The use of GeneXpert *Mycobacterium tuberculosis*/rifampicin assay in diagnosis of pulmonary tuberculosis in children

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Introduction
Rapid diagno sis of tuberculosis (TB) is essential for early disease management. GeneXpert (GX) test is a novel rapid diagnostic modality. In this study, we aimed to evaluate the diagnostic role of GX test in diagnosing pulmonary tuberculosis (PTB) in children as well as detection of rifampicin (RIF) resistance.

Patients and methods
This was a cross-sectional study that included 80 patients suspected to have PTB based on clinical data together with at least one of the followings: a positive tuberculin skin test or QuantiFERON-TB Gold In-Tube test result, a positive blood TB PCR result, or a positive Ziehl–Neelsen staining result.

Results
PTB was more common in older females. Extrapulmonary manifestations and hilar lymphadenopathy were more frequent in younger age. Both positive tuberculin skin test and positive GX were more frequent in older age. Positive GX was found in 30 (37.5%) cases, and RIF resistance was detected in two (6.6%) of them. Positive GX results were more frequent in sputum than gastric aspirate samples. Positive GX results were more frequent with absent Bacillus Calmette–Guérin scar, and with hemoptysis, failure to thrive, cavitary lung lesions, and lower mean hemoglobin.

Conclusion
GX detects many more cases than blood PCR and Ziehl–Neelsen stain. GX had 70% sensitivity and 95% specificity in diagnosing PTB, with overall diagnostic accuracy of 86.67%, with better performance in older children who could expectorate. RIF resistance was ~6.6%.

Keywords:
blood PCR, GeneXpert, pediatric, pulmonary tuberculosis

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Introduction

Incidence of tuberculosis (TB) in Egypt was estimated to be 13 cases (per 100 000 population), which is the lowest in Africa and among the lowest in WHO Eastern Mediterranean region. However, the rising incidence of drug-resistant TB complicates the battle against TB [1].

Children younger than 5 years represent a special population regarding risk, presentation, and diagnosis of TB. Despite being the oldest among TB investigations, chest radiograph and tuberculin skin test (TST) still have a valuable role beside microbiologic confirmation, which was rarely attempted in children mainly owing to the incorrect perception that respiratory specimens are difficult or impossible to obtain in children. Moreover, even when samples were obtained, the yield of the organism was very low [2]. The GeneXpert (GX) *Mycobacterium tuberculosis* (MTB)/rifampicin (RIF) assay represents a candidate solution for this problem. GX uses a real-time reverse transcription PCR technology using unprocessed clinical specimens, regardless of their smear status. GX probes for the rpoB gene of MTB, which encodes for most cases with RIF resistance. Thus, it can simultaneously identify the organism and RIF resistance, which is a strong indicator of concurrent multidrug resistant TB, within 2 h. GX uses molecular beacon technology to detect DNA sequences using five different nucleic acid hybridization probes in the same multiplex reaction. The assay is conducted within a simple, almost fully automated cartridge-based system [3].

Aim

The aim was to evaluate the diagnostic role of GX MTB/RIF assay in pulmonary tuberculosis (PTB) in children as well as detection of RIF resistance.

Patients and methods

The study included 80 patients suspected to have PTB who attended to Assiut University Children Hospital from April 2017 to March 2019. Their ages ranged from 1 to 18 years. They comprised 35 males and 45 females. Patients were divided in our study into two groups:

Group 1: infants and children up to 5 years old ($N = 23$).
Group 2: children and adolescents up to 18 years old ($N = 57$).

All studied patients were subjected to thorough clinical evaluation, besides complete blood count, TST, and blood PCR assay for MTB. Respiratory samples [sputum/gastric aspirate (GA)] were obtained in the morning after overnight fasting and subjected to the following: (a) Ziehl–Neelsen (ZN) staining, following WHO guidelines [4]; (b) culture on Lowenstein–Jensen media was done for 30 (37.5%) cases only, following WHO guidelines [5]; and (c) GX assay, following WHO guidelines [6].

Inclusion criteria

Patients suspected to have PTB based on clinical data, such as prolonged cough (>1 month) not responding to nonspecific treatment and TB toxemia (night fever, night sweating, anorexia, or weight loss), together with at least one of the followings were included:

(1) Positive TST or QuantiFERON-TB Gold In-Tube test.
(2) Positive blood TB PCR.
(3) Positive ZN smear for acid-fast bacilli.

