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CUT-OFF VALUE OF MANTOUX INDURATION IN TUBERCULOUS CHILDREN IN R. MACEDONIA AND THEIR NUTRITIONAL STATUS

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A b s t r a c t: *Introduction:* The interpretation of the Mantoux test, as well as knowledge of factors that affect the test's induration size, is significant for the early diagnosis of tuberculosis in children.

Aims: To determine the Mantoux test induration cut-off size which distinguishes between positive and negative results in Macedonian children suffering from tuberculosis.

To find whether there is any correlation between the nutritional status (malnutrition) and the Mantoux induration size.

Materials and methods: A retrospective and descriptive study was carried out in 270 patients up to 14 years old with diagnosed tuberculosis, treated during the period of 2003–2007 in the Pediatric Tuberculosis Department at the Kozle Institute for Respiratory Diseases in Children, Skopje, Macedonia.

The cut-off value of the Mantoux test induration size was determined by analysis of the test results distribution. The nutritional status was calculated as the weight for age standard (z-score). Correlation between the TST results and the nutritional status was calculated with Pearson's coefficient of correlation.

Results: Two hundred and seventy (270) children with tuberculosis were included in the study. The lung form of tuberculosis was present in 87.8% of the children. 53.7% of the patients were male, and 46.3% were female. The age group of 1–2 years was the most numerous (15.2%), followed by the group aged 7–8 years (10.4%). 33 children (12.2%) were undernourished (z-score < –2). The Mantoux test induration size was in the range of 0 to 32 mm, with an average of 13.4 mm. The distribution of the frequencies of the indurations had a bimodal form, with the primary mode at 0 mm, the

secondary mode at 15–19 mm, and antimode at 5–9 mm. The Mantoux test had a sensitivity of 82.5%, specificity of 62%, positive predictive value of 68.46% and negative predictive value of 78.61%. Pearson's coefficient of correlation (r) of 0.175 showed a weak positive correlation between the results of TST (induration size) and the nutriational status.

Conclusions: The cut-off value of the induration which distinguishes negative from positive Mantoux reaction was 5 mm. The interpretation of the test is recommended to be carried out depending on the risk factors for TB development, which surround the children. The Mantoux test in malnourished children should be interpreted with caution.

Key words: tuberculosis, Mantoux test, induration, cut-off value, weight for age z-score.

Introduction

The tuberculin skin test, developed by Koch over a century ago as a means to treat tuberculosis, has emerged as the definite means to identify infection with Mycobacterium tuberculosis. The tuberculin skin test has been used extensively to identify individuals who have been infected with Mycobacterium tuberculosis and who would most likely benefit from treatment of latent tuberculous infection [1]. The tuberculin skin test using the Mantoux technique is the standard tuberculin test in most countries, including ours. However, BCG vaccination can confound TST reaction in the diagnosis of latent tuberculous infection. Information provided by the purified protein derivative (PPD) reaction becomes unreliable because it is unable to distinguish between natural infection and the bacille Calmette–Guerin effect [2]. In fact, there is a new, *in vitro* whole blood test (Quanti–Ferron- TB-Gold = QFT-G) which measures the amount of Interferon-Gamma (IFN-Gamma) produced by CD4-T cells previously exposed to the peptides ESAT-6 and CFP-10, which are present in the antigens of M.tuberculosis, but absent in *Mycobacterium bovis* (BCG) and in non-tuberculous mycobacteria. But the assay is expensive and not suitable for routine use, though its specificity is superior to that of the tuberculin skin test (TST) [3].

The World Health Organization (WHO) does not recommended a unique cut-off value of Mantoux induration valid for the whole world. Instead, it recommends different cut-off points for different regions, rep. groups, taking into the account the prevalence of tuberculosis and the immunobiological characteristics of the group (e.g. HIV infection).

The cut-off value of the Mantoux induration is 5 mm. transverse diameter, which demarcates positive from negative responders in our paediatric population [5].

