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Original article

Interferon-gamma release assay performance in northeastern Brazil: influence of the *IFNG* + 874 A>T polymorphism

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ABSTRACT

Introduction: Latent tuberculosis infection diagnosis based on the release of interferon-gamma in cultures of peripheral blood cells stimulated with *Mycobacterium tuberculosis* antigens has replaced the tuberculin skin test in many countries with low tuberculosis prevalence. The IFN-γ production can be influenced by genetic polymorphisms, of which the *IFNG* + 874 (rs62559044) locus is the most studied. We investigated the possible influence of the *IFNG* + 874 A/T polymorphism on interferon-gamma test performance.

Methods: Patients diagnosed with pulmonary tuberculosis (75), volunteers with positive tuberculin skin test (70) and healthy volunteers with negative tuberculin skin test and no history of contact with tuberculosis (57) were evaluated regarding the *IFNG* + 874 genotype and the IFN-γ levels in whole blood cultures performed using an interferon-gamma commercial kit (QuantiFERON-TB® Gold In-Tube).

Results: IFN-γ production was not influenced by the *IFNG* + 874 genotype, regardless of antigen or mitogen-based stimulation, which suggests that other genes may influence IFN-γ production in response to mycobacteria. The *IFNG* + 874 polymorphism was found to exert no influence over QFT-IT test sensitivity in our study.

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Conclusions: The *IFNG* + 874 polymorphism was not shown to influence QuantiFERON-TB® Gold In-Tube test performance in an admixed population from northeastern Brazil.

Introduction

Mycobacterium tuberculosis (Mtb) infection is present in one-third of the world's population. While some of these individuals are in a subclinical stage of tuberculosis (TB), most infected people will remain asymptomatic throughout their lives.[1] These individuals are considered to have latent tuberculosis (LTBI) and only a fraction will develop active TB. This latent pool complicates disease control, as reactivation can occur at any time and chemoprophylaxis is administered only in individuals of known recent contact with an index case.[2,3] LTBI screening is performed using tests based on an immune response to mycobacterial antigens. The tuberculin skin test (TST) has been used for over a century and offers good sensitivity and specificity in endemic conditions. However, it may present cross-reactivity to sensitization with the BCG vaccine as well as environmental mycobacteria.[4,5] IFN-γ release assays (IGRAs) were first described in 2000[6,7] and present an alternative to TST.[8] To date, most studies interpret IGRAs similarly to TST, except in populations vaccinated with BCG after childhood, or in individuals receiving multiple doses of this vaccine, as well as in locations where environmental mycobacteria infections are common.[4,5] In these situations, IGRAs do seem to present a better positive predictive value due to greater specificity.[9,10] Cost-effectiveness is another criterion to select IGRA as the test of choice, e.g. in countries with low TB prevalence.[11–13]

Both TST and IGRA tests are not able to distinguish active from latent infection. This is partly because these tests are based on the immune response, and those at increased risk to develop active TB are immunocompromised individuals who also present the highest rates of false-negative results. Moreover, the antigens expressed by Mtb during latent infection may not be the same as those expressed during the active replication stage.[2,14] As a result, a lower specificity of TB diagnosis is expected in countries with high TB burdens.[15–17]

Studies focusing on the impact of genetic background with regard to IGRA testing are surprisingly scarce. IFN-γ production may be influenced by the presence of polymorphisms in the gene that encodes this cytokine, of which the most studied is the *IFNG* + 874 A/T polymorphism. The *IFNG* + 874 A/T polymorphism determines the emergence of an NF-κB binding site that can increase the transcription of the human IFN-γ gene.[18] The AA genotype at this locus has been linked to TB susceptibility and low IFN-γ production, including in the Brazilian population.[19] In a study among South African families in a hyperendemic area for tuberculosis, the influence of heredity on quantitative responses to IFN-γ release was estimated to be between 43% and 58%, depending on the nature of the antigen used as a stimulus[20] It is possible, therefore, that the *IFNG* + 874 A/T polymorphism influences the sensitivity and specificity values of IGRA assays. In this context, the aim of this study was to evaluate the influence of the *IFNG* + 874 A/T polymorphism in *M. tuberculosis* infected individuals residing in an endemic area.

Methods

Recruitment and study design

The present study included 202 volunteers, divided into three groups: (i) patients with a diagnosis of pulmonary tuberculosis by positive culture and/or BAAR staining who were naïve to antituberculosis treatment (TB, $n = 75$); (ii) volunteers with a positive TST greater than 10 mm, with known contact with TB patients, who tested negative under culture and BAAR staining (TST+, $n = 70$); (iii) volunteers with a negative TST, an absence of tuberculosis symptoms and no history of contact with individuals with tuberculosis (TST−, $n = 57$). All individuals were recruited from two local reference institutions for TB diagnosis located in the city of Salvador, Bahia-Brazil. Written informed consent was obtained from all participants. This study was approved by the Institutional Review Board of the Bahiana School of Medicine and Public Health (registered under protocol: CAAE 57662016.8.1001.5662) and complied with the Declaration of Helsinki, as well as with Brazilian regulations pertaining to research ethics involving human beings (Resolution: CNS 466/2012).

Latent TB infection diagnosis

Latent TB infection was diagnosed by TST (PPD RT 23 2TU, Statens Serum Institute, Denmark) and IGRA using a QuantiFERON®-TB Gold in tube (QFT-IT) (Cellestis Ltd., VIC, Australia) assay in accordance with manufacturers' recommendations. Individuals were considered positive when presenting an increment of at least 0.35 IU/mL in IFN-γ production in the Ag tube (specific antigen-stimulated culture) as compared to the Nil tube (cultures in the absence of any antigen), and an increment in IFN-γ production of at least 0.5 IU/mL when comparing the PHA tube (mitogen-stimulated culture) with the Nil tube. Individuals were considered positive by TST when presenting skin induration measuring at least 10 mm. QFT-IT was repeated for samples with discordant TST and IGRA results.

Genotyping and IFN-γ production

Following whole blood sample collection, genomic DNA was extracted using a PureLink® Genomic DNA Kit (Invitrogen, Carlsbad, CA, USA) in accordance with manufacturer's instructions. Genotyping for the IFN-γ polymorphism was performed

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