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IMMUNOLOGICAL ASPECTS

Treatment end point determinants for pulmonary tuberculosis: Human resistin as a surrogate biomarker

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SUMMARY

Treatment of tuberculosis (TB), which takes one human life every 15 s, globally, requires a prolonged (>6 months) antitubercular treatment (ATT) which, is known to have hepatotoxic side effects. This study was designed to explore the utility of human resistin, a proinflammatory hormone, as a sensitive biomarker to determine TB treatment end points. Patients for pulmonary tuberculosis enrolled under the directly observed treatment, short-course (DOTS) program were followed-up for six months and were monitored by sputum analysis, body weight and ELISA-based serum resistin and C-reactive protein (CRP) levels at 0, 2, 4 and 6 months, along with close family contacts of TB patients and healthy controls. The mean circulating resistin levels were found to be significantly higher ($P < 0.001$) in patients ($n = 48$, 25.74 ± 9.45 ng/ml) reporting for the first time for treatment (T0) as compared to healthy subjects ($n = 45$, 7.18 ± 2.40 ng/ml). Resistin levels in contacts ($n = 48$, 19.61 ± 7.88 ng/ml) also were found to be significantly ($P < 0.001$) elevated as compared to healthy controls. Significant increase in body weight after four months ($P = 0.006$) and at 6 months ($P < 0.001$) of treatment inversely correlated with resistin levels. Our data suggest resistin could be a surrogate marker for TB treatment in addition to its utility as an early prognostic biomarker for monitoring TB disease onset.

1. Introduction

Tuberculosis (TB), caused by the pathogen *Mycobacterium tuberculosis,* is a disease of global significance, with alarmingly increasing incidences in some of the hotsopt countries. The Indian subcontinent, a global hotspot for the growth and spread of the TB epidemic in recent times[1] with India alone accounting for 22% of the global burden, has served as the corridor of early world-wide dissemination of *M. tuberculosis* during the ancient era.[2] Although, a large catchment of tuberculosis endemic regions is being covered for anti-tubercular treatment (ATT) under directly observed treatment, short-course (DOTS) program of World Health Organization (WHO) and positive outcomes of successful treatment have been documented in cases of stringent compliance of the treatment regimen, in many cases treatment is extended for more than six months in order to circumvent possibilities of relapse or the development of drug resistance. Also, since anti-tubercular drugs have known hepatotoxic side effects[3,4] it is indeed important to have end point determinants, based on sensitive biomarkers for continuation or otherwise of ATT. Use of such biomarkers for early evaluation of drug response will not only greatly improve clinical management of the disease but will also aid in assessing novel anti-tubercular drugs which are very much needed due to spread of MDR and XDR cases. There is therefore, a need to identify and develop biomarkers not only for stratification of patients as a function of treatment outcome but also measure relapse rate within the first 2 year of ATT.[5]

There has been considerable interest about the host defenses in *M. tuberculosis* infection and about the mechanisms and correlates

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of bacterial containment or clearance leading to clinical recovery from TB. The utility of the CRP, an acute phase protein and a non-specific marker of systemic inflammation, as a marker of bacterial infection of the lower respiratory tract has been extensively studied.[6] In tuberculosis, serum CRP has been reported to increase significantly in patients with active tuberculosis, particularly in patients with advanced disease.[7–10] Furthermore, CRP levels were shown to decline with therapy and reduction of CRP directly correlated with clinical response.[7–10]

Resistin, a cysteine-rich secretory protein, has been positively associated with inflammation[11–13] and several acute inflammatory and chronic diseases such as diabetes, endotoxemia, asthma, severe sepsis or septic shock, atherosclerosis, coronary artery disease, arthritis, malignant tumors, inflammatory bowel disease, non-alcoholic fatty liver disease and chronic kidney disease.[14–21] The ability to induce a proinflammatory Th1 response[11,22] argues for a role of this hormone in containing infectious agents *via* innate immune mechanisms employing proinflammatory signaling pathways. It is not clear if hyper-resistinemia, also associated with bacterial infections,[23] is a sequel or pathognomonic of every infectious episode and whether it reverses after the cause of infection is eliminated. Also, there are no data showing upregulation of resistin by mycobacterial infection or as an additional consequence of underlying cachexia and nutritional limitation as seen in some of the chronic wasting diseases such as inflammatory bowel disease and some cancers. Similarly, serum resistin levels have not yet been monitored in a chronological fashion to establish the biological co-ordinates of pathogen clearance or treatment outcome.

