Tuberculosis xxx (2016) 1–7

Contents lists available at ScienceDirect

Tuberculosis

journal homepage: http://intl.elsevierhealth.com/journals/tube

IMMUNOLOGICAL ASPECTS

An adverse immune-endocrine profile in patients with tuberculosis and type 2 diabetes

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ARTICLE INFO

Article history:
Received 8 July 2016
Received in revised form
22 August 2016
Accepted 4 September 2016

Keywords:
Tuberculosis
Diabetes mellitus
Immune-endocrine-metabolic alterations

SUMMARY

Diabetes is a risk factor for the development of pulmonary tuberculosis (TB) and both diseases present endocrine alterations likely to play a role in certain immuno-endocrine-metabolic associated disorders. Patients with TB, or with TB and type 2 diabetes (TB + T2DM) and healthy controls (HCo) were assessed for plasma levels of cortisol, dehydroepiandrosterone (DHEA), estradiol, testosterone, growth hormone (GH), prolactin, insulin-like growth factor-1 (IGF-1), cytokines (IL-6, IL-10, IFN-γ) and the specific lymphoproliferative capacity of peripheral blood mononuclear cells. All patients had higher levels of cortisol with a reduction in DHEA, thus resulting in an increased cortisol/DHEA ratio (Cort/DHEA). Increased prolactin and particularly GH levels were found in both groups of TB patients. This was not paralleled by increased concentrations of IGF, which remained within the levels of HCo. Estradiol levels were significantly augmented in patients TB, and significantly more in TB + T2DM, whereas testosterone levels were decreased in both groups of patients. IFN- γ and IL-6 concentrations were significantly increased in all TB, even further in TB + T2DM; while IL-10 was equally increased in both groups of TB patients. The *in vitro* specific proliferative capacity was decreased in both groups of patients as compared to that of HCo. The adverse immune-endocrine profile of TB seems to be slightly more pronounced in patients who also have T2DM.

1. Introduction

Pulmonary tuberculosis (TB) is a major cause of mortality around the world. In 2014, 1.5 million people died of TB (0.4 million HIV-positive) and 9.6 million people worldwide are estimated to have contracted the disease during this period [1]. The clinical manifestations are greatly influenced by the immune response to *Mycobacterium tuberculosis*, its etiologic agent, but the mechanisms underlying the outcome of the disease are not fully understood [2]. Endocrine responses during chronic infections such as lung tuberculosis are worth studying since some of the cytokines produced during this disease are likely to affect endocrine mechanisms that, in turn, influence the course of the infectious process [3,4]. In fact, proinflammatory cytokines released from affected tissues that reach the central nervous system are known to influence the secretory activity of the hypothalamic–pituitary–adrenal (HPA) axis. The adrenal gland is responsible for the release of glucocorticoids (GCs), which generally inhibit or modulate inflammation, as well as dehydroepiandrosterone (DHEA), a steroid that counteracts GCs effects on cytokine production, but also exerts itself potent anti-inflammatory effects [3,5]. Interactions between the endocrine and the immune system also involve the hypothalamic–pituitary–gonadal (HPG) axis, since macrophages and lymphocytes have receptors for gonadal steroids and these hormones can affect macrophage and lymphocyte development and function [6,7].

By evaluating hormonal and cytokine levels in patients with TB, we have previously shown imbalanced immune-endocrine responses in which levels of pro-inflammatory cytokines, cortisol and estradiol concentrations were increased whereas testosterone and

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http://dx.doi.org/10.1016/j.tube.2016.09.001
1472-9792/

DHEA amounts were diminished in patients, together with an increased Cort/DHEA ratio even more pronounced in those with a severe disease [8].

Endocrine disturbances can also contribute to the pathology of patients with TB, as illustrated by the detrimental influence of type 2 diabetes mellitus (T2DM) on TB development. Several studies have shown that T2DM may be associated with an increased risk of developing active TB, whereas TB patients with concomitant diabetes are at higher rates of treatment failure and death [9–11].

Type 2 DM is characterized by the failure of beta cells to compensate for insulin resistance, with inflammatory or immunological factors being implied in such alterations [12]. Thus, the simultaneous occurrence of both diseases may impose a particular set of alterations in immune and endocrine parameters that is worth exploring.

On these bases, we analyzed the blood levels of several cytokines, hormones and the specific immune response to mycobacteria in the context of the TB and diabetes association. Studies included the assessment of the plasma levels of IL-6, IFN-γ and IL-10, adrenal (cortisol, DHEA) and gonadal (estradiol, testosterone) steroids, hormones involved in immune-metabolic effects like growth hormone (GH), prolactin and insulin-like growth factor-1 (IGF-1), in parallel to the specific lymphoproliferative capacity of peripheral blood mononuclear cells.

2. Materials and methods

2.1. Sample population

Patients (6 females and 29 males) with no HIV co-infection and newly diagnosed pulmonary TB of moderate to severe degree were included. Pulmonary TB diagnosis was based on positive clinical symptoms and radiological chest results as well as sputum smear positivity for acid fast bacillus (AFB) by Ziehl Neelsen staining and a confirmatory positive culture for Mtb on Lowenstein–Jensen medium. Fourteen of these patients were diagnosed as also having T2DM (TB + T2DM). Criteria for diabetes diagnosis were hyperglycemia (based on two fasting glucose levels greater than 125 mg/dL or a random glucose level equal to or higher than 200 mg/dL) evaluated on EDTA-anticoagulated blood specimens. According to current guidelines and considering the patient age, we estimated that all TB + T2DM patients had T2DM [13–15]. Most of them had a previous diabetes diagnosis and were under conventional treatment.