Recently, a group of authors from Uganda [6] has developed a new method for determining the cut-off value of the Mantoux induration, which separates the

positive from the negative reaction. Using the same technique, we wanted to determine the critical cut-off value of Mantoux induration in Macedonian children suffering from tuberculosis and who had previously been vaccinated with BCG.

In addition, we wanted to see if malnourisment has any effect on the results of the tuberculin skin test.

Material and Methods

A retrospective study was carried out in 270 children and adolescents aged 0 to 14 years, treated for TB at the Kozle Institute for Respiratory Diseases in Children, Skopje, in the period from January 2003 to December 2007. The Institute is a specialized paediatric hospital for the treatment of childhood TB in R. Macedonia. The number of children treated at the Institute represents the prevalence and incidence of TB in children of R. Macedonia [17].

The sources of information were the patients' records (case histories) and the patients' data were filled in a questionnaire specially designed for this study. A total of 420 case history records were reviewed, of which 270 were retained for the study. Inclusion criteria for this study were: newly registered TB cases, Mantoux test result, age under 14 years and weight in kilograms (kg) noted in the case history of the patient.

Variables taken into consideration for this study were: gender, age in months, Mantoux induration size and nutritional status (z-score) of the patients. The tuberculin skin test (Mantoux method) was administered by injecting 0.1 ml of 5-TU PPD–T 23 intradermally into the volar surface of the left forearm [20].

The injection is made using a one quarter to one half inch, 27-gauge needle and a tuberculin syringe. A discrete, pale elevation of the skin (a weal) 6–10 mm in diameter should be produced when the injection is done correctly. Tests are read between 48 and 72 hours, when the induration is maximum. The basis of the reading is the presence or absence of induration, which should be determined by palpation. For standardization, the diameter of induration should be measured transversally to the long axis of the forearm and recorded in millimetres. For the purpose of diagnosis, indurations of ≥ 5 mm were interpreted as positive, regardless the nutritional status or history of BCG vaccination.

The nutritional status was calculated as the weight for age standard (z-score), using the Epi Info 3.5 software.

A z-score of < -2 shows undernourishment.

A z-score of –2 to 2+ shows normal weight.

A z-score of > 2 shows overweight.

The statistical analysis of the variables was done by means of the SPSS statistical software, version 16.0 (SPSS Inc. Chicago). The level of significance was set at $p < 0.05$ for all analyses. Distribution of frequencies was analyzed for each of the variables: age in months, gender, induration size and z-score. Correlation between the TST results and nutritional status was calculated with Pearson's coefficient of correlation. In order to define the cut-off value of the Mantoux induration size, which demarcates positive from negative reactions, the results of Mantoux induration size were plotted on a frequency distribution graph, grouped in the range of 5 mm. A curve was superimposed onto the graph and the modes and antimode were identified. The antimode was taken to represent the cut-off point between negative and positive results.

The study was retrospective and did not affect the treatment of the patients.

Results

A total of 270 children treated for tuberculosis at the Kozle Institute for Respiratory Diseases in Children, Skopje, satisfied the inclusion criteria and were included in the study.

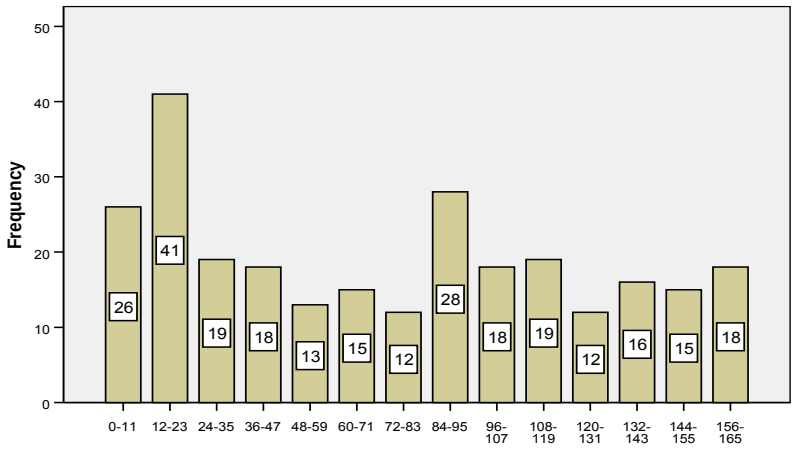

Figure 1 – Age frequency distribution of children

One hundred and forty five (145) (53.7%) of the patients were male and one hundred and twenty five (125) (46.3%) female. The ratio male/female was 1.6 : 1 for the males. The age of the patients ranged from 1 month to 165 months

