COMBINATION BIOMARKERS FOR DIAGNOSIS OF LATENT TUBERCULOSIS INFECTION

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT FOR THE AWARD OF M.Sc. DEGREE IN MEDICAL MICROBIOLOGY OF THE UNIVERSITY OF NAIROBI

ABSTRACT

Background

Pulmonary tuberculosis is a worldwide public health problem affecting about 10 million individuals globally, causing 1.6 million deaths annually, despite availability of inexpensive effective ant TB therapy. Sub Saharan part of Africa is the most affected especially as a result of HIV/AIDS pandemic as evidenced by high HIV prevalence among TB patients.

Kenya has been ranked as a high burden TB country, with TB as a major cause of morbidity and mortality especially in the most productive age group of 15-44 years. Some socio economic factors like poor housing in peri urban slums contribute to disease transmission.

The etiologic agent, *Mycobacterium tuberculosis,* is transmitted through droplets of respiratory secretions from infected persons to other individuals. About 90% of individuals exposed to the bacilli contain the infection for decades and therefore become latently infected. Latently infected persons are at risk of reactivation resulting to clinical disease.

Studies on mRNA gene expression of IL-4δ_2 and Rab- 33A in latently infected individuals clinical TB patients and healthy controls have previously shown differential expression of these genes. This suggests their involvement in an additional adaptive immune mechanism to TB.

The aim of this study was to investigate a combination of these protective immune markers in latently infected persons for possible utilization in TB control programs.

Objectives

- To quantify changes in fold mRNA gene expression of small GTPase Rab33A and IL- 4 delta 2 as diagnostic markers of latent tuberculosis infection.
- To compare changes in fold mRNA gene expression of small GTPase Rab33A with IL- 4 delta 2 in diagnosis of latent tuberculosis infection.
- To establish the predictor of diagnosis of latent tuberculosis using changes in fold mRNA gene expression of Rab 33A and IL-4δ_2.
- To establish the association of exposure to development of latent TB infection.

Materials and Methods

Using a cross sectional study design,64 sputum smear positive patients consecutively diagnosed of T.B. at Mbagathi District Hospital TB clinic ,39 of their household contacts and 30 healthy controls from University of Nairobi level 2 medical students were recruited into the study.

A structured questionnaire was used to collect socio demograghic, exposure and clinical data. Four milliliters of venous blood samples were also taken aseptically using vacutainers at recruitment. Total RNA was separated from the whole blood samples using QIAmp RNA blood minikit (Qiagen), and then quantified using NanoDrop1000 UV. Primers and probes were designed using published sequences and purchased from Bioneer. Assays were done using Agpath ID RT-PCR kit and Power SYBR Green RT-PCR master mix (ABI).

Relative quantification method was used to determine change in gene expression of Rab 33A, IL-4, and IL-4δ_2 using Ct values.

Data entry was done using Excel software and then analyzed using STATA 11.

Results

The study found significant up regulation of interleukin 4 (IL-4) mRNA gene expression in tuberculosis patients as compared to their close contacts and controls with median rank 77.97 ($p < 0.001$).

Interleukin 4delta 2 (IL-4δ_2.) mRNA gene expression was significantly higher in contacts as compared to tuberculosis patients and controls with median rank 101.18, ($p < 0.001$).

Rab 33A protein mRNA gene expression was higher in contacts than tuberculosis patients and controls although the difference was not significant. (p 0.127)

Exposure characteristics had no significant association with increased IL-4δ_2.mRNA gene expression in contacts.

Conclusion

Elevated production of interleukin 4 delta2 (IL-4δ_2) can be used as a pointer of robust immunity against TB and as index of suspicion of latent TB infection. It is not sufficient alone as a diagnostic tool for latent TB.