Differential B-Cell responses are induced by Mycobacterium tuberculosis Ag85A synthetic peptides in two populations from Venezuela

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Background: The aim of the present study was to assess progress made in the diagnosis of pulmonary tuberculosis when evaluating B-cell responses to 16 Ag85A synthetic peptides, the recombinant antigen 85 (rAg85) and the non-recombinant PPD antigen.

Methods: The B-cell responses of tuberculosis patients and healthy individuals were evaluated by an IgG-ELISA. A total of 120 individuals were included in this study. Patient groups were composed of 20 Warao indigenous (WP) and 20 Creole non-indigenous (CP), whilst healthy control groups were composed of 40 Warao indigenous (WC) and 40 Creole non-indigenous (CC). Both control groups included 20 positive and 20 negative individuals for the tuberculin skin test (TST). Association of positive tests for each antigen, defined with receiver operator characteristics (ROC) analysis, was assessed for each population.

Results: Different patterns of the B-cell responses were displayed by each population. The anti-29878 IgG method reached highest sensitivity of 95.0% (negative predictive value (NPV) = 94.4) within the Warao population, but was lowly specific, 42.5% (positive predictive value (PPV) = 45.2), compared to highest specificity showed by the anti-29879 IgG method (100.0%, PPV = 100). Regarding the Creole population, anti-11006 IgG showed highest sensitivity of 95.0% (NPV = 90) but was lowly specific (22.5%, PPV = 45.2), compared to highest specificity showed by the anti-29879 IgG method (100.0%, PPV = 100). Anti-10998 IgG was found to be the most specific (100.0%, PPV = 100), followed by the anti-PPD IgG method (90.0%, PPV = 66.7). These findings indicate that population-to-population heterogeneity of peptide antigen recognition, rather than recognition of particular antigens, is a characteristic feature of antibody responses in these two populations. Furthermore, responses to anti-29879 IgG and anti-10998 IgG were associated to inactive TB.

Conclusion: Ag85A peptides were more specific than sensitive, showing that these peptides’ high specificity does not stimulate primed T cells in TST+ individuals, suggesting that
they might be useful in identifying population at a higher risk of latent TB reactivation.

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Characterization of rpo B Gene for Detection of Rifampicin Drug Resistance by SSCP and Sequence Analysis

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Background: Due to the emergence of multidrug resistant TB (MDR-TB) in recent time, the rapid detection of resistance to first line antituberculosis drug such as Rifampicin (which is generally considered a marker for determination of MDR strains) was felt worldwide.

Methods: Rifampicin susceptibility of 22 rifampicin resistant and 11 rifampicin susceptible Mycobacterium tuberculosis strains was analyzed by PCR-Single Strand Conformation Polymorphism(SSCP) and validated by DNA sequencing within the 157 bp region of the rpo B gene(Ala500 to Val550) for checking its utility as a rapid screening test for determination of MDR drug resistance.

Results: RMP resistance was detected successfully by PCR-SSCP in 20/22(90.90%) of the RMP-resistant strain. 2 RPMr strains showed identical PCR-SSCP pattern with wild type H37Rv. There were seen seven different mutation in the amplified region of rpo B gene of 20 RPMr: codon 513(CAA→CCA), 516(GAC→GTC), 507(GGC→GAC), 526(CAC→GAC, TAC), 531(TCG→TTG, TGG), 522(TCG→TGG), 533(GTG→CCG). Thus this study demonstrated the high specificity(100%) and sensitivity(90.90%) of PCR-SSCP method for detection of mutation in rpo B gene; 77.27% of RPMr strain showed a single mutation and 9.09% had no mutation.

Conclusion: Our data support the common notion that rifampicin resistance genotypes are generally present in codons 516, 526, and 531, most frequently found in M. tuberculosis population regardless of geographic origin and can be rapidly detected by PCR-SSCP making it as a useful tool in primary screening of drug resistance.

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Mutations in the rpoB Gene of Rifampicin - Resistant Mycobacterium tuberculosis Isolates from Poland

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Background: Resistance to rifampicin is considered as a surrogate marker for MDR-TB. This putative rifampicin resistance is associated with mutations that occur within a 69-bp region of the rpoB gene, which encodes the β subunit of RNA polymerase. This study was undertaken for the first time in Poland to detect and characterise the rpoB gene mutation associated with rifampicin resistance in M. tuberculosis isolates by INNO-LiPA Rif. Tb assay.

Methods: A total of 44 clinical samples collected from patients with pulmonary and extra pulmonary tuberculosis were used for study. The clinical samples were tested simultaneously by LiPA test and by conventional methods. Drug susceptibility tests were performed by standard proportion method. All of M. tuberculosis positive samples (in culture or/and in LiPA test) were then evaluated by using LiPA kit for any specific mutational pattern of rpoB gene. As a control, H37Rv strain was used.

Results: Of the 44 clinical samples tested 23 of the specimens were culture positive for M. tuberculosis. The LiPA assay identified 44 of them as M. tuberculosis complex and simultaneously detected the susceptibility pattern to rifampicin. Among 44 strains, 32 rifampicin resistant strains and 12 rifampicin sensitive strains were detected by using LiPA kit. Of 32 resistant strains: 27 showed R types mutations accounted for 61,3%, 4 strains demonstrated S type mutations and only 1 strain revealed double mutations. Twelve strains with no mutations were defined as RMPs. The RPMr strains revealed 7 different LiPA profiles. Of R type mutations the R4b (H526D), R5 (S531L) and R2 (D516V) were observed with followed frequencies -31,8%, 25,0% and 4,5%. S type mutations occurred in 11,4% of analysed strains.

Conclusions: The results obtained by LiPA assay show that the mutations of the rpoB gene at codons 516, 526 and 531 are predominant in M. tuberculosis Polish strains.

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Comparison of 5% Sodium Hypochlorite and Phenol Ammonium Sulphate Concentration Techniques in Tuberculosis Diagnosis

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The recently developed phenol ammonium sulphate (PHAS) sputum concentration method was compared to the established sodium hypochlorite (NaOCl) method in diagnosis of pulmonary TB. Three hundred sputum specimens were collected from patients with clinical symptoms of TB in Harare, Zimbabwe. Specimens were emulsified using both methods in parallel and concentrated by centrifugation. Pellets were smeared on slides and stained by the ZN method for AFB. A total of 80 (27%) were AFB positive by both concentration methods. An additional 16(20%) were positive by the NaOCl method. The greater sensitivity of NaOCl was statistically significant (X² = 70.605, d.f. = 1, p = 0.000). More AFB were seen per high power field using the NaOCl method(ranked as 1+ or greater)than for PHAS. Also, the sputum samples that had a low count of AFB (<1 per field) using NaOCl were negative using PHAS. The NaOCl concentration method showed greater sensitivity in detecting pulmonary TB than the PHAS method.

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