(14 years) with an average of 75 months (6 years and 3 months ± 1SD of 4.208). The median value was 77.5 months.

The age distribution curve showed a bimodal form, the most numerous age group being from 12–23 months (41 patients), followed by the group aged between 84 and 95 months (28 patients).

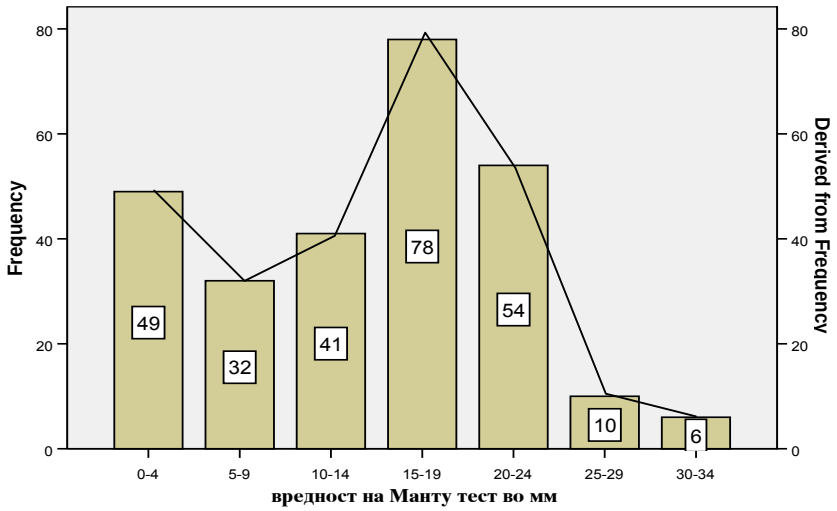

Figure 2 – Frequency distribution of Mantoux test induration results

The Mantoux test results ranged from 0 to 34 mm, with a mean of 14.6 mm. 49 (18.1%) patients had no detectable induration or reaction with a transverse induration size of < 5 mm. In the majority of patients (221) (81.9%) a positive reaction was recorded, with induration size of ≥ 5mm.

Figure 2 shows a graphic presentation of the frequency distribution of Mantoux test results. The distribution shows a bi-modal pattern, with the anterior mode at 0 mm and the posterior mode between 15 and 19 mm. The anti-mode between the two modes was at 5–9 mm. From this distribution, the 5 mm cut-off point demarcated the negative from the positive reactions.

