Is there interference in the interpretation of the tuberculin skin test in children with intestinal parasitic infestation?

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Background: Infestation by intestinal parasites could be a cause of a false-negative tuberculin skin test (TST) result.

Objective: To evaluate TST results in a population of immigrants and internationally adopted children and to analyze whether intestinal parasitic infestation may modify or not TST results.

Methods: A cross-sectional observational study which includes adopted children or immigrants evaluated in our hospital between January 2003 and December 2008. The TST was considered as the dependent variable and independent variables were gender, age, geographical origin, bacille Calmette–Guérin scar, nutritional status, immune status, and intestinal parasitism.

Results: One thousand and seventy-four children were included, of whom 69.6% were female. There was a bacillus Calmette–Guérin scar in 79% of the children and in 20.3% intestinal parasites were found. There were no differences in TST results among infested and non-infested children.

Conclusions: Intestinal parasitic infestation did not change TST results in our study and these results coincide with recent articles regarding questionable interference that intestinal parasitic infestations may produce on TST results.

Keywords: Tuberculin test, Parasites, Child, Tuberculosis

Introduction

The tuberculin skin test (TST) remains as the most useful method for the diagnosis of tuberculosis (TB) disease and latent tuberculosis infection, both in children and adolescents. It is based on a delayed hypersensitivity to certain antigenic components of *Mycobacterium tuberculosis*.^{1,2}

Most of consensus documents concerning TST interpretation agree that any intestinal parasitic infestation could cause a false-negative result, especially if there was infestation 2 months before the TST was performed.^{2,3} The last Spanish consensus document on the diagnosis of TB in pediatrics, issued by the Spanish Society of Pediatric Infectious Diseases, concludes that comorbidity could modify TST results, without specifying intestinal parasitic infestation.¹ Scientific publications in this regard, which are detailed later, show conflicting results. This research matters

because of the risk that false-negative TST might prevent children from receiving preventive therapy.

The aims of the study were:

1. to evaluate TST results in a population of immigrants and internationally adopted children from several geographical areas;
2. to analyze whether intestinal parasitic infestation may modify or not the TST results.

Methods

The Pediatric Infectious and Tropical Diseases Unit in which the study was performed is a national reference clinic for the evaluation of internationally adopted children and immigrants. The Microbiology Laboratory has a specific Parasitology Unit employing highly specialized techniques and a widespread practice of the diagnosis of tropical diseases.

A cross-sectional observational study was performed, which was approved by the ethics committee of our hospital. Patient enrollment was commenced in January 2003 and finished in December 2008.

Inclusion criteria were:

1. immigrants or adopted children aged 0–14 years, whose guardians or parents had requested a health

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examination at the Pediatric Infectious and Tropical diseases Unit;

2. acceptance by guardians or parents and by adolescents over 12 years, to be included in the study after reading and signing an informed consent document.

Exclusion criteria were:

1. children who did not complete the requested study;
2. children with signs or symptoms of TB or non-TB mycobacteria, or with recent contact with TB patients;
3. children who had received any dose of live-attenuated virus vaccine during the 2 months prior to the study;
4. HIV-infected children or children with chronic diseases, diagnosed either before or during the study;
5. children under treatment with immunosuppressive drugs (not including inhaled corticosteroids, non-immunosuppressive doses of corticosteroids or steroids administered over a period of <1 week).

The TST result was considered as the dependent variable and was performed by an internationally approved technique [intradermal injection of 2 TU (0.1 ml) of PPD RT 23]. Results were studied by a pediatrician 72 hours after the injection by measuring the induration diameter in millimeters. Only children without known contact with TB were studied, so any result ≥ 10 mm was considered as a positive TST result.

