Abstract:** *Background:* Mantoux test, a type IV hypersensitivity reaction, is commonly used to screen children for possible tuberculosis. It is applied on the volar surface of forearm. Sunlight ultra-violet rays (UV) have recently been shown to induce immunosuppression that alters the skin response to Mantoux test negatively.

*Objective:* The study aimed to evaluate the inner thigh as a possible site for Mantoux test in children attending Federal Medical Centre Owerri whom their primary attending Physicians requested a Mantoux test.

*Methods:* This was a cross sectional study conducted between June and October 2011. Concomitant intradermal injection of 0.1ml of purified protein derivative on the volar aspect of the forearm and the inner thigh was carried out. Induration was measured concurrently in 106 consecutive children, between the ages of 3 months and 15 years. Using volar surface of forearm as gold

standard, values of inner thigh induration at a set cut-off was determined. Student's t test, Pearson correlation coefficient and the Chi square were used to compute data.

*Results:* The mean diameter of induration on the inner thigh ( $12.51 \pm 3.44$ mm) was higher than mean diameter of induration on the volar surface of the arm ( $11.03 \pm 6.34$ mm). This is however not statistically significant. There were (29) children who had reactive induration on the inner thigh whilst 19 had similar induration on the volar surface of the forearm. In the presence of factor(s) that negatively affects the response of the skin to Mantoux test; the inner thigh induration yield was higher. There was a strong positive correlation between the two site measurements.

*Conclusions:* The inner thigh as a site for Mantoux test in children has shown potential for higher yield.

**Keywords:** Sunlight, Inner thigh, Mantoux test, TB, Children.

### **Introduction**

The diagnosis, treatment, and prevention of tuberculosis; is made difficult by the huge challenge of worldwide human immunodeficiency virus (HIV) pandemic. Early and accurate tuberculosis diagnosis is essential to improve treatment outcomes for patients. In the diagnosis of childhood TB, a criteria based on clinical and radiological findings have been developed by the World Health Organization (WHO).<sup>1,2</sup> The laboratory diagnosis of tuberculosis involves culture of sputum and other clinical specimens in Lowenstein-Jensen medium, Ziehl-Neelsen staining for acid fast and alcohol fast bacilli, Lipoarabinomannan detection assay, Microscopic observation of drug susceptibility assay, QuantiFERON-TB Gold and Mantoux test. In the less developed countries of the world, screening of children for possible TB is by tuberculin skin testing. Mantoux test is expected to de-

tect children and adolescents with latent TB infection. It is applied on the volar surface of the forearm (for ease of assessing the presence or absence of induration). Mantoux test may be affected negatively by such factors as severe malnutrition, steroid, and cytotoxic therapy.<sup>3,4</sup> Immunosuppression induced by ultraviolet rays (UVR) has been shown to alter the skin response to the Mantoux test negatively.<sup>5-9</sup> Furthermore, It has been observed that even modest doses of UV rays can significantly reduce local delayed type IV hypersensitivity (DTH) response to recall antigens, and that recent sun exposure should be considered a possible cause of false negative Mantoux response.<sup>5</sup>

Kuchel and Barnetson<sup>8</sup> documented a clear reduction in the Mantoux response at UV irradiated site when compared to the un-irradiated site. They suggested the use of the lower back; a part of the body not exposed to

sunlight UVR as site for Mantoux testing, especially for children in the tropics who are exposed to enormous amount of sunlight.

Recently, Wammanda et al in a study in Zaria Nigeria highlighted the potential for an enhanced Mantoux test result if parts of the body that are not exposed to high doses of ultraviolet radiation are used.<sup>10</sup>

This cross sectional study which was aimed at evaluating the inner thigh as a potential site for Mantoux test in children was undertaken in Owerri South Eastern Nigeria. Mantoux test was requested for suspected TB cases as part of their evaluation for tuberculosis by their primary attending physicians.

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## Materials and Methods

This cross sectional study was carried out at the Federal Medical Centre, Owerri (FMCO) a tertiary Health facility in Imo State, South Eastern Nigeria. It was carried out from June to October 2011. One hundred and six children were studied, their age ranged from 3 months to 15 years. Sample size was calculated using prevalence from a previous study in Owerri<sup>11</sup>

Ethical approval was sought and obtained from Ethics Committee of the Federal Medical Centre. Parents/care givers of the subjects gave informed consent before inclusion in the study. However, any subject that violated the protocol at any point during the course of the study or those on cytotoxic, steroid therapy at least 6 week before enrolment were excluded.

A structured interviewer administered questionnaire was used to obtain pertinent socio-demographic and clinical information. The height in centimetre (cm) and weight in kilograms (Kg) of subjects was measured using appropriate equipments (An infantometer and a stadiometer to which a weighing scale (ZT-120 Health scale) was attached). For the very young children less than 2 years old, lengths were measured using an infantometer with the help of an assistant. Elimination of zero error was done before each weight was obtained. For older children, weight and height were measured using a stadiometer to which a weighing scale (ZT-120 Health scale) was attached. Standardization using standard weights was done at intervals. A straight adjustable wooden blade was used as a headpiece to mark off the child's height. The results of height and weight were compared with the standard National Centre for Health Statistics Charts (NCHS charts).