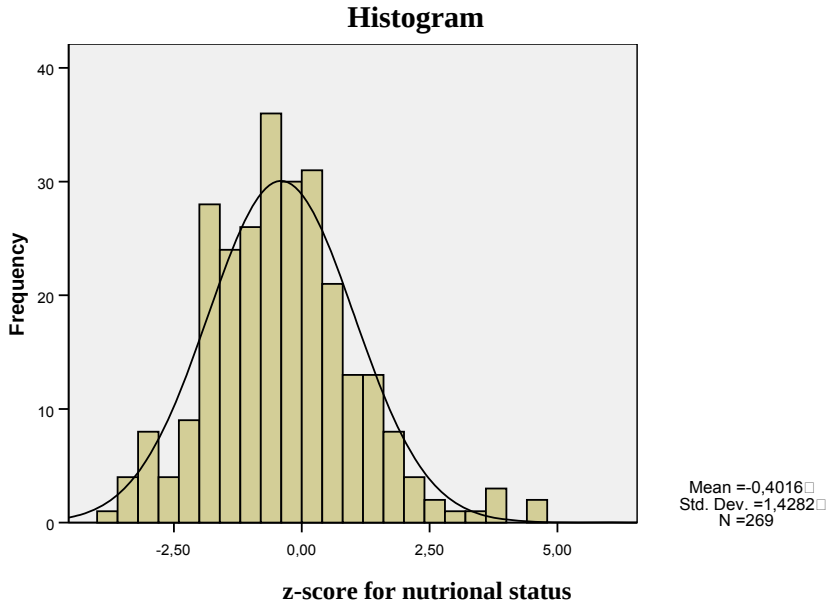

Figure 3 – Weight for age z-score frequency distribution

The weight for age (z-score) frequency distribution ranged from –3.63 to +4.68, with a median of –0.46. The mean value of the weight for age z-score was –0.4016 with a SD of 1.4282.

Thirty-three (12.26%) of the patients had a < –2.00 z-score, while 236 (87.73%) patients had a weight for age z-score of > –2.00.

Figure 3 shows that the weight for age z-score nutritional status of the patients does not show a normal distribution of frequencies, but there is a movement of the average value of the weight for age z-score of -0.4016 to the left, in relation to 0.00 on the abscisse (x system) from the coordination system. This shows malnutrition in 12.26% of the study population.

The linear regression of the correlation between the Mantoux reaction and the nutritional status of the patients had $r^2 = 0.031$; B constante = 0.193; ß coefficient = 0.175, with p = 0.004. The confidence interval was in the limits between 0.062 and 0.323 (95% CI) and showed a partial positive correlation between these two variables. More precisely, by moving the z-score of the nutritional status towards the negative scores (malnutrition), the results of the Mantoux test in mm were also moving to the negative scores.

The Pearson's coefficient of correlation of 0.175 for the Mantoux results and nutritional status of the patients (weight for age z-score) showed a weak positive correlation between these two variables.

The American Academy of Pediatrics [4] accepts 15 mm or more induration as positive in children who are over 4 years of age and who do not carry any risk. Ten (10 mm) or 5 mm induration could be accepted as positive for those who are at risk. The American Academy of Pediatrics accepts a maximum of 10 mm induration in BCG vaccinated people and it also states that no effect would be seen 3–4 years following vaccination [10].

Some other authors (Menzies D, and Amdekar YK) also advise ignoring the BCG vaccination status while interpreting a tuberculin skin test. They state that more than 50% of children demonstrate a negative tuberculin test after BCG vaccination. Of those who are positive initially, the majority become negative within a year of vaccination, while the remaining go within the next 2–3 years.

Discussion

Tuberculosis is one of the deadliest diseases in the world. The World Health Organization (WHO) estimates that each year more than 8 milion new cases of tuberculosis occur and approximately 3 milion persons die from the disease [7]. With the increasing incidence of tuberculosis worldwide, childhood cases now constitute 40% of the total [8].

Short of demonstrating viable organisms in body tissues and fluids, the tuberculin skin test is the only method of detecting *Mycobacterium tuberculosis* infection in an individual and is used in the diagnosis of tuberculosis in individual patients as well as in epidemiological settings to measure the prevalence of tuberculous infection in a population [9]. However, various factors, both in the host and inherent in the test, lower its specificity and sensitivity [10].

Interpretation of the Mantoux test in our paediatric population is of great importance as the large majority of them (95–97%) have been vaccinated with BCG at birth. For the test to be useful in a clinical study, an appropriate cut-off needs to be defined to demarcate positive and negative reactions. In this study, we aimed to examine the distribution of reactions to the standard Mantoux test in a group of children suffering from tuberculosis, and to use this to estimate the cut-off value of the induration. Results from this group of patients showed a bimodal distribution, with a large proportion having no, or mild reaction (0–4 mm), and the rest having stronger, more clearly positive reactions, ranging from 5 to 34 mm. The antimode was at 5 mm, indicating that this would be a reasonable cut-off for this patient group. The estimated 5 mm cut-off point corresponds to the arbitrarily chosen 5 mm cut-off value of Mantoux induration,

already used in the paediatric population of R. Macedonia, in accordance with the criteria of the American Thoracic Society.