Exclusion criteria

The following were the inclusion criteria:

(1) Neonates and infants (<1 year).
(2) Children with chronic cough owing to other causes, such as bronchial asthma, interstitial pulmonary fibrosis, or cystic fibrosis.
(3) Patients suspected to have extrapulmonary TB (EPTB) only.
(4) Patients who have started anti-TB treatment.
(5) Human immunodeficiency virus-positive cases.

Statistical analysis

Continuous data were expressed in the form of mean ± SD (range), whereas categorical variables were described by frequencies [n (%)]. X^2 test was used to compare between categorical variables, whereas Student t test was used to compare the means of groups. A two-tailed P value less than 0.05 was considered statistically significant. All analyses were performed with the IBM SPSS 20.0 software (IBM Corp., Armonk, New York, USA).

Ethical consideration

The study was approved and monitored by the Medical Ethics Committee, Assiut Faculty of Medicine, IRB No. 17100059. The investigators explained the steps and value of the research to the parents/guardians of all eligible participants. Those who agreed to participate signed a fully informed consent form.

The parameters to be assessed were as follows:

(1) Frequency of positive GX versus results of ZN stain, blood PCR, TST, and culture.
(2) Sensitivity and specificity of GX (with culture as reference standard).
(3) Frequency of RIF resistance as detected by GX.

Results

The number of patients older than 5 years was significantly higher than younger children. The number of female cases was higher in older age group, but no significant difference was noted regarding frequencies of symptoms or clinical diagnoses. Frequency of extrapulmonary manifestations was higher in younger age, and the commonest was cervical lymphadenopathy.

Radiologic and laboratory findings of our patients are detailed in Table 1.

In cases with positive GX, RIF resistance was detected in two (6.6%) cases.

Regarding sample type in GX and ZN stain, in GX assay, 24 (48%) sputum samples were positive and six (20%) GA samples were positive, whereas in ZN stain, 15 (30%) sputum samples were positive and three (10%) GA samples were positive. The frequencies of positive GX and positive ZN stain were both higher in sputum than GA samples.

Frequency of positive GX was higher with older age, with absent Bacillus Calmette–Guérin (BCG) scar and with positive contact history. Frequency of positive GX results was higher with hemoptysis and with failure to thrive.

Table 2 demonstrates the frequency of GX positivity versus different radiologic and laboratory findings.

The most common radiologic finding in both positive and negative cases was hilar lymphadenopathy followed by consolidation. Frequency of positive GX was higher with cavitary lesions.

Table 1 Radiologic and laboratory findings of the included patients

Variables	Group ≤5 years (*n*=23)	Group >5 years (*n*=57)	*P*
CXR			
Hilar lymphadenopathy	22 (95.6)	44 (77.2)	0.049*
Parenchymal disease			
Noncavitary lesions (consolidation)	20 (87.0)	42 (73.7)	0.20
Cavitary lesion	0	4 (7.0)	0.65
Pleural effusion	1 (4.4)	9 (15.8)	0.162
Normal CXR	2 (8.7)	4 (5.3)	0.95
TST	13 (56.5)	46(80.7)	0.026*
Blood PCR	6 (26.1)	9 (15.8)	0.29
ZN stain	3 (13.0)	15 (26.3)	0.20
GX	3 (13)	27 (47.4)	0.004*
Culture (*n*=30)	(*n*=5) 1 (20)	(*n*=25) 9 (36)	0.49

CXR, chest radiograph; GX, GeneXpert; TST, tuberculin skin test; ZN, Ziehl–Neelsen. (*) is a marker for statistically significant *P* values, a *P* value less than 0.05 was considered statistically significant.

Table 2 Frequency of GeneXpert positivity in contrast with different radiologic and laboratory findings

Variables	Positive GX (*n*=30)	Negative GX (*n*=50)	*P*
CXR			
Hilar lymphadenopathy	24 (80)	42 (84)	0.648
Parenchymal disease			
Noncavitary lesions (consolidation)	21 (70)	41 (82)	0.213
Cavitary lesion	4 (13.3)	0	0.043*
Pleural effusion	3 (10)	7 (14)	0.61
Normal CXR	2 (6.67)	4 (8)	0.721
TST	25 (83.3)	34 (68)	0.21
Blood PCR	10 (33.3)	5 (10)	0.009*
ZN stain	12 (53.3)	6 (12)	0.004*
Culture	(*n*=10) 7 (70)	(*n*=20) 3 (15)	0.009*

CXR, chest radiograph; GX, GeneXpert; TST, tuberculin skin test; ZN, Ziehl–Neelsen. (*) is a marker for statistically significant *P* values, a *P* value less than 0.05 was considered statistically significant.