A dose of 0.1ml PPD (BB-NCIPD Ltd, Sofia, Bulgaria), containing five tuberculin units constituted shortly before use; was concurrently administered intradermally over the volar aspect of the forearm and the inner thigh (one finger breath medial to the spatial line running down the middle of the thigh). This was done using a 27 gauge needle by the principal investigator with an assistant after training and standardization of the process. Throughout the data collection process standard aseptic procedure was observed. The injection site

was differentiated with an ink marker, using a circle about 2-4 centimetres diameter with the site of the injection in the centre. The parents and subjects were advised not to stroke or sponge the injection site during a bath (to avoid false positive results), and to have the children dressed in clothes that will cover the thigh (to remove exposure to UV light that may lead to a false negative result).

The sites for Mantoux test were examined for the presence or absence of induration after 48-72 hours. In subjects with induration, it was measured along the transverse axis of forearm and thigh in millimeters<sup>12</sup> with a transparent meter rule, using the ball point pen technique of Sokal.<sup>13</sup> Reading was done in good light<sup>4</sup> by a researcher. To be regarded positive, induration ought to measure 10 mm.

## Statistical analysis

The collected data was analysed using the Statistical Package for Social Sciences (SPSS) for Windows (Inc. Chicago USA, 2001). Paired t test was used to compare the mean induration measurements from the two sites. Correlation was determined by the Pearson correlation coefficient. The level of significance was taken to be  $p < 0.05$ .

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## Results

The subjects' age ranged from 3 months -15 years. Out of the 106 children, 60 (56.6%) were male while 46 (43.4%) were female with a male/female ratio of 1.3:1. The mean age was  $6.5 \pm 4.2$  years. Of the 106 studied children, 85 (80.2%) had normal weight for age while 11 (10.4%) were underweight for age, none of which was overtly malnourished. The overweight for age children were 10 (9.4%). There was a progressive increase in the number of inner thigh induration measurements with increasing age, but among those 15+ years there was a reduction in percentage of induration measurements. This was statistically significant.  $\chi^2 = 12.89$ ,  $p = 0.012$  ( $p < 0.05$ ).

The diameters of induration of the Mantoux response on the volar aspect of the forearm for all studied children ranged from 0-26 mm, (mean  $4.15 \pm 5.72$ mm) while the diameters of the Mantoux response on the inner thigh ranged from 0-22 mm (mean  $4.91 \pm 5.64$ mm). This difference in mean was significant statistically,  $t = 2.48$ ,  $p$  value = 0.015. ( $p < 0.05$ ).

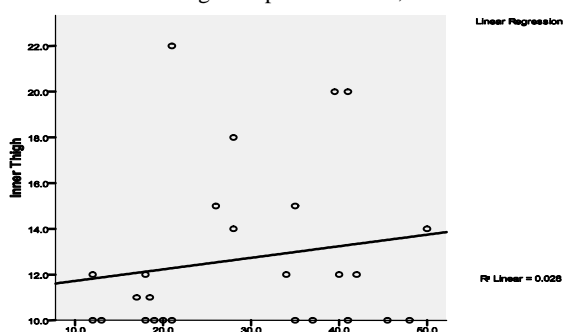
The mean induration measurement on the volar surface of the forearm ( $12.51 \pm 3.44$ mm) was not statistically significantly higher than the mean Mantoux induration measurements on the inner thigh ( $11.03 \pm 6.34$ mm). A total of 29 (27.4%) children had positive Mantoux test. In all the 29 (27.4%) children with a positive Mantoux induration, the mantoux test was positive on the inner thigh, while in 19 (17.9%) of these subjects the concomitant Mantoux induration was positive (induration of

10mm) on the volar surface of the forearm. This difference in the site of Mantoux positivity was statistically significant. The average difference between the mean of volar surface and inner thigh was  $1.47 \pm 7.10$ mm.

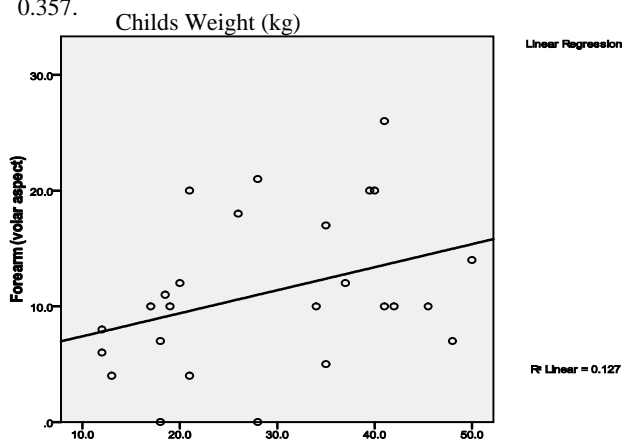
When the nutritional status was considered, 85 had normal weight for age, 11 were underweight and 10 overweight. Of the 11 underweight subjects, 3 (27.3%) had positive Mantoux induration on the inner thigh as against 2 (18.2%) subjects with positive Mantoux induration on the volar surface of the forearm.

