Original Article

The use of topical zinc in tuberculin test

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Abstract

Background Diagnosing tuberculosis in children is difficult, with under-diagnosis or over-diagnosis commonly occurring. The tuberculin test as a supporting examination for tuberculosis infection has a false negative value rate of 10-25%. Also, a majority of children with tuberculosis have lower plasma zinc levels than healthy children. Zinc deficiency may impair the immune response, leading to more false negative skin-test results.

Objective To evaluate the effectiveness of topical zinc for augmenting the diameter of tuberculin induration in children with tuberculosis.

Methods This unblinded, clinical trial was performed from October to December 2012 in subjects matched with themselves. Subjects were children with tuberculosis aged 2-18 years at the Department of Child Health at Cipto Mangunkusumo Hospital and Persahabatan Hospital. Every subject was injected tuberculin in volar region of left and right arm. Test sites then were covered with topical zinc cream on the right arm and placebo cream on the left arm. Indurations were measured after 72 hours. The difference of tuberculin diameter of 3 mm was considered as clinically significant. Data analysis was performed with Wilcoxon signed rank test.

Results There were 47 subjects in this study. The majority of subjects were well-nourished (53%). Subjects' median treatment duration was < 6 months, median age was 72 months and 47% of subjects were under the age of five years. Sixteen subjects had a median 1 mm difference in tuberculin induration diameter between the zinc arm and placebo arm (P<0.001), but this was clinically insignificant. Twenty-two subjects (43%) had Koch reaction after zinc cream application, but the placebo cream did not cause Koch reaction.

Conclusion Application of topical zinc does not increase tuberculin induration compared to placebo.
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Keywords: topical zinc, tuberculin test, children, tuberculosis

Diagnosis of tuberculosis (TB) in children is difficult. Microbiological examination as the gold standard often shows negative results due to the limited quantity of bacilli present and difficulty extracting specimens from distant lung parenchyma sites. Also, as a diagnostic tool for TB infection, the tuberculin test has a high false negative rate of 10-25%. The tuberculin test is influenced by cellular immunity. As such, factors influencing cellular immunity may contribute to false negative results including malnutrition, immunodeficiency, immunocompromised state, severe TB, as well as procedural errors in preparation or injection of the tuberculin test.[1-3]

Micronutrient deficiency is common in both adults and children with TB. Since zinc is involved in cellular immunity, zinc deficiency may cause false negative result of tuberculin test. Fortunately, this condition is reversible upon supplementation.[4] Children with TB have lower plasma zinc levels than healthy children.[5] In addition, TB patients with negative tuberculin tests have lower plasma zinc levels than those with positive tests.[6] Furthermore, a study of 30 children with TB found that plasma zinc levels were lowest prior to initiation of tuberculosis

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medication, but increased along with administration of anti-tuberculosis drugs.[6] To date, studies using topical zinc cream in tuberculin test is limited for adult TB patients.[7,8] We aimed to evaluate the effectiveness of topical zinc for increasing the diameter of tuberculin induration in children with tuberculosis.

Methods

This unblinded, clinical trial was conducted from October to December 2012 in the Department of Child Health at Cipto Mangunkusumo and Persahabatan Hospitals. Subjects were children with TB aged 2-18 years, with positive tuberculin tests at the time of diagnosis, and who had undergone ≤1 year of tuberculosis therapy. We excluded patients with immunocompromised conditions (severe malnutrition, HIV, malignancy, in steroid therapy for more than 2 weeks or in chemotherapy), those who were within 6 weeks post-viral disease (varicella or measles) or within 6 weeks post-vaccination (varicella or measles). Subjects were matched with themselves, by comparing the right and left arm results. The minimum required sample size was calculated to be 43, along with an estimated 10% dropping out, so we arrived at a total sample size of 47 subjects. Subjects were recruited by consecutive sampling.

This study was approved by the Ethics Commitee of the University of Indonesia Medical School. All patients and parents were given a complete explanation of the study. Subjects' parents provided written informed consent. After parental approval, baseline characteristics were measured and recorded. In an unblinded manner, tuberculin tests (0.1 mL PPD RT 23 2 TU *Biofarma®*) were injected in the left arm and covered with 1 spoon of placebo cream (manufactured by FMIPA/Faculty of Mathematics and Science University of Indonesia), while the tuberculin test injected in the right arm was covered with 1 spoon of zinc sulfate dissolved in aqueous cream at a concentration of 10% elemental zinc (manufactured by FMIPA/Faculty of Mathematics and Science University of Indonesia). One spoon contains 20 mg either placebo or zinc cream that were applied directly at the injection site and its surrounding skin. Tuberculin test sites were immediately covered with an occlusive dressing (*Tegaderm®*). On the right arm, zinc sulfate cream was additionally applied 5