The control group was composed of 20 healthy controls (HCo), sharing the same socioeconomic conditions of TB patients, without any known prior contact with TB patients, as well as no clinical or radiological evidence of pulmonary TB. Patients and HCo had no other respiratory disease, nor immunocompromising diseases.

All patients started anti-TB treatment shortly after blood sample collection (1–3 days later), for which they were untreated at the time that studies were carried out.

Samples were obtained at 8 a.m. to avoid differences due to circadian variations. Exclusion criteria included disease states that affect the adrenal glands, the HPA or HPG axes, or requiring corticosteroid treatment, pregnancy, and age below 18 years. The body mass index (BMI) was also calculated (weight/square of height). The protocol was approved by the Bioethic Committee of the School of Medical Sciences, National University of Rosario. All participants gave their consent to participate in the study.

2.2. Lymphoproliferation

Peripheral blood mononuclear cells (PBMC) were obtained from fresh EDTA-treated blood. After centrifugation, the buffy coat was separated and diluted 1:1 in RPMI 1640 (PAA Laboratories GmbH, Austria), containing standard concentrations of L-glutamin, penicillin, and streptomycin (culture medium, CM). The cell suspension was layered over a Ficoll-Paque Plus gradient (density 1.077, Amersham Biosciences, NJ, USA), and centrifuged at 400 *g* for 30 min at room temperature (19–22 °C). PBMC recovered from the interface were washed three times with CM, and resuspended in CM containing 10% heat-inactivated pooled normal AB human sera (PAALaboratories GmbH, Germany). Cells were cultured in quadruplicate in flat-bottomed microtiter plates (2×10^5 cells/well in 200 μl) with or without addition of γ-irradiated H37Rv *M. tuberculosis* strain, (Mtb; 8 μg/ml) kindly provided by Dr J. Belisle (Colorado State University, Fort Collins, CO, U.S.A.) PBMC cultures were incubated for 5 days at 37 °C, in a 5%, CO_2 humidified atmosphere and pulsed with ^{3}H-thymidine for 18 h before cell harvesting. The average counts per minute (cpm) of stimulated and non-stimulated cultures were calculated.

2.3. Quantification of cytokines and hormones in plasma

Plasma was obtained from EDTA-treated blood. Samples were centrifuged at 2000 rpm during 30 min and the plasma stored at −20 °C. Cortisol (DRG Diagnostics, detection limit 2.5 ng/ml), DHEA (DRG Diagnostics, detection limit 0.108 ng/ml), IFN-γ (BD Pharmingen, detection limit 4.7 pg/ml), IL-10 (BD Pharmingen, detection limit 3.9 pg/ml) and IL-6 (DRG Diagnostics, detection limit 2 pg/ml), prolactin (DRG Diagnostics, detection limit 0.35 ng/ml), hGH (DRG Diagnostics, detection limit 0.17 μIU/ml), insulin-like growth factor-1 (Quantikine, R&D Systems, detection limit 0.026 ng/ml), testosterone (DRG Diagnostics, detection limit 0.083 ng/ml) and estradiol (DRG Diagnostics, detection limit 9.714 pg/ml) plasma concentrations were determined using commercially available ELISA kits according to the manufacturer instructions. All samples were processed individually and assayed in duplicate.

2.4. Statistical analysis

Comparisons between groups were made by nonparametric methods: Kruskall-Wallis followed by Dunn's test for multiple comparisons, if applicable. Qualitative variables were compared by the chi square test. Associations between variables were analyzed using the Spearman correlation test. A value of $p < 0.05$ was considered as indication of significant differences.

3. Results

The subject profile is shown in Table 1. There were no between-group differences in age and sex distribution, while the presence of the BCG scar was less prevalent among TB patients. Both groups of TB patients (with or without T2DM) had a BMI lower than HCo ($p < 0.001$, Table 1). Data from *in vitro* proliferation of PBMC from patients and HCo are also presented in Table 1. PBMC from HCo had higher median proliferative responses to Mtb than both groups of TB patients ($p < 0.02$), which was more decreased in TB patients without T2DM. As commented, TB and TB + T2DM patients had a similar degree of disease severity. Measurements on HbA1c levels indicated that TB + T2DM had poorly controlled diabetes (means ± standard error of the mean of % HbA1c): HCo = 5.54 ± 0.09; TB = 5.78 ± 0.15; TB + T2DM = 9.58 ± 1.06 ($p < 0.001$).

Results from the analysis of cytokine levels in plasma are depicted in Figure 1. TB patients had increased amounts of IFN-γ respect to HCo, which was more pronounced in those with concomitant T2DM and statistically significant from HCo and TB

Please cite this article in press as: Fernández R, et al., An adverse immune-endocrine profile in patients with tuberculosis and type 2 diabetes, Tuberculosis (2016), http://dx.doi.org/10.1016/j.tube.2016.09.001