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Rapid Molecular Detection of Tuberculosis and Rifampicine Resistance in Ecuador

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Abstract

Background: In Ecuador, tuberculosis (TB) remains a serious problem that is complicated by the emergence of multidrug-resistant TB (MDR-TB). To evaluate this problem, this study was carried out at the Social Security Hospital (IESS) in Guayaquil, Ecuador from 2013 to 2015. **Methods:** The Xpert TB/RIF system was used to detect TB and MDR-TB and a survey was carried out to identify the factors that are potentially causing MDR-TB. **Findings:** 200 TB patients were confirmed on 5649 suspected patients and 20 (10%) with MDR-TB. It was observed that the annual prevalence of TB and MDR-TB had declining during study period. Trends have been declining but co-infection has doubled since 2009 with 16% of patients co-infected with HIV. Potential resistance factors identified were: disruption in drug supply, lack of resources and lack of credibility of treatment.

Keywords

Pulmonary Tuberculosis, HIV, Rifampicin Resistance, GeneXpert

1. Introduction

TB is an old disease that is far from being resolved. In fact, in 1993 WHO declared that it was a global emergency. In 2012, there were 9 million TB cases in the world and 1.3 million TB deaths [1], but the problem is more complex because many countries are reporting an increase in multidrug-resistant tuberculosis (MDR-TB) rates.

MDR-TB was defined as that occurs in TB patients with resistance to the two most effective first-line agents: isoniazid and rifampicin. It was generally estimated in 2014 that MDR-TB represents 3.3% of new cases and 20% of retreatment cases [2]. Overall, only 48% of patients with MDR-TB in the cohort of cases detected in 2010 were treated successfully [1]. It is known that resistance to rifampicin rarely occurs alone and is usually an indicator of resistance to other drugs [3]. In some cases, a more drug-resistant variant of TB may be developed: drug-resistant TB (TB-XDR). XDR-TB is a resistant form with additional resistance to more drugs. It has been reported in 105 countries around the world. On average, it is estimated that 9.6% of MDR-TB cases may present XDR-TB.

In the Americas, there were 280,000 new cases and 19,000 TB deaths. As indicated in the PAHO/WHO Report of 2014, the estimated incidence rate was 29 cases per 100,000 inhabitants and the case detection rate was 79% [4]. TB in the Andean region countries is also of concern, as is the case of Colombia where 70% of patients are cured with supervised treatment, but this is considered a low percentage. Causes of therapeutic failure include non-adherence, lack of medication, and bacterial resistance. Currently, Ecuador has reported an increase in the cure rate that went from 61% in 2007 to 73% in 2012, but it is still a serious problem.

Is it possible that Mycobacterium tuberculosis (MTB) that has been studied since the beginning of microbiology as a science is still a microorganism so difficult to diagnose and treat? Among other causes, the genetic characterization of MTB is the main difficulty, because the MTB genome comprises a wide range of genes with a high GC content and its study will allow a better understanding of the mechanisms of pathogenesis and drug resistance according to Fontalvo *et al.* [5]. An additional problem is interaction with the human immunodeficiency virus (HIV) since its interaction has led to changes in the epidemiology and clinical aspects of both diseases. MTB facilitates the progression and immune deterioration of the patient with AIDS and HIV in turn to create a favourable environment for the progression of the latent form to the clinical form of TB as referred by Reyes *et al.* [6] as evidence of this, in 2006 worldwide more than 350 thousand deaths occurred in patients co-infected with HIV [7]. At the same time, TB was the leading cause of death among HIV patients. Based on this situation, PAHO/WHO has recommended intensifying the search for TB cases among people with HIV [4] and to optimize these treatments since anti-TB drugs co-administered with antiretroviral may interact and HIV is constituted in a factor that favours the appearance of resistance as previously reported [6].

In response to these problems in 2010, WHO adopted the Xpert® MTB/RIF system (Cepheid, Sunnyvale, CA, USA). The Xpert® MTB/RIF molecular diagnostic test is being rapidly adopted by countries. By the end of June 2013, 88 of the 145 countries had purchased 1402 for test devices and 3.2 million cartridges with reagents. This assay has shown high sensitivity and specificity as reported by Weyer *et al.* [8], "For smear-positive culture-positive TB, the pooled sensitivity of Xpert MTB/RIF was 98% (95% CrI, 97% - 99%) (23 studies, 1952 partici-

pants) and when used to detect rifampicin resistance, Xpert MTB/RIF achieved a pooled sensitivity of 95% (95% CrI, 90% - 97%) (17 studies, 555/2624 total specimens) and a pooled specificity of 98% (95% CrI, 97% - 99%) (24 studies, 2414 specimens, including true negatives and false positives)."

It is an *in vitro* diagnostic test semi-quantitative PCR, integrated in real time and is based on the simultaneous detection of the MTB gene and rPOB gene mutations associated with RIF resistance, accelerating the diagnosis of TB and MDR-TB. The introduction of Xpert MTB/RIF increased reporting rates for TB cases, as demonstrated by Sachdeva *et al.* [9] in a study developed in India. In another study conducted in Lima, Peru, Carriquiry *et al.* [10] reported that MTB/RIF showed excellent performance in patients with advanced immuno-suppressant and a high clinical suspicion of TB.