Regarding hematological parameters, patients with positive GX had lower hemoglobin levels.

In patients with negative culture, ZN examination was positive in one (5%) and negative in 19 (95%), but in those with positive cultures, ZN examination was positive in six (60%) and negative in four (40%). Based on culture results, GX had 70% sensitivity and 95% specificity in diagnosing PTB with overall diagnostic accuracy of 86.67%. On the contrary, ZN examination had 60% sensitivity and 95% specificity in diagnosing PTB, with overall diagnostic accuracy of 83.33%.

Discussion

TB was significantly higher in older group owing to increased exposure to infection and waned protection from BCG vaccine, which declines with time and can last for up to 10 years [7]. Adolescents have many contacts and are at high-risk for contracting TB [8].

Moreover, the number of females was significantly higher in older group (63.3%) than younger group (39%). This agrees with Margarit *et al.* [9]. Women nowadays socialize as men do, but their gatherings are often in confined spaces, unlike males.

We found that the associating extrapulmonary manifestations were more frequent in younger age. Feja and Saiman[10] attributed this observation to immunologic host factors. However, Cano *et al.*[11] reported that age did not influence disease presentation as PTB or EPTB.

We found hilar lymphadenopathy as the most common radiographic finding (82.5%). Hilar lymphadenopathy was more frequent in young age. This agrees with Boloursaz *et al.* [12]. This can be explained by the nature of disease process of TB.

TST was positive in most patients (73.75%), and TST positivity was more frequent in older group. This agrees with Beshir *et al.* [13]. Cano *et al.*[11] reported similar frequency of TST positive cases (72.3%); however, such frequency was slightly higher in younger age.

PCR was positive in 18.75% of our patients, with no significant difference with age. This is consistent with Lima *et al.* [14]. There is scarcity of studies about blood PCR in pediatric PTB.

ZN stain was positive in our 18 (22.5%) patients. This can be explained by the paucibacillary nature of TB in children. However, other studies reported variable results [11,15]. Jain *et al.*[16] reported that microbiological positivity was associated with higher mean age.

Regarding GX results, 30 (37.5%) patients were positive, with significantly higher frequency in older children. This is comparable to Kasa *et al.* [17]. Several studies showed variation in frequency of positive cases above or below our figures, mainly owing to variation in inclusion and exclusion criteria.

Our study showed that GX positivity was more frequent in older group. This agrees with Sekadde *et al.*[18] and Detjen *et al.* [19]. However, Yin *et al.*[20] reported contradicting results.

In positive GX cases, we found RIF resistance in two (6.6%) patients: one (3.7%) among new TB cases and one (33.3%) among previously treated cases. This agrees with the latest Egyptian Drug Resistance Survey in 2010 [21].

Regarding sample type, both GX and ZN stain had better performance in sputum samples. There is evidence that supports that GA has inferior yield compared with other respiratory samples in diagnosing PTB, such as Hepple *et al.*[22] and Singh and Tiwari [23].

A much larger bulk of evidence, however, supports the reliability of GA in diagnosing PTB, such as Pang *et al.*[24] and Detjen *et al.* [19]. The latter reported that the pooled sensitivity and specificity of GX for TB detection were 62 and 98%, respectively, with the use of sputum, and 66 and 98%, respectively, with the use of GA. GX sensitivity was 40% higher than microscopy [19].

GX was significantly more frequently positive in those with absent BCG scar. This agrees with Yin *et al.*[20] and Jain *et al.* [16]. This can be explained by BCG-induced immunity enhances containment of TB organisms, preventing their appearance in respiratory samples.

GX was significantly more frequently positive in those with contact history of TB. This agrees with Sekadde *et al.*[18] and Yin *et al.* [20]. However, contact history is a risk factor for TB in both microbiologically positive and negative patients [25].

Positive GX was more frequent in those with hemoptysis. Campos *et al.*[26] reported that hemoptysis was less frequent in smear-negative patients as hemoptysis is associated with cavitation, which is associated with more bacillary yield.

Positive GX was more frequent in those with failure to thrive, as weight loss is a consequence of severity and/or long duration of TB illness. Both of which were found to predict high bacteriologic yield [27]. Jain *et al.*[16] reported that low BMI was associated with microbiological confirmation in children with PTB.

Cavitary lesions were significantly more common in those with positive GX results. Palaci *et al.*[28] reported that patients with cavitary disease had higher colony forming unit levels.

Patients with positive GX had significantly lower hemoglobin levels. This agrees with Kerkhoff *et al.*[29] and Jain *et al.* [16]. Lower hemoglobin may reflect severity and/or duration of TB illness.

