The Egyptian Journal of Medical Human Genetics (2016) 17, 191–196

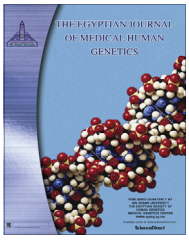
HOSTED BY

ELSEVIER

Ain Shams University

The Egyptian Journal of Medical Human Genetics

www.ejmhg.eg.net
www.sciencedirect.com



ORIGINAL ARTICLE

Sirtuin 1 gene rs2273773 C > T single nucleotide polymorphism and protein oxidation markers in asthmatic patients

Aida Abdeen Mahmoud [a,*], **Abdellah Hamed Khalil Ali** [b], **Essam Nour Eldin** [c]

[a] *Department of Medical Biochemistry, Faculty of Medicine, Sohag University, Egypt*
[b] *Department of Respiratory Medicine, Faculty of Medicine, Sohag University, Egypt*
[c] *Department of Medical Biochemistry, Faculty of Medicine, Umm Al-Qura University, Saudi Arabia*

Received 18 November 2015; accepted 6 December 2015
Available online 24 December 2015

KEYWORDS

Asthma;
Gene polymorphism;
Protein oxidation;
Sirtuin 1;
Sulfhydryl group

Abstract *Background:* Sirtuin-1 (SIRT-1), a protein has been found to protect the cells against oxidative stress due to its deacetylase activity.

In this investigation, we aimed to study SIRT-1 gene rs2273773 C > T single nucleotide polymorphism and markers of serum protein oxidation (protein carbonyl and sulfhydryl groups) in asthmatic patients.

Subjects and methods: 120 asthmatic patients and 120 healthy controls were genotyped for SIRT-1 gene rs2273773 C > T SNP using polymerase chain reaction – confronting two pair primer method (PCR-CTPP). Serum protein carbonyl and sulfhydryl groups were measured using colorimetric methods.

Results: SIRT-1 gene rs2273773 C > T SNP genotyping revealed that the TT genotype was significantly higher in the patients compared to the controls ($P < 0.05$), while there were no significant differences regarding the genotypes TC and CC between the patients and the controls ($P > 0.05$). T allele was significantly higher in the patients compared to the controls ($P = 0.017$). The distribution of the genotypes didn't differ among the atopic and the non-atopic asthmatic patients, also no difference was found in the genotype distribution according to the severity of asthma ($P > 0.05$). Serum protein carbonyl group concentration was significantly higher in the patients compared to the controls ($P < 0.001$), while serum protein sulfhydryl group content decreased significantly in the patients compared to the controls ($P < 0.0001$). No differences in markers of protein oxidation according to SIRT-1 gene rs2273773 C > T genotype were found.

Conclusion: SIRT-1 gene rs2273773 C > T SNP was associated with asthma but not with protein oxidation markers in Egyptian population.

* Corresponding author.

Peer review under responsibility of Ain Shams University.

http://dx.doi.org/10.1016/j.ejmhg.2015.12.002

1. Introduction

Asthma is a chronic inflammatory disease of the airways with a complex etiology [1]. The interaction of genes and environmental factors affects the development of asthma and determines the expression or progression of the disease [2]. Epigenetic mechanisms such as DNA methylation and histone modification control the accessibility of the genome and manage gene transcription in response to the environment in a heritable fashion. Recent evidence suggests that these mechanisms play a role in allergy and asthma [3]. SIRT-1 is a NAD + −dependent nuclear deacetylase of 747 residues, involved in various important metabolic pathways. It down-regulates p53 activity, rising lifespan, and cell survival and deacetylases peroxisome proliferator-activated receptor-gamma and its coactivator 1 alpha, promoting lipid mobilization [4]. SIRT-1 can directly modify chromatin, silence transcription and modulate the cellular proliferation. It possesses a probable antiaging effect through increasing the genomic stability suppressing recombinant DNA recombination [5,6]. SIRT-1 gene is located to chromosome 10 (10q21.3) and has many single nucleotide polymorphisms (SNPs), of which the SNP rs2273773 C > T, a silent mutation in exon 5, was found to be associated with many diseases including chronic obstructive pulmonary disease, hypertension, type 2 diabetes and cardiovascular disease [7–9]. Oxidative stress was found to play an important role in asthma pathogenesis [10]. Reactive oxygen species (ROS) attack proteins causing their oxidation by forming carbonyl groups. The amino acids; lysine, arginine, proline, and histidine are the most liable to modification [11]. The bulk of protein sulfhydryl in plasma is represented by Cys-34 of albumin. Protein sulfhydryl acts as an antioxidant reacting with ROS, protecting cells against the damage induced by them [12].