The objective of our study was to evaluate the frequency of TB and MDR-TB in adult patients with high clinical suspicion and to establish the relationship between patients co-infected with HIV and the occurrence of resistance.

2. Methods

2.1. Study Design

In order to evaluate this problem, a descriptive and transversal study was designed at a hospital in the city of Guayaquil (Ecuador) from 2013 to 2015.

2.2. Study Population

Patients older than 18 years with a high clinical suspicion of pulmonary TB who did not receive treatment with anti-TB drugs were included.

A high clinical suspicion of pulmonary TB was defined by Heemskerk *et al.* [11] as those clinical cases that included cough for ten or more days with abnormal chest x-ray (cavity, focal opacity, pleural effusion, nodule or lymphadenopathy) and at least one of the following symptoms: difficulty respiratory, fever, fatigue, night sweats, haemoptysis, coughing up blood, excessive sweating, especially during the night and chest pain, wheezing or weight loss.

2.3. Technical Information

The detection of patients with positive smear microscopy was confirmed by PCR techniques. In addition, we evaluated the frequency of patients co-infected with HIV and resistance to anti-TB drugs.

Patients resistant to rifampin voluntarily agreed to respond to a survey to identify factors that could have contributed to the generation of resistance. An analytical type survey was conducted in face-to-face interviews and a questionnaire with 10 closed questions was used to collect data.

In this study, descriptive statistics were used as a method to describe the variables, using the mean and standard deviation for quantitative variables and frequency and percentage for qualitative variables, which were represented in tables and graphs. All calculations were performed in the statistical program

SPSS version 22.0.

All biological samples should be treated with the usual precautions of the health institution. Guidelines for sample management are available from the US Center for Disease Control and Prevention (CDC) [12] and the Clinical and Laboratory Standards Institute (CLSI) [13].

Clinical samples were analyzed using the Xpert MTB/RIF system according the following procedure: 3 ml of sputum were transferred to a 50 ml tube to be decontaminated with N-acetyl-L-cysteine and sodium hydroxide; centrifuge and discard the supernatant and take an aliquot of the decontaminated pellet. 0.5 ml was used for smear staining with Ziehl-Neelsen. For direct MTB/RIF, the sputum sample was carefully mixed to make it homogeneous, then sample reagent was added to 1 ml of untreated sputum on a 2:1 ratio, mixed twice manually during an incubation period of 15 minutes at room temperature, and then 2 ml were transferred to the MTB/RIF cartridge as described in [14].The cartridge was closed and placed into the GeneXpert System.

The GeneXpert system integrates and automates the processing of samples; nucleic acid amplification and detection of target sequences in simple or complex samples. The primers of Xpert MTB/RIF amplify a part of the rPOB gene containing the "core" region of 81 base pairs. Probes can differentiate between natural conserved sequence and mutations in the central region that are associated with resistance.

This system is designed for use only with samples of untreated patients showing clinical signs of TB. The characteristics and method of use were adjusted to the manufacturer's recommendations. The use of Xpert MTB/RIF for the detection of MTB or for determining sensitivity to rifampin has not been validated for patients than are receiving treatment for TB. The study was carried out in a total of 5649 patients with suspected pulmonary TB between January 2013 and June 2015.

2.4. Ethical Considerations

The study was approved by the Executive Director of the Hospital. Informed written consent was obtained from all patients included, and their data were treated anonymously, without any bioethical conflicts of any kind.

3. Results

As a result, 200 cases were positive for TB and 20 (10%) of them were positive for TB-MR. For diagnosis, 343 smears were analyzed for the presence of MTB and confirmed by PCR. 76% of TB patients were men with a mean age of 42 years (IQR 28 - 59), 75% under 60 years and 50% under 42 years. On the other hand, patients co-infected with HIV have been 32 and represent 16% of TB positive cases with a male-female ratio of 3.57:1 and an average age of 33 years. As mentioned above, patients with MDR-TB were 20 (10%) and were classified according to sex and HIV co-infection as shown in Table 1.

Based on these findings, the annual values of Prevalence of TB obtained were 4.45% (2013); 3.46% (2014) and 2.42% (first half of 2015) and resistance values to rifampicin were 0.63% (2013); 0.33% (2014) and 0% (first half of 2015) (Figure 1).

The results of the analytical survey to identify the main factors that may have contributed to the generation of resistance can be seen in Table 2.
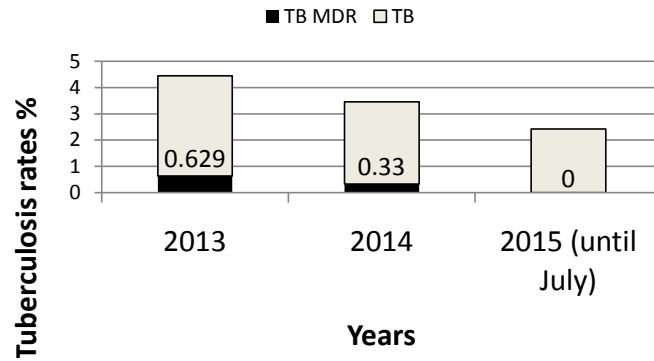

Figure 1. Annual evolution of the rates of TB and MDR-TB expressed in patients with confirmed TB every 100 patients at the Hospital TMC. The prevalence rates of TB and MDR-TB showed a decreasing annual trend consistent with trends reported by Ecuador to WHO.