The independent variables were gender, date of birth, age at first consultation in the hospital, country of origin and length of stay in the country of origin before arrival in our country, availability of immunization cards, nutritional status according to McLaren's classification, immune status according to the percentage of CD4 lymphocytes, and intestinal parasitic infestation. McLaren's nutritional classification⁴ was calculated as actual weight $\times$ actual height/percentile 50 weight $\times$ percentile 50 height (percentiles according to the World Health Organization growth charts). The results are very similar to Waterloo's nutritional classification. Stool samples were collected on three non-consecutive days. Microscopic examination (temporary wet mounts) and concentration techniques (sedimentation and flotation procedures) were used as parasitological examination methods.

We performed univariate and multivariate (log *R*) analyses with SPSS 14.0, with statistical significance being assumed for values of $P < 0.05$. The bivariate analysis was tested using Pearson's chi-squared or Fisher's exact tests. Unconditional logistic regression analysis was used in order to include diverse co-variables associated with the dependent variable, thus making the autocorrelation more accurate and controlling any possibly confounding factors.

In the Community of Madrid, the initial health examination of internationally adopted and immigrant children is primarily conducted at two pediatric

hospitals. During the study period, the distribution of the children was random between both hospitals, so it was believed that this did not cause any bias in the origin or characteristics of the patients included in the study.

A limitation of the study was that it was not always possible to establish when the vaccine was administered in children with a bacillus Calmette-Guérin (BCG) scar, especially in those without a valid immunization card. However, in most of the origin countries, the vaccine is usually administered at birth.

Results

Between January 2003 and December 2008, 1148 immigrants or internationally adopted children were evaluated at our Pediatrics Infectious and Tropical Diseases Unit and of these, 1074 were included in the final data analysis after the exclusion of 74 children who fulfilled the exclusion criteria.

Out of the 1074 children included in the study, 747 (69.6%) were female. Of the total study population, 373 (34.7%) came from China, 224 (20.8%) from Latin America, 208 (19.4%) from India and Nepal, 169 (15.7%) from Eastern Europe, and 100 (9.3%) from Africa. The most common countries were China (34.7%), India (15.2%), Russia (9.4%), Ethiopia (5.5%), and Bolivia (5%).

The average age of the children at the time of the study was 42.61 months, ranging between 1 and 192 months with a standard deviation of 40.66 and the median age was 24 months. The average time spent in their country of origin was 39.5 months, ranging between 1 and 172 months with a standard deviation of 36.6. The variable length of stay in their country of origin was very similar to the age at the time of the study, since the majority of immigrants or adopted children made their first visit shortly after arriving in our country. The correlation between both variables is high, correlation coefficient $r = 0.97$ ($P < 0.001$), so for the rest of the study, we used only the variable age at the time of the study.

Although valid immunization cards showed BCG vaccine administration in 709 children (66%), a BCG scar was found on physical examination in a total of 848 (79%). Due to the unreliability of the immunization cards regarding the BCG vaccine in all countries, and because 99% of vaccinated children develop a scar,^{5,6} we only took the presence of a BCG scar on physical examination into account when assessing the possible effect of the vaccine on TST results.

The results of the TST 72 hours after the injection were:

- Mantoux = 0 mm in 907 [84.4%; 95% confidence interval (CI): 82.2–86.7%];
- Mantoux < 5 mm in 19 (1.8%; 95% CI: 0.9–2.6%);
- Mantoux 5–9 mm in 25 (2.3%; 95% CI 1.4–3.3%);

Table 1 Intestinal parasites by geographical areas

Area/parasites	GL	HN	EH	TT	AL	SS	Total
China	30	0	3	1	2	0	34
Latin America	29	4	8	7	4	1	45
India and Nepal	41	25	5	8	2	10	66
Eastern Europe	29	1	5	0	0	0	35
Africa	22	5	6	8	7	1	38
Total	151	35	27	24	15	12	218

Note: GL: *Giardia lamblia*; HN: *Hymenolepis nana*; EH: *Entamoeba histolytica*; TT: *Trichuris trichiura*; AL: *Ascaris lumbricoides*; SS: *Strongyloides stercoralis*.