There is no specific agreement on the influence of the BCG vaccine in the interpretation of the Mantoux results. The reaction of the TST in a vaccinated person depends on the potency of the vaccine, the time passed from the moment of vaccination (it is the strongest in the first years after immunization), as well as on the prevalence of TB in the area where such a person lives. Since these data are usually not known, the diameters of 10 mm and 15 mm are arbitrarily used as upper limits [9, 11].

However, the 10 mm and 15 mm cut-off are not general criteria for differentiation between postvaccinal reaction and reaction as a result of an infection with *Mycobacterium tuberculosis* [12]. Furthermore, different regions and populations have different cut-off values for the TST [8, 12].

For a population not vaccinated with BCG, The American Thoracic Society [10] has recommended three cut-off points for defining a positive tuberculin reaction, on the basis of the sensitivity, specificity and prevalence of tuberculosis in different groups:

a.) A cut-off point of ≥ 5 mm for individuals who are at great risk of developing tuberculosis disease if they are infected with *M.tuberculosis*. Tuberculin reactions in persons who have had recent close contact with a patient with infectious tuberculosis and in persons with abnormal chest radiographs consistent with tuberculosis, are more likely to represent infection with *M. tuberculosis* than a cross-reaction. Persons who are immunosuppressed because of disease (e.g. HIV infection) or drugs (e.g. corticosteroids) are more likely to progress to tuberculosis disease if they are infected with *M. tuberculosis*.

b.) A cut-off point of ≥ 10 mm is suggested for persons who have normal or mildly impaired immunity and a high likelihood of being infected with *M. tuberculosis* but are without other risk factors which would increase their likelihood of developing active disease.

c.) Persons who are not likely to be infected with *M. tuberculosis* should, generally, not be tuberculin tested, as the predictive value of a positive test in a low prevalence population is very low. However, if the skin test should be done (e.g. at entry into a work-place where some risk of exposure to tuberculosis is anticipated), a higher cut-off point of ≥ 15 mm is suggested in order to improve the specificity of the test.

The specificity of the TST is the percentage of people free of the disease, who have a negative test. It is about 99% in populations who are not exposed to non- tuberculous mycobacteria and are not vaccinated with BCG. False positive results decrease the specificity of the test.

In populations with a high prevalence of tuberculosis, sensitivity and specificity of the test are approximtely 90%, which results in a high positive predictive value of the test [2]. In our study, the real positivity of the test (a positive test in patients having TB disease) was 68.46%, which points to a moderate prevalence of TB in the population of R. Macedonia.

The sensitivity of the Mantoux test reflects its ability to identify children suffering from tuberculosis [10]. In our study the sensitivity of the test was 82.5%, a significantly high result, which confirms the importance of the Mantoux test in discovering tuberculosis in children.

Since malnutrition and TB are very often common for certain regions, the negative TST could lead to misdiagnosis of TB infection in such populations [16]. In two surveys conducted in R. Macedonia, in the periods 1982–1991 and 1992–1996, malnutrition was observed in 43.8% and 48.1% of the children with TB, respectively [17].

In our study, covering the period 2003–2007, malnourishment (z-score < -2) was observed in 12.26% of the patients with TB, which is lower than the 63% underweight children with TB in the recent study of Kiwanuka [6].

There is no agreement as to the influence of malnutrition on the results of the tuberculin skin test.

While the results of the majority of authors show no influence [6, 18, 19], a small number of authors show with their results that undernourishment suppresses the tuberculin skin test, as well as the whole immune response of the organism. Recently, this group of authors has received substantial support from a group of Peruvian authors who have produced evidence that protein-malnutrition, but not caloric, suppresses the immune reaction to TB antigen. The association between relative protein deficiency and negative TST implies antigen-specific energy to tuberculin in those with lower body protein reserves [16].