TST was much more frequently positive than GX without significant association between them. This agrees with Hanrahan *et al.* [30]. However, Sekadde *et al.*[18] reported that a positive TST was associated with a positive GX test.

Positive GX was more frequent in those with positive blood PCR. As we address PTB, it is expected that respiratory samples to be of more yield than blood samples. Lima *et al.*[14] reported that the sensitivity of blood PCR was higher for EPTB (55.56%) than PTB (18.18%).

GX could confirm much more cases than ZN smear. This agrees with Dzodanu *et al.*[31] and others. GX requires smaller number of organisms for detection than acid-fast bacilli staining [32].

Conclusion

In conclusion, GX detected much more cases than blood PCR and ZN stain. GX had 70% sensitivity and 95% specificity in diagnosing PTB with overall diagnostic accuracy of 86.67% (with culture as gold standard), with better performance in older children who could expectorate. RIF resistance rate was 6.6%.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 World Health Organization. Global Tuberculosis Report 2018. Geneva: World Health Organization; 2018. Available at: *http://apps.who.int/medicinedocs/en/m/abstract/Js23553en/*[Accessed 24 February 2020].

2 Zar H, Hanslo D, Apolles P, Swingler G, Hussey G. Induced sputum versus gastric lavage for microbiological confirmation of pulmonary tuberculosis in infants and young children: a prospective study. Lancet 2005; 365:130–134.

3 Lawn S, Nicol M. Xpert®MTB/RIF assay: development, evaluation and implementation of a new rapid molecular diagnostic for tuberculosis and rifampicin resistance. Future Microbiol 2011; 6:1067–1082.

4 Laszlo A. Sputum examination for tuberculosis by direct microscopy in low income countries. IUATLD Technical Guide International Union against Tuberculosis and Lung Disease, Paris, France 2000; 7–15.

5 Chihota VN, Grant AD, Fielding K, Ndibongo B, Van Zyl A, Muirhead D, *et al.* Liquid vs. solid culture for tuberculosis: performance and cost in a resource-constrained setting. Int J Tuberc Lung Dis 2010; 14:1024–1031.

6 World Health Organization. *Xpert MTB/RIF implementation manual: technical and operational 'how-to'; practical considerations. No. WHO/HTM/TB/2014.1: Geneva*. World Health Organization; 2014.

7 Abubakar I, Pimpin L, Ariti C, Beynon R, Mangtani P, Sterne JAC, *et al.* Systematic review and meta-analysis of the current evidence on the duration of protection by Bacillus Calmette-Guérin vaccination against tuberculosis. Health Technol Assess 2013; 17:1.

8 Friedrich, MJ 'Guidelines for Ending Childhood and Adolescent Tuberculosis. JAMA 2018; 320:1969–1969.

9 Margarit A, Simó S, Rozas L, Deyà-Martínez À, Barrabeig I, Gené A, *et al.* Adolescent tuberculosis: a challenge and opportunity to prevent community transmission. An Pediatr (Barc).2017; 86:110–114.

10 Feja K, Saiman L. Tuberculosis in children. Clin Chest Med 2005; 26:295–312.

11 Cano APG, Romaneli MTN, Pereira RM, Tresoldi AT. Tuberculosis in pediaTric paTienTs: how has the diagnosis been made? Rev Paul Pediatr

2017; 35:165–170.

12 Boloursaz MR, Khalilzadeh S, Baghaie N, Khodayari AA, Velayati AA. Radiologic manifestation of pulmonary tuberculosis in children admitted in pediatric ward-Massih Daneshvari Hospital: a 5-year retrospective study. Acta Med Iran 2010; 48:244–249.

13 Beshir MR, Zidan AE, El-Saadny HF, Ramadan RA, Karam NA, Amin EK, *et al*. Evaluation of the immune response to interferon gamma release assay and tuberculin skin test among BCG vaccinated children in east of Egypt: a cross-sectional study. Medicine (Baltimore. 2016; 95:e3470.

14 Lima JF, Montenegro LM, Montenegro Rde A, Cabral MM, Lima AS, Abath FG, *et al*. Performance of nested PCR in the specific detection of Mycobacterium tuberculosis complex in blood samples of pediatric patients. J Bras Pneumol 2009; 35:690–697.

15 Elhassan MM, Elmekki MA, Osman AL, Hamid ME. Challenges in diagnosing tuberculosis in children: a comparative study from Sudan. Int J Infect Dis 2016; 43:25–29.