cm from the site of tuberculin injection and also covered with occlusive dressing, while no placebo cream was applied on the left arm except at the tuberculin test injection site. After 72 hours, the dressing and cream were removed, and the "ballpoint pen" test was used to measure indurations. Koch reaction was defined as induration of tuberculin that accompanied by pain or skin reaction (vesicles, bullaes, necrosis). We made 2 comparisons: zinc arm induration size compared to indurations size at the time of diagnosis and zinc arm induration size compared to placebo arm induration size. The difference of tuberculin diameter of 3 mm was considered as clinically significant. Wilcoxon test was used to analyze differences in diameters of tuberculin test indurations between the two arms whereas Mcnemar test was used to analyze Koch reaction. A P value of < 0.05 was considered as statistically significant. Statistical analysis was performed using *SPSS 15.0.*

Results

One hundred patients fulfilled the study criteria, with 10 who refused to enroll and 43 who were not recruited as 47 subjects were already enrolled **(Figure 1)**. Subjects' median age was 72 (range 24-192) months and 47% subjects were under five years of age. The majority of subjects were well-nourished (53%), had lung TB (81%) and had received BCG vaccinations (85%). The median duration of TB treatment in our subjects was 6 months **(Table 1)**.
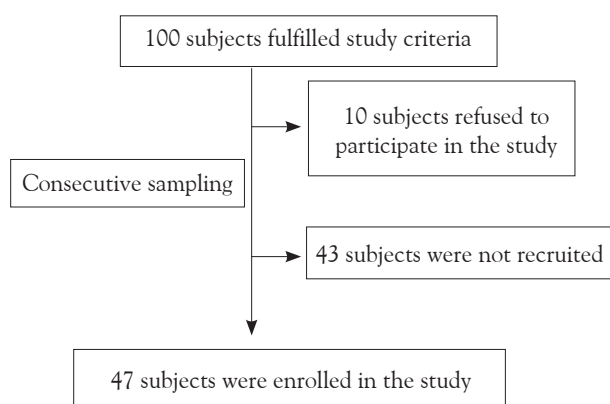

Figure 1. Flow chart of subjects selection

We observed induration augmentation in the arm that received topical zinc cream in 16 subjects, but not in the other 31 subjects. Diameters of tuberculin induration in zinc arms were all equal to or greater than those of the placebo arms. The median difference in tuberculin induration for all subjects was 1 mm (P<0.001, Wilcoxon test, **Table 2**), but was clinically insignificant. Comparison of induration diameter at the time of diagnosis to the diameter of the placebo arm yielded the following results: 35 subjects with similar diameter, 8 subjects with increased diameter in the placebo arm and 4 subjects with decreased diameter in the placebo arm. Of the 16 subjects with tuberculin augmentation in the zinc arm, 9 were undernourished and 12 had undergone tuberculosis therapy for ≤6 months.

The application of topical zinc cream 5 cm from the tuberculin test site did not cause hypersensitivity reactions. Nevertheless, application of topical zinc cream after injection of tuberculin test caused Koch reaction (vesicles or pustules) at the injection site in 20 subjects (43%) **(Table 3).** Of those subjects, 11/20 were undernourished and 17/20 had durations of anti-tuberculosis therapy of ≤6 months. Koch reactions were self-limiting along with waning of tuberculin induration over 1-2 weeks. Application of placebo cream after injection of tuberculin test did not cause Koch reaction in any subjects.

Table 1. Baseline characteristics of subjects

Characteristics	N=47
Gender, n (%)	
Male	23 (49)
Female	24 (51)
Median age (range), months	72 (24-192)
Nutritional status, n (%)	
Overweight	2 (4)
Well-nourished	25 (53)
Undernourished	20 (43)
BCG vaccination, n (%)	
Yes	40 (85)
No	7 (15)
Type of TB, n (%)	
Lung TB	38 (81)
Meningitis TB	2 (4)
Spondylitis TB	3 (6)
Others TB (scleral TB, ovarian TB, TB lymphadenitis and TB osteomyelitis)	4 (9)
Median TB treatment duration (range), months	6 (2-11)
Median induration diameter at the time of diagnosis (range), mm	14 (10-23)