- Mantoux ≥ 10 mm in 123 (11.4%; 95% CI: 9.5–13.4%).

Chest radiography was performed in all cases with an induration ≥ 10 mm. The X-ray results were normal or otherwise these children would have been diagnosed of TB, thus fulfilling an exclusion criterion. All of them were considered to have latent tuberculosis infection and were treated with isoniazid for 9 months, according to the current recommendations of the Spanish Society of Pediatric Infectious Diseases.^{1,7}

Stool samples were investigated for intestinal parasites in all the children, parasites which do not cause disease being excluded. Intestinal parasites were found in 218 children (20.3%), with the following distribution:

- *Giardia lamblia*: 151 cases (14.1% of the total; 69.3% of all parasites);
- *Hymenolepis nana*: 35 (3.3% of the total; 16.1% of all parasites);
- *Entamoeba histolytica*: 27 (2.5% of the total; 12.4% of all parasites);
- *Trichuris trichiura*: 24 (2.2% of the total; 11% of all parasites);
- *Ascaris lumbricoides*: 15 (1.4% of the total; 6.9% of all parasites);
- *Strongyloides stercoralis*: 12 (1.1% of the total; 5.5% of all parasites);
- mixed infestation: 40 children (3.7% of the total; 18.3% of all parasites).

Table 1 shows the distribution of intestinal parasites by geographical area of origin. Children were dewormed

according to parasites isolated: metronidazole or tinidazole (*G. lamblia*), praziquantel or niclosamide (*H. nana*), metronidazole and paromomycin (*E. histolytica*), mebendazole or albendazole (*T. trichiura*), mebendazole or pyrantel pamoate (*A. lumbricoides*), and ivermectin or albendazole (*S. stercoralis*).

Two key study groups were defined as PPD0 and PPD10. In the first group, we selected those children with PPD reactivity equal to 0 mm compared to any other result. In the second group, children were divided according to the TST result: <10 mm compared to ≥ 10 mm. The different study groups and their characteristics concerning parasitic infestation are shown in Tables 2 and 3.

In the univariate analysis, only two variables, namely age and presence of a BCG scar, influenced the TST result. The remaining variables, including intestinal parasitic infestation, did not change the result of PPD reactivity in any of the study groups. The results of the multivariate analysis in both groups were similar to the findings described above. The only variables that influenced the TST result were age and the presence of a BCG scar. The obtained results did not change by stratifying the sample according to the age of the children, nor when single parasites were analyzed separately (Tables 4 and 5).

Discussion

Articles published 10–20 years ago emphatically assert the relationship between the TST false-negative results and the existence of intestinal parasites. In 1978, Buck *et al.*⁸ described the interaction between the presence of different intestinal parasites and the diagnostic capability of several immunological assays. The authors concluded that any intestinal parasitic infestation may cause malnutrition and this, in turn, causes false-negative results when using diagnostic techniques based on immunological reactions, such as the TST. In

Table 2 Intestinal parasites in groups: TST=0 mm versus TST ≥ 1 mm (n=1074; univariate analysis)

PPD0		TST=0 mm	TST ≥ 1 mm	OR (95% CI)	P
		n=907	n=167		
All intestinal parasites	Yes	178/907 (19.6%)	40/167 (24%)	1.29 (0.87–1.90)	0.20
	No	729/907 (80.4%)	127/167 (76%)		
<i>Giardia lamblia</i>	Yes	125/907 (13.8%)	26/167 (15.6%)	1.15 (0.73–1.82)	0.54
	No	782/907 (86.2%)	141/167 (84.4%)		
<i>Hymenolepis nana</i>	Yes	27/907 (3%)	8/167 (4.8%)	1.64 (0.73–3.67)	0.22
	No	880/907 (97%)	159/167 (95.2%)		
<i>Entamoeba histolytica</i>	Yes	22/907 (2.4%)	5/167 (3%)	1.24 (0.46–3.33)	0.60
	No	885/907 (97.6%)	162/167 (97%)		
<i>Trichuris trichiura</i>	Yes	21/907 (2.3%)	3/167 (1.8%)	0.77 (0.23–2.61)	1
	No	886/907 (97.7%)	164/167 (98.2%)		
<i>Ascaris lumbricoides</i>	Yes	10/907 (1.1%)	5/167 (3%)	2.77 (0.93–8.20)	0.07
	No	897/907 (98.9%)	127/167 (97%)		
<i>Strongyloides stercoralis</i>	Yes	11/907 (1.2%)	1/167 (0.6%)	0.49 (0.63–3.83)	0.70
	No	896/907 (98.8%)	166/167 (99.4%)		