This study was accordingly designed to estimate resistin levels and compare the same in patients with TB, their contacts and unrelated healthy controls. Resistin was measured before the start of DOTS (T0), 2 months (T2), 4 months (T4) and 6 months (T6) after DOTS, variations in resistin levels were correlated with clinical parameters of recovery namely body weight gain and sputum clearance. CRP was used as an adjunct marker for comparison with resistin.

2. Materials and methods

2.1. Study groups

This study comprised of three different groups, i) TB patients – 48 freshly diagnosed as having active pulmonary TB by clinical symptoms [fever, cough and abundance of acid-fast bacilli (AFB) in sputum] enrolled for treatment under the DOTS program, ii) Contacts – 48 family members, one from each family of the TB case who were in close contact with the patient, iii) Healthy controls – 45 unrelated individuals who did not have any known respiratory disease. None of the subjects selected had any clinical evidence of co-infection with HIV, myocardial infarction, respiratory disorders, diabetes, renal failure, hypertension, pregnancy or jaundice.

Upon enrollment and during follow-up visits, epidemiological and demographic information was collected through a structured questionnaire. TB patients were administered standard anti-tuberculosis drugs under the DOTS program and were motivated to attend the clinics for a period of six months at different intervals for follow-up study. During therapy and follow-up visits, patients were assessed for clinical evidence of active tuberculosis and staging of the same for which sputum specimens were collected and tested by sputum smear staining for the presence of *M. tuberculosis*. Blood (5 ml) was collected from all subjects (TB patients and contacts) at their first visit and at every two months interval (0, 2, 4 and 6 months). Sera were separated and stored at −70 °C until assayed for resistin and CRP levels by enzyme-linked immunosorbent assay (ELISA). Samples for estimation of resistin levels were coded so as to avoid any bias.

Written informed consent was obtained from all the subjects [and from parents/guardian in case of minors] prior to their enrolment in the study as per institutional ethical guidelines. The study design, patient recruitment procedures and sample collection were approved by institutional ethics and bio-safety committee.

2.2. Sputum examination of the patients

Sputum of the patients was collected and was stained by Ziehl Neelsen stain. The Acid Fast stained bacilli were observed under microscope. Grading was done as per the Revised National Tuberculosis Control Program (RNTCP) guideline.[24]

2.3. ELISA for estimating serum resistin levels

Serum resistin levels were spectrophotometrically measured using commercially available human resistin ELISA kit (AdipoGen, Seoul, Korea) as per instructions of the manufacturer. All the sera (1:20 dilution) were tested in duplicate to ensure greater inter-assay reproducibility. Mean of the values were taken for calculation of correlations. As there was <10% variations in all duplicates, correlation with single values were not calculated. Resistin concentration was calculated by interpolation of regression curve formula as recommended by the manufacturer, with a detection limit of 100 pg/ml.

2.4. ELISA for estimating serum C-reactive protein (CRP) levels

Serum CRP levels were spectrophotometrically measured using commercially available high sensitive CRP (hs-CRP) ELISA kit (Diagnostic Biochem Canada Inc) as per instructions of the manufacturer. All the sera (1:20 dilution) were tested in duplicate to ensure greater inter-assay reproducibility. CRP concentration was calculated by log–log graph recommended by the manufacturer. The sensitivity of hs-CRP ELISA kit is 10 ng/ml.

2.5. Statistical analysis

Group means, standard deviations, standard error of mean,'t' tests, ANOVA, correlations, box plots and simple percentages were used for the analyses of data. For all analyses $P \leq 0.05$ was considered as statistically significant.

3. Results

3.1. Age and gender

TB group had 48 TB patients with mean age of 25 years (range, 14–55) with 20 males and 28 females. Contacts group had 48 healthy contacts with mean age 25 years (range, 15–50) consisting of 23 males and 25 females. Healthy group had 45 individuals consisting of 29 males and 16 females with mean age 26 years (range, 15–40).

3.2. Serum resistin levels in TB patients, contacts and healthy controls

Serum resistin levels were estimated at the time of enrollment in contacts and healthy controls whereas in patients, at various time points during treatment [0 month (T0), 2 months (T2), 4 months (T4) and 6 months (T6)] (Figure 1). Mean value of serum resistin levels (Mean ± SD) in TB patients was 25.74 ± 9.45 ng/ml, in contacts 19.61 ±7.88 ng/ml and in healthy controls 7.18 ± 2.40 ng/ml. Resistin level in TB patients (T0) ranged from 3.92 to 40.63 ng/ml, in contact the levels ranged from 3.75 to 38.18 ng/ml and in healthy controls the levels were 0.64–13.8 ng/ml. Mean value of serum resistin level in patient (T0) was higher than the healthy controls ($P < 0.001$) and contacts ($P < 0.001$) (Table 1). Mean value of serum resistin level in