The regression analysis of induration and weight among subjects with induration showed that the ability of the weight to predict the Mantoux response for the inner thigh was  $p$ -value 0.412, beta-value 0.168, while for the volar surface of the forearm  $p$ -value was 0.074 and beta-value 0.357 (Figures 1 and 2).

**Fig 1:** Regression analysis of inner thigh induration and weight among subjects with induration. The ability to predict response for the inner thigh was  $p$ -value 0.412, beta-value 0.168.

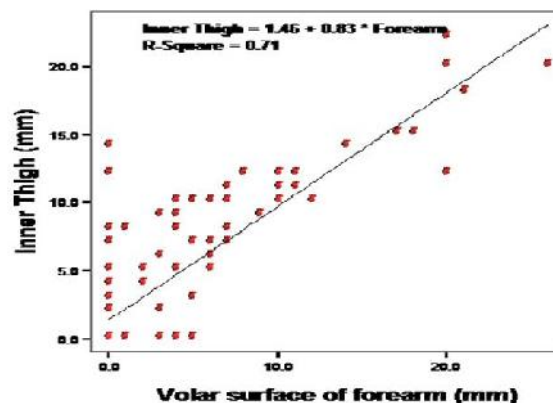


**Fig 2:** Regression analysis of volar surface of forearm induration and weight among subjects with induration. The ability to predict response for the inner thigh was 0.074 and beta-value 0.357.



A significant positive correlation between volar surface induration measurement and the inner thigh induration measurement ( $r = 0.71$ ;  $p < 0.001$ ) was established. As shown in Figure 3, high volar surface measurements were associated with high inner thigh Mantoux measurements.

**Fig 3:** The relationship between volar surface and inner thigh measurements.



## Discussion

This study has demonstrated the potential of the inner thigh as a site for an enhanced reactive Mantoux test result in children. The number of children with reactive induration on the inner thigh was higher (32%) than the number of children with similar induration on the volar surface of the forearm (12.3%). Even when 10 mm is taken as cutoff for positive Mantoux induration, the number of children with reactive induration of 10 mm on the inner thigh was higher (27.4%) than the number of children who had similar reactive induration on the volar surface of the forearm (17.9%). This difference was statistically significant. A similar result was obtained by Moyal,<sup>5</sup> Kuchel,<sup>6</sup> Damian<sup>8</sup> and Wammamda<sup>10</sup> *et al* when they examined Mantoux induration measurements without a set cutoff point. Since the Mantoux tests on the inner thigh and the volar surface of the forearm were concurrently applied, the most likely explanation for this observed difference would be the presence of a factor that could negatively influence the delayed type of hypersensitivity reaction in the children in a differential way. While malnutrition, steroid and cytotoxic therapy will result in global immunosuppression in a child, sunlight-induced immunosuppression will depend on the exposure of the skin to sunlight. Several other authors<sup>7-9,10,14</sup> have also previously documented the role of UV light in significantly reducing local DTH response to recall antigen.

Apart from the rate of reactive Mantoux response, this study has shown that the mean diameter of the reactive Mantoux test was statistically significantly higher; when the Mantoux test was applied on the inner thigh compared to that obtained on the volar surface of the forearm. The mean inner thigh induration measurement was higher than volar surface induration measurement with a difference of  $1.47 \pm 7.10$ mm. This difference though small and not statistically significant is critical when measuring Mantoux induration in the children, where accuracy is crucial for important management decisions especially in tropical countries with dearth of facilities.

The result of this study also revealed that the use of the inner thigh as a possible site for the Mantoux test may

be more appropriate than the volar surface of the forearm, the traditional site for this test, in situations where the presence of factor(s) that negatively affects the response of the skin to Mantoux test prevail. Out of the 11 children who were underweight at the time of the study, 3(27.3%) had positive induration. All the 3(27.3%) children had positive induration on the inner thigh as against 1(9.0%) who had positive reaction on the volar surface of the forearm. Since the number of this group of children was small, the statistical significance of this difference was not assessed. But the result suggest more reactive Mantoux test result on the inner thigh compared with the traditional volar surface of the forearm in children who are underweight. This observed difference was also highlighted by the Zaria study.<sup>10</sup>

A limitation of this study was that it was a hospital based study and therefore it will be hard to generalize the result except a community based study is carried out.

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### Conclusion

It is concluded that a higher number of subjects had reactive induration on the inner thigh compared to the

number of children with similar induration on the volar surface of the forearm. Also in the presence of factor that negatively (underweight) affects the response of the skin to Mantoux test, the inner thigh as a site for PPD administration in children has been shown to have good potential. Additional studies will be needed to further evaluate its benefit in the community especially in resource poor countries.

**Conflict of interest:** None

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