Note: TST: tuberculin skin test; OR: odds ratio; CI: confidence interval.

Table 3 Intestinal parasites in groups: TST<10 mm versus TST≥10 mm (n=1074; univariate analysis)

		TST<10 mm		TST≥10 mm	OR (95% CI)	P
PPD10		n=951		n=123		
All intestinal parasites	Yes	191/951 (20.1%)		27/123 (22%)	1.12 (0.71–1.76)	0.62
	No	760/951 (79.9%)		96/123 (78%)		
<i>Giardia lamblia</i>	Yes	132/951 (13.9%)		19/123 (15.4%)	1.13 (0.67–1.91)	0.64
	No	819/951 (86.1%)		104/123 (84.6%)		
<i>Hymenolepis nana</i>	Yes	30/951 (3.2%)		5/123 (4.1%)	1.30 (0.49–3.42)	0.59
	No	921/951 (96.8%)		118/123 (95.9%)		
<i>Entamoeba histolytica</i>	Yes	22/951 (2.3%)		5/123 (4.1%)	1.79 (0.67–4.81)	0.24
	No	929/951 (97.7%)		118/123 (95.9%)		
<i>Trichuris trichiura</i>	Yes	22/951 (2.3%)		2/123 (1.6%)	0.70 (0.16–3.00)	1
	No	929/951 (97.7%)		121/123 (98.4%)		
<i>Ascaris lumbricoides</i>	Yes	13/951 (1.4%)		2/123 (1.6%)	1.19 (0.27–5.35)	0.68
	No	938/951 (98.6%)		121/123 (98.4%)		
<i>Strongyloides stercoralis</i>	Yes	11/951 (1.2%)		1/123 (0.8%)	0.70 (0.09–5.47)	1
	No	940/951 (98.8%)		122/123 (99.2%)		

Note: TST: tuberculin skin test; OR: odds ratio; CI: confidence interval.

2000, Borkow *et al.*⁹ showed that chronic helminthiasis causes a persistent activation of the immune system. This leads to the generation of anergy, which distorts the results of diagnostic tests based on delayed hypersensitivity, as is the case of the TST. One year later, Elias *et al.*¹⁰ added that these findings may explain, at least in part, the limited efficacy of BCG against *M. tuberculosis* infection in those geographic areas with high endemic helminthiasis. A recent study published in 2009, conducted in 96 adults from Egypt,¹¹ concludes that helminthic infection is associated with a lower diameter of PPD reactivity induction compared to non-infested individuals (10.89 mm versus 8.65 mm; $P<0.05$).

However, recent publications on studies with a greater number of subjects question whether intestinal infestations could really modify TST results. In 2003, 944 Ecuadorian children aged between 8 and 11 years were studied.¹² The prevalence of infested children was very high (70.3%), mostly with *T. trichiura* (52.3%) and *A. lumbricoides* (52.1%). No significant differences were found in the TST results between infested and non-infested children. Another study of 212 Peruvian adults¹³ also showed no relationship between the existence of intestinal parasites and PPD reactivity. In 2006, Lipner *et al.*¹⁴ published a study in 5096 subjects aged between 6