Table 2. Differences in diameter induration between the zinc arm and placebo arm

Variables	Zinc arm N=-47	Placebo arm N=47	P value[1]
Median tuberculin induration (min-max), mm	16 (10-21)	15 (10-20)	<0.001
Tuberculin induration based on nutritional status (min-max), mm			
Overweight	17.5 (15-20)	15 (15-17)	0.317
Well-nourished	15 (10-21)	15 (10-20)	0.026
Undernourished	17 (10-21)	15 (10-20)	0.007

[1] Wilcoxon test

Table 3. Koch reaction in all tuberculin tests (n=94)

Variables	Koch reaction		Total	P value[1]
	+	-		
Zinc arm	20	27	47	<0.001
Placebo arm	0	47	47	
Total	20	74	94	

[1]McNemar test

Discussion

Studies using topical zinc cream to augment tuberculin induration are limited to adults only. Children with TB are known to have lower plasma zinc levels than healthy children, and those with negative tuberculin tests also have lower plasma zinc than those with positive tests. This study is the one of the first to use topical zinc cream to attempt to augment tuberculin induration in children. Limitations of this study were its unblinded design, measurement bias, limitation of consecutive sampling, unknown subjects' plasma zinc levels, and no restriction of diet containing zinc.

The median age of subjects was 72 months, with 43% of our subjects in the under-five-year-old group, similar to subjects in previous studies.[6,9] The majority of the subjects were well-nourished because subject recruitment was anytime during their tuberculosis treatment, similar to Soegiarto's study,[6] but different from the study by Ray *et al*.[5] We observed that 80% of our subjects had lung tuberculosis, which is to be expected since the lung is the main port of entry for tuberculosis infection, similar to a previous study.[10]

How zinc influences cutaneous cellular immunity remains unclear. In general, zinc is a cofactor of thymulin, a hormone produced in the thymus, which affects T-lymphocyte maturity. Zinc deficiency reduces the chemotactic response of neutrophils, monocytes, and macrophages. Cytokines, as modulators of the immune system, are also influenced by zinc. Secretion of interleukin-1, interleukin-6, tumor necrosis factor-alpha, and interferon-gamma by leukocytes is stimulated by zinc.[11-13] In the skin, it has been proposed that zinc can augment the cutaneous immune response after tuberculin injection. Zinc affects the interaction between antigen-presenting cells and T-helper cells at the injection site. Cytokines secretion and leukocytes recruitment in delayed type hypersensitivity after tuberculin injection are stimulated by zinc.[14,15]

The cellular immune response to tuberculin test may wane in the years after the first test. Variability of test results following repeated tuberculin testing may be due to biological phenomenon, variability in reading and administration of the test. Inter-reader variability resulted in standard deviations of readings of 2.3 mm or 2.5 mm. Variability was less with the same reader (intra-reader), however, standard deviations of 1.3 to 1.9 mm were still seen. All factors contributed to result variability to 3 mm.[17]

We found that application of topical zinc cream on the site of the tuberculin test yielded a median induration 1 mm larger than the application of placebo cream (P<0.001). We had previously determined a 3-mm difference of transversal induration diameter to be clinically significant. Wilcoxon test revealed the 1 mm difference to be statistically significant, but it was clinically insignificant. Previous studies did not explicitly state the difference in mm of diameter induration.[7,8,16]

Among 16 subjects who showed augmentation of tuberculin induration in the zinc arm most were undernourished and had undergone tuberculosis therapy for ≤6 months. Specifically, subjects who were undernourished had a higher median augmentation of 2 mm. Undernourished children generally have lower plasma zinc levels. Based on previous studies,[6] zinc plasma level after 2 months of tuberculosis treatment has reached normal level so application of topical zinc cream possibly didn't cause augmentation of tuberculin induration beyond that period.

In our study, application of topical zinc on the site of tuberculin injection sometimes yielded Koch reaction (vesicles or pustules). However, application of placebo cream on the site of tuberculin injection or application of topical zinc cream alone did not cause any Koch reaction. One study reported no hypersensitivity reaction after topical zinc application,[7] while two other studies gave no report on hypersensitivity reactions in their subjects.[8,16] Koch reaction is due to zinc's role in increasing cellular immunity resulting in greater inflammatory responses. Koch reaction and positive tuberculin test often indicate natural tuberculosis infections. Sensitivity and specificity of Koch reaction for tuberculosis infection were reported to be 77.2% and 98%, respectively.[18]

In conclusion, topical zinc application is clinically insignificant to augment the diameter of tuberculin test induration. Koch reaction after topical zinc application is due to zinc's role in increasing cellular immunity and will resolve by itself. We propose further follow-up studies with better methodology, such as a blinded study design, larger number of subjects, subjects with undernourished nutritional status, and plasma zinc level examinations.

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