In this investigation, we aimed to study SIRT-1 gene rs2273773 C > T SNP and serum markers of protein oxidation (protein carbonyl group concentration and protein sulfhydryl group content) in asthmatic patients. Also, we studied the association between SIRT-1 gene rs2273773 C > T SNP and the type and severity of asthma.

2. Materials and methods

2.1. Study population

The study was approved by the Ethics committee of Sohag Faculty of Medicine and was in accordance with the Helsinki Declaration of 1975. An informed written consent was obtained from all individuals included in the study. 120 asthmatic patients and 120 healthy controls were enrolled in the study. The patients were recruited from the chest disease department of Sohag University Hospital, the diagnosis of asthma was according to the clinical history, physical examination, and pulmonary function tests, including reversibility testing and measurement of bronchial reactivity. Patients were classified into atopic and non-atopic and further as having mild intermittent, mild moderate or severe persistent asthma. Atopic asthma and non-atopic asthma were defined based on the presence or absence of atopy (any skin prick test $\geqslant$3 mm). In addition, IgE antibodies and eosinophil count were evaluated for all the participants to differentiate between atopic and non-atopic asthma [13]. The exclusion criteria were the presence of hypertension, diabetes mellitus or any other chronic disease, as SIRT-1 gene rs2273773 C > T SNP has been established to be associated with blood pressure and hyperglycemia in previous studies [7,9].

2.2. Blood samples and laboratory investigations

Fasting blood samples (about 5 ml) were collected from the patients and the controls via the venipuncture of an antecubital vein. Samples were divided into two parts; one taken in tubes containing Na2-EDTA (final concentration 1 mg/ml) for genomic DNA extraction and the other part was taken into plain tubes and the serum of it was used for the routine laboratory investigations (blood glucose and lipid profile) and for the estimation of IgE antibodies, protein carbonyl and sulfhydryl groups. Total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), triglycerides (TG) and fasting glucose were determined using an enzymatic method on Cobas C 311 analyzer (Roche diagnostics, Germany). Low-density lipoprotein-cholesterol (LDL-C) was calculated using the Friedewald formula. IgE was measured using an ELIZA kit (ab108650), according to the enclosed instructions. The assay range of the kit was from 5 to 800 IU/ml.

2.3. Assay of serum protein carbonyl group concentration

Protein carbonyl group concentration was measured according to the method of Reznick and Packer [14] with some modifications; as follows, two tubes of 200 µL serum were taken, one as a test and the other as a control. After that, 1.0 ml of 10 mM 2,4 dinitrophenyl-hydrazine (DNPH) prepared in 1.25 M HCl was added to the test sample and 1.0 ml of 2.5 M HCl alone was added to the control sample. The contents were mixed and incubated in the dark at room temperature for 1 h and shaken intermittently every 15 min. Then 1.25 ml of 20% trichloroacetic acid (TCA) (w/v) was added to both tubes and the mixture left in ice for 10 min. The samples then underwent centrifugation at 3500 rpm for 20 min to get the protein pellet and the supernatant carefully discarded. This was followed by a second wash with 10% TCA. Finally the precipitates were washed three times with 1 ml of ethanol: ethyl acetate (1:1, v/v) to remove unreacted DNPH and lipid remnants. The final protein pellet was dissolved in 1 ml of 6 M guanidine hydrochloride (GuHCL) and incubated at 37 °C for 10 min. The insoluble materials were removed by centrifugation. Carbonyl concentrations were determined from the difference in absorbance at 370 nm between the test and the control samples, with $\varepsilon 370 = 22{,}000\ M^{-1}\ cm^{-1}$ (the molar extinction coefficient of DNPH). Carbonyl levels were expressed as nmol/mg protein.

2.4. Assay of serum protein sulfhydryl group content

It is determined according to thiol/disulfide reaction of thiol and Ellman's reagent (5,5′-dithiobisnitrobenzoic acid, DTNB) [15]. 50 µL of serum was mixed with 1 ml 0.1 M Tris, 10 mM EDTA PH 8.2, constituting the blank reaction and assessed at 412 nm. After that, we added 40 µL 10 mM DTNB in methanol and the absorption was read at 412 nm after stable color formation (1–3 min). The concentrations of protein sulfhydryl groups were calculated using a molar extinction