and 65 years and born in India. Some type of helminth infection was revealed in 32% of the stool samples and 9% filarial antigen positive were detected. Of the total study population, 54% had a positive TST. None of the parasites studied was associated with a higher percentage of TST negative result, so the authors concluded that helminth infection does not significantly influence the delayed hypersensitivity response to tuberculin. Three years later, a laboratory of parasitology in Brazil¹⁵ investigated the existence of intestinal parasites in 327 patients with TB. Nearly 20% had some type of infestation, but again there were no significant differences in TST results between infested and non-infested subjects. They concluded that the cellular response against TB was not affected by the presence of intestinal parasites, adding that TB disease progression was not worse in patients who showed infestation. Finally, Zevallos *et al.*¹⁶ studied 195 Peruvian Indians from a region near the Amazon in 2010. Worms were found in 98% of the subjects with co-infestations in 24%. TST results were not modified either by the concentration of parasite eggs in stool samples, or by the type of parasite, or by the presence of co-infestation, or by treatment with albendazole, whether it was effective or not.

In our series, intestinal parasites were documented in 20.3% of the children (of which 18.3% had

Table 4 Multivariate analysis of effect of parasites on TST (PPD0 group)

PPD0	OR (95% CI)	P
All intestinal parasites	1.58 (0.47–5.31)	0.46
<i>Giardia lamblia</i>	0.78 (0.24–2.50)	0.68
<i>Hymenolepis nana</i>	1.16 (0.38–3.57)	0.79
<i>Entamoeba histolytica</i>	0.75 (0.17–3.27)	0.70
<i>Trichuris trichiura</i>	0.27 (0.05–1.47)	0.13
<i>Ascaris lumbricoides</i>	3.96 (0.94–16.65)	0.06
<i>Strongyloides stercoralis</i>	0.51 (0.06–4.27)	0.53

Note: TST: tuberculin skin test; OR: odds ratio; CI: confidence interval.

Table 5 Multivariate analysis of effect of parasites on TST (PPD10 group)

PPD10	OR (95% CI)	P
All intestinal parasites	0.49 (0.12–2.02)	0.32
<i>Giardia lamblia</i>	2.13 (0.56–8.11)	0.27
<i>Hymenolepis nana</i>	1.74 (0.50–6.06)	0.38
<i>Entamoeba histolytica</i>	2.82 (0.62–12.97)	0.22
<i>Trichuris trichiura</i>	0.81 (0.13–5.10)	0.83
<i>Ascaris lumbricoides</i>	1.74 (0.29–10.51)	0.54
<i>Strongyloides stercoralis</i>	0.84 (0.10–7.27)	0.87

Note: TST: tuberculin skin test; OR: odds ratio; CI: confidence interval.

co-infestation). *G. lamblia* was the most common parasite. In the PPD0 study group, 19.6% of children with a PPD reactivity equal to 0 mm were infested by some intestinal parasite, compared to 24% of the children who had an induration ≥ 1 mm. In the PPD10 group, there is an even greater similarity in percentages. Among the infected children, 20% had a TST negative result (induration < 10 mm) compared to 22% with a TST positive result. In neither of the two study groups were there statistical differences ($P=0.20$ in PPD0 and $P=0.62$ in PPD10). The parasites founded were mainly *G. lamblia* and in second place *H. nana*. Although our results did not change when single parasites were analyzed separately, the other groups are small and it is difficult to extract conclusions about the other parasites.

As a potential bias in our study, TB and parasites both generally principally affect poorer people. Maybe children in poorer families are more likely to have TB, worms, and false-negative TST and it is possible that these effects together cause no apparent effect. This possibility could be investigated in future studies by assessing poverty and TB risk and adjusting this in the data analysis.

In conclusion, the presence of intestinal parasites (mainly *G. lamblia*) did not change the TST results in our study. The only two variables which influenced the TST result were age and presence of a BCG scar. The results of our study, based on a large number of children from several geographical areas, coincide with most recent publications regarding the questionable interference that intestinal parasitic infestations may cause on the interpretation of the TST.

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