The nutritional status of children with TB in our study was not directly linearly related to the size of reaction to tuberculin ($r = 0.175$; $r^2 = 0.031$). The results were similar to the results obtained by Kiwanuka J. ($r = 0.27$; $r^2 = 0.079$ [6] and Ganapathy KT. ($r = 0.3$) [19].

However, underweight children in our study were significantly more likely to have a negative tuberculin test.

Conclusion

Following the WHO recommendations, we carried out this investigation in order to determine the cut-off Mantoux induration size, demarcating positive from negative reactions, which would be valid for our childhood population.

The obtained 5 mm cut-off point coincides with that already arbitrarily chosen, in accordance with the criteria of the American Thoracic Society and routinely used in interpreting the results of the tuberculin skin test (Mantoux) in children in R. Macedonia.

The Mantoux test in malnourished children with tuberculosis is often negative and should be read with care by health providers.

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Резиме

ГРАНИЧНА ВРЕДНОСТ НА MANTOUX ИНДУРАЦИЈА КАЈ МАКЕДОНСКИТЕ ДЕЦА БОЛНИ ОД ТУБЕРКУЛОЗА И НИВНИОТ НУТРИТИВЕН СТАТУС

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Вовед: Интерпретацијата на Mantoux тестот, како и познавањето на факторите кои влијаат врз големината на индурацијата на тестот од значење се за раната дијагноза на туберкулозата кај децата.

Цел: Одредување на големината на индурацијата на Mantoux тестот, која ја разграничува позитивната од негативната реакција и е валидна за македонските деца болни од туберкулоза.

Материјал и методи: Изведена е ретроспективна и дескриптивна студија кај 270 деца на возраст до 14 години, лекувани од туберкулоза на Институтот за респираторни заболувања кај децата „Козле“, Скопје, Македонија, во периодот од 2003 до 2007 година.

Граничната големина на Mantoux индурацијата беше определена по пат на анализа на дистрибуцијата на резултатите од тестот. Нутритивниот статус беше пресметан како стандард тежина за возраст (z-score). Корелацијата помеѓу резултатот од ТСТ и нутритивниот статус беше пресметана со Pearson-овиот коефициент на корелација.

Резултати: 270 деца, болни од туберкулоза, беа вклучени во испитувањето. Белодробната форма на туберкулозата беше присутна во 87,8% од испитаниците. 53,7% од пациентите беа од машки пол а 46,3% беа од женски пол. Најбројната старосна група беше најмладата, на возраст од 1 до 2 години, со учество од 15,2%. Следна по бројност беше старосната група од 7 до 8 години, со учество од 10,4%, а 33 деца (12,2%) беа потхранети (z-score < –2). Големината на индурацијата на Mantoux тестот беше во границите од 0 до 32 мм, со средна вредност од 13,4 мм. Дистрибуцијата на фреквенциите на инциденцијата имаше бимодална форма, со примарниот мод врз 0 мм и вториот мод врз 15–19 мм, а антимодот врз 5–9 мм. Mantoux тестот имаше сензитивност од 82,5%, специфичност од 62%, позитивна предиктивна вредност од 68,46% и негативна предиктивна вредност од 78,6%. Pearson-овиот коефициент на корелација (r) од 0,175 покажа слаба позитивна корелација помеѓу индурацијата на ТСТ и нутритивниот статус.

Заклучок: Граничната вредност (големина) на индурацијата на Mantoux тестот, која ја разграничува позитивната од негативната реакција, беше определена на 5 мм. Се препорачува интерпретацијата на тестот да се изведува водејќи сметка за факторите на ризик за развивање на туберкулозата, кои ги опкружуваат испитаниците.

Mantoux тестот кај деца со малнутриција треба да се интерпретира со големо внимание.

Клучни зборови: туберкулоза, Mantoux-тест, индурација, cut-off вредност, тежина за возраст z-score.

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