Table 1. Rifampicin resistant patients classified by sex and HIV coinfection.

	HIV negative	HIV positive	Total
Female	4	2	6
Male	9	5	14a
Total	13	7b	20

aMDR-TB resistance is more common in men than women at a ratio of 2.3 to 1; bSeven MDR-TB patients (33%) had HIV co-infection.

Table 2. Factors that may contribute to the generation of drug resistance.

Factor	affirmative	%
Interruption in the supply of drugs	19	95
Lack of resources	18	90
Lack of credibility regarding the effectiveness for treatment	10	50
Messy life style/substance abuse	9	45
Lack of credibility with the diagnosis	5	25
Misunderstanding	3	15
Intolerance/toxicity	1	5
Cultural beliefs	0	0
Pregnancy	0	0
Neuropsychiatric disease	0	0

4. Discussions

A perfect correlation (100%) was observed between Acid fast Bacilli (AFB) and RT-PCR. However, the additional advantage of the molecular technique is that it allows simultaneously diagnose TB and MDR-TB and provides rapid and useful information when evaluating treatments.

Our results over pulmonary TB detection in Ecuador, determined that there is predominance in male patients (3.16 men per woman) and mainly affects the economically active population. Results show an increase compared to previous data, as demonstrated in studies conducted in 2009 where men/women diagnosed with TB positive ratio was 1.7 men for every woman [15].

This value, represents a 62.96% males and it is according previous reports of WHO worldwide, exists an estimated 60% of TB cases are men, a situation that repeats itself when he analyses the TB morbidity and mortality [1]. In relation to age range affected, the National TB Control Program reports [15] that the age range is mainly between 15 and 34 years (men) and coincides with our study (50% of TB cases are under 42 years of age). This finding is worrying because it is an economically active age group and this constitutes an economic problem at the country level.

The study of the prevalence rates, their evolution and the MDR-TB component can be seen in Figure 1, where a decrease in the values of 2014 compared to the year 2013 coincides with the tendency to the low level reported by the WHO [1]. This decline is probably due to the political control of TB that has been implemented without interruption since 2010. The strategy includes the DOTS (Directly Observed Treatment Short Course) program and is generating excellent results in the control of TB as described by Santos-Preciado *et al.* [16] In 2015 the data are only in the first half and show a tendency to increase the value of the TB rate, but there are no cases of MDR-TB.

The incidence of total TB in Ecuador decreased from 132 in 1990 to 54 in 2014, expressed in cases per 100,000 population, according to World Bank data [17]. Ecuador is the only country in the world that has introduced a subsidy payment that has reduced treatment abandonment from 28.2% to 7% in the first year (to ensure compliance with treatment) year of implementation (2011-2012) [1].

Patients with rifampin resistance mutations contributed to the overall indices, as can be seen in Figure 1, where a marked decrease (50%) was observed in the MDR-TB cases detected from 2013 to 2014. Globally, Proportion of new cases of MDR-TB was 3.5% in 2013 and has not changed in recent years, approaching the proportional relationship determined in other reports [1]. In 100% of the patients studied with a confirmed diagnosis of TB, 20 patients (10%) were found to have resistance mutations.

This value is similar to the resistance values described in previous studies in Ecuador between 2008 and 2009 [14]. Overall, MDR-TB rates were similar to those found in our study [1] [18], it was found that the occurrence of resistance

is more common in men as can be seen in Table 1.

Our contribution to the diagnosis of TB cases in the Hospital represents a significant fraction in a district (Guayas) that historically has contributed with 48.25% of cases throughout the country [14].

A much higher percentage of cases of HIV co-infection have been observed than those reported in studies conducted in 2008 [14], whose values reached 8.8%, whereas in our study HIV patients accounted for 16%. People infected with HIV are 20 times more likely to develop TB than uninfected people. In addition, 7/20 patients with MDR-TB (33%) had co-infection with HIV (Table 1). Although there are no data in Ecuador on the MDR-TB and HIV ratio, it is significant that the frequency doubles with respect to that detected in TB and HIV co-infection.

5. Conclusion

We conclude that although the prevalence rates of TB and MDR-TB shows a decrease, HIV co-infection has doubled since 2009 and this problem is increasing in cases of MDR-TB. We emphasize that TB and MDR-TB affect the male population in the economically active age and the responses of patients with MDR-TB allowed the identification of the main factors that may contribute to the emergence of resistance, the interruption in the supply of drugs, lack of resources (>90%) and lack of credibility in the effectiveness or need for treatment (>50).

Acknowledgements

Conflict of Interest

All authors report no conflict of interest in this study.

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