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ORIGINAL ARTICLE

Sputum–plasma ratio of soluble receptor for advanced glycation end-products in patients with chronic obstructive pulmonary disease

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KEYWORDS

COPD;
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Abstract *Background:* Chronic obstructive pulmonary disease (COPD) is associated with systemic inflammatory consequences. Receptor for advanced glycation end products (RAGE) acts as an important progression factor amplifying the immune and inflammatory responses in several pathophysiological conditions. The soluble form of RAGE (sRAGE) acts as a decoy for the receptor ligands and is thus thought to protect against excessive inflammation. Conflicting reports exist about sRAGE value in stable and exacerbating COPD.

Objective: To assess the sputum to plasma ratio of sRAGE in stable COPD patients.

Subjects and methods: The study included 44 adult patients of both sexes who were presented to Alexandria Main University Hospital between March and July 2015. Patients were categorized into three groups; 15 stable COPD patients (Group I), 15 smokers without COPD (Group II), and 14 healthy non-smokers (Group III). Measurement of sRAGE level in induced sputum and plasma was performed using ELISA technique.

Results: The study included 38 male patients and 6 female patients, whose median ages were 50, 42 and 35.5 years in Groups I, II, and III respectively ($p < 0.001$). Median FEV_1% predicted were 35, 96, and 105% in Groups I, II, and III respectively ($p < 0.001$). No statistical significant difference was found among all groups regarding sRAGE level in induced sputum, plasma or sputum/-plasma ratio ($p = 0.092, 0.372, 0.154$, respectively). Although levels of sRAGE is apparently higher in induced sputum rather than in plasma, it lacked significance ($r = 0.27$, $p = 0.08$). Furthermore, no significant correlation was found between either plasma or sputum sRAGE level and predicted FEV_1% ($r = -0.11$, $p = 0.48$ and $r = -0.12$, $p = 0.28$, respectively).

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Conclusions: sRAGE level either in induced sputum, plasma or sputum plasma ratio is not significantly different between stable COPD patients, smokers and healthy controls. Thus, sRAGE cannot be considered as a marker of either diagnosis or severity of COPD.

Introduction

Chronic obstructive pulmonary disease (COPD) is a common preventable and treatable disease with ongoing systemic inflammation [1,2]. Many of the systemic manifestations of COPD are mediated through increased systemic levels of inflammatory proteins such as interleukin-6, interleukin-8, tumor necrosis factor-α and C-reactive protein [3].

Receptor for advanced glycation end products (RAGE) acts as an important progression factor amplifying the immune and inflammatory responses in several pathophysiological conditions [4].

Soluble RAGE or sRAGE, a secreted isoform, lacks a transmembrane domain and acts as a decoy for the receptor ligands and is thus thought to protect against excessive inflammation [5]. sRAGE represents a naturally occurring competitive inhibitor of RAGE-mediated signaling pathways [6]. Conflicting reports exist about sRAGE value in stable and exacerbating COPD [7]. Thus, our objective was to assess the sputum to plasma ratio of soluble receptor for advanced glycation end product (sRAGE) in COPD patients.

Subjects and methods

The study was carried out on 44 adult patients of both sexes (38 male patients and 6 female patients), who were presented to Alexandria Main University Hospital. Patients were categorized into three groups; 15 stable COPD patients (Group I), 15 smokers without COPD (Group II), and 14 healthy non-smokers (Group III). All COPD patients and smoker controls must be current active cigarette smokers.

Patients who have renal or cardiac diseases, diabetes mellitus, bronchial asthma, granulomatous lung diseases, malignancy, autoimmune diseases or are receiving long-term oral corticosteroid therapy were excluded from the study.

Diagnosis of COPD was made according to Global Initiative for chronic obstructive lung disease (GOLD) criteria [1]. Spirometry was done for all patients in the three groups using clinical spirometer (CHESTGRAPH HI-701; Chest M. I. Inc., Hongo, Bunkyo-Ku-Tokyo, Japan).

Sputum induction was performed in all patients by receiving bronchodilator treatment 15 min prior to the procedure, and then inhaled 3% hypertonic saline solution was delivered by an ultrasonic nebulizer device (3A Health Care S.r.l. Lonato del Garda (BS), Italy) for 15 min. Sputum and serum levels of sRAGE was determined using enzyme-linked immunosorbent assay (ELISA) (MyBioSource, San Diego, CA 92195-3308, USA. Catalog #: MBS032029).

Samples collection and storage

Five milliliters of blood were withdrawn from each patient on ethylene-diamine-tetraacetic acid (EDTA) coated vacutainer tubes. Samples were then centrifuged for 20 min at 3000 rpm to separate plasma. Plasma was collected in eppendorf tubes and stored at −20 °C till time of assay. Sputum was collected in sterile containers, the volume of sputum samples was measured, and the samples were vortexed with phosphate-buffered solution (PBS). Sputum was then ultracentrifuged at 60,000*g* for 60 min at 4 °C to remove contaminating cellular debris. This was stored at −20 °C for time of assay [8].

Assay procedure

On the day of the assay, all reagents were prepared and brought to room temperature together with the samples 30 min before starting assay procedure. Standard wells, sample wells and blank (Control) wells were set, 50 µl of standard were added to each standard well, and 50 µl of sample were added to each sample well. This was followed by addition of 50 µl sample diluent to each Blank/Control well. Hundred microliters of HRP-conjugate reagent were added to each well, then covered with an adhesive strip and incubated for 60 min at 37 °C. The microtiter plate was washed 4 times. Fifty microliters of each of chromogen solution A and Chromogen Solution B were added to each well successively. This was gently mixed and then protected from light and incubated for 15 min at 37 °C. Fifty microliter stop solution was added to each well. The color in the wells changed from blue to yellow. The Optical Density (O.D.) was read at 450 nm using a microtiter well reader (ELISA Humareader single).

Statistical analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (IBM SPSS Statistics for Windows, Version 20.0., IBM Corp., Armonk, NY) qualitative data were described using number and percent. Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. Comparison between different groups regarding categorical variables was tested using Chi-square test. When more than 20% of the cells have expected count less than 5, correction for chi-square was conducted using Monte Carlo correction. The distributions of quantitative variables were tested for normality using Kolmogorov–Smirnov test, Shapiro–Wilk test and D'Agostino test, if it reveals normal data distribution, parametric tests was applied. If the data were abnormally distributed, non-parametric tests were used. For normally distributed