16 Jain R, Mukherjee A, Singla M, Verma Y, Gautam H, Lodha R, *et al*. Predictors of microbiologically confirmed intrathoracic tuberculosis. Indian J Pediatr 2017; 84:843–847.

17 Kasa Tom S, Welch H, Kilalang C, Tefuarani N, Vince J, Lavu E, *et al*. Evaluation of Xpert MTB/RIF assay in children with presumed pulmonary tuberculosis in Papua New Guinea. Paediatr Int Child Health 2018; 38:97–105.

18 Sekadde MP, Wobudeya E, Joloba ML, Ssengooba W, Kisembo H, Bakeera-Kitaka S, *et al*. Evaluation of the Xpert MTB/RIF test for the diagnosis of childhood pulmonary tuberculosis in Uganda: a cross-sectional diagnostic study. BMC Infect Dis 2013; 13:133.

19 Detjen AK, DiNardo AR, Leyden J, Steingart KR, Menzies D, Schiller I, *et al*. Xpert MTB/RIF assay for the diagnosis of pulmonary tuberculosis in children: a systematic review and meta-analysis. Lancet Respir Med 2015; 3:451–461.

20 Yin QQ, Jiao WW, Han R, Jiao AX, Sun L, Tian JL, *et al*. Rapid diagnosis of childhood pulmonary tuberculosis by Xpert MTB/RIF assay using bronchoalveolar lavage fluid. Biomed Res Int 2014; 2014:310194.

21 Ministry of Health and Population. National tuberculosis control program. Tuberculosis control guidelines, Egypt. 2017. Available at: *http://www.mohp.gov.eg/UserFiles/Userfiles/61/6813d74d-edfc-4a07-b447-7beb3318ded7.pdf*. [Accessed on 18 February 2020].

22 Hepple P, Ford N, McNerney R. Microscopy compared to culture for the diagnosis of tuberculosis in induced sputum samples: a systematic review. Int J Tuberc Lung Dis 2012; 16:579–588.

23 Singh V, Tiwari S. Getting a better bacteriological diagnosis in children with tuberculosis. Pediatr Infect Dis 2012; 4:75–80.

24 Pang Y, Wang Y, Zhao S, Liu J, Zhao Y, Li H. Evaluation of the Xpert MTB/RIF assay in gastric lavage aspirates for diagnosis of smear-negative childhood pulmonary tuberculosis. Pediatr Infect Dis J 2014; 33:1047–1051.

25 Muttamba W, Kirenga B, Ssengooba W, Sekibira R, Katamba A, Joloba ML. Prevalence of tuberculosis risk factors among bacteriologically negative and bacteriologically confirmed tuberculosis patients from five regional referral hospitals in Uganda. Am J Trop Med Hyg 2019; 100:386–391.

26 Campos LC, Rocha MV, Willers DM, Silva DR. Characteristics of patients with smear-negative pulmonary tuberculosis (TB) in a region with high TB and HIV prevalence. PLoS ONE 2016; 11:e0147933.

27 Marais BJ, Hesseling AC, Gie RP, Schaaf HS, Enarson DA, Beyers N. The bacteriologic yield in children with intrathoracic tuberculosis. Clin Infect Dis 2006; 42:e69–e71.

28 Palaci M, Dietze R, Hadad DJ, Ribeiro FKC, Peres RL, Vinhas SA, *et al*. Cavitary disease and quantitative sputum bacillary load in cases of pulmonary tuberculosis. J Clin Microbiol 2007; 45:4064–4066.

29 Kerkhoff AD, Wood R, Vogt M, Lawn SD. Predictive value of anaemia for tuberculosis in HIV-infected patients in sub-Saharan Africa: an indication for routine microbiological investigation using new rapid assays. J Acquir Immune Defic Syndr 2014; 66:33.

30 Hanrahan CF, Dansey H, Mutunga L, France H, Omar SV, Ismail N, *et al*. Diagnostic strategies for childhood tuberculosis in the context of primary care in a high burden setting: the value of alternative sampling methods. Paediatr Int Child Health 2019; 39:88–94.

31 Dzodanu EG, Afrifa J, Acheampong DO, Dadzie I. Diagnostic yield of fluorescence and Ziehl-Neelsen staining techniques in the diagnosis of pulmonary tuberculosis: a comparative study in a District Health Facility. Tuberc Res Treat 2019; 2019:4091937.

32 Denkinger CM, Schumacher SG, Boehme CC, Dendukuri N, Pai M, Steingart KR. Xpert MTB/RIF assay for the diagnosis of extrapulmonary tuberculosis: a systematic review and meta-analysis. Eur Respir J 2014; 44:435–446.