Intracellular cytokine flow cytometry (ICCFC) for measurements of TB-specific T cells

Abstract:

Background: Tuberculosis (TB) is a communicable disease that is a major cause of ill health, one of the top 10 causes of death worldwide, and the leading cause of death from a single infectious agent (ranking above HIV/AIDS). Multifunctional T cells, defined by their ability to coexpress two or more cytokines, e.g., (TNF-α and IFN-γ), have shown a good diagnostic yield to detect TB infection.

The aim of the study: Assessment of the diagnostic sensitivity of intracellular cytokine flow cytometry analysis of multifunctional specific CD4+ T cells coexpressing TNF-α and IFN-γ after stimulation with phytohemagglutinin for the diagnosis of active pulmonary TB.

Patients and methods: This study was performed on 30 active pulmonary tuberculosis (APTB) patients who were admitted to Fayoum Chest Hospital and 30 healthy controls. CD4+ T cells were stimulated by phytohemagglutinin (PHA) and then were measured using the intracellular cytokine staining technique by flow cytometry to detect CD4+ T cells expressing TNF-α and IFN-γ on whole-blood samples.

Results: Receiver-operator characteristic (ROC) analysis was done to determine the optimum cutoff value for the studied diagnostic markers, which was the difference between the percentages of CD4+ lymphocytes coexpressing TNF+α and IFN+γ before phytohemagglutinin (PHA) stimulation (inactivated CD4+) and after phytohemagglutinin (PHA) stimulation (activated CD4+) in the cases and controls, which was 4.9 with sensitivity 90% and specificity 50%. Using this cutoff, it scored 27 (90%) as positive from 30 APTB of active TB patients and 15 (50%) as negative from 30 of healthy controls.

Conclusion: Multifunctional flow cytometry analysis of specific CD4+ T-cell response may represent a relatively simple and rapid immune-based approach to distinguish between MTB-infected and - uninfected patients.