Nat. Volatiles & Essent. Oils, 2022; 9(1): 450-455

NVEO
Natural Volatiles &
Essential Oils

Investigation Of The Molecular Role Of Cell-Free Nuclear DNA And Cell-Free Mitochondrial DNA In Asthmatic Patients

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Abstract

Asthma is a chronic reversible inflammatory disease of airways characterized by chronic inflammation and airway hyper-reactivity. The genetic and immunological factors besides environmental agents as virus infection have an essential role in the pathophysiology of bronchial asthma in different populations. Limited data are available concerning the clinical relevance of cf DNA in asthmatic patients. This study aims to investigate the clinical significance of cf DNA in patients with asthma. Forty asthmatic patients (20 males and 20 females) and the control group consisted of 20 healthy individuals (10 males and 10 females). The calculation of gene expression fold changed was made using relative quantification. This depends on the normalization of Ct values calculating the ΔCt. Results of ΔCt for the control group displayed a mean of 6.8, whereas for patients group 11.5 with a significant difference p=0.001 when compared patients to controls. Results of ΔΔ Ct showed a significant difference for the patient's group with a mean of 4.68. The fold of gene expression in the patient's group of cf-mt DNA was 0.039 lower than that of the control group. These results indicate that cf-mt DNA down regulated significantly in asthmatic patients.

Conclusion: The results reveal that both cf-n DNA and cf-mt DNA could be significant biomarkers for asthma.

Keywords: Cell-free DNA; plasma, cf-mt DNA, real-time PCR, association with asthmatic patients.

Introduction

Asthma defined according to The Global Initiative for Asthma management and Prevention (GINA); is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role (Tang., 2019). Chronic inflammation is associated with airway hyper-responsiveness (AHR), obstruction, mucus hyper-production, and airway wall remodeling, which leads to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night or in the early morning. These episodes are usually associated with widespread, but variable, airflow obstruction within the lung that is often reversible either spontaneously or with treatment (Wardzyńska et al., 2019). Asthma is a serious health and socioeconomic issue all over theworld, affecting more than 300 million individuals and affecting all age groups (Zierau et al., 2018). Free circulating nucleic acids, cell-free deoxyribonucleic acid (cf-DNA), were discovered in human plasma in 1948 by (Mandel and Metais., 1948). But it would take until 1966 for changes in circulating DNA levels to be correlated with human disease in studies of systemic lupus erythematosus patients (Tan et

al., 1966). Cell-free DNA is a double-stranded molecule that appears in both plasma and serum and is made up of tiny fragments (70 to 200 base pairs) and bigger fragments with molecular weights of up to 21-kilo bases (Jahr et al., 2001). Necrosis, Apoptosis, and active cellular secretion are three potential causes for the occurrence of cf-DNA (Bronkhorst et al., 2019). Some studies showed the damaged mitochondria release their DNA content into the systemic circulation (Oka et al., 2012; Zhang et al., 2010). Thus, cell-free mitochondrial deoxyribonucleic acid (cf-mt DNA) is easily detected in plasma and has been explored as a biomarker of various diseases (Nakahira et al., 2013; Cao et al., 2014). Recently, increased plasma levels of cf-mt DNA have been reported to correlate with the severity of injury in patients sustaining polytrauma (Lo et al., 2000). And the severity of stroke (Rainer and Lam., 2006). Be a prognostic marker of acute myocardial infarction (Antonatos et al., 2006). And intensive care unit patients (Rhodes et al., 2006; Saukkonen et al., 2007). Alterations of cf-mtDNA in the blood also might be associated with several systemic diseases, including primary mitochondrial disorders, carcinogenesis, and hematologic diseases (Urata et al., 2008; Hurtado-Roca et al., 2016).

Limited data are available concerning the clinical relevance of cf DNA in asthmatic patients. This study aims to investigate the clinical significance of cf DNA in patients with asthma .

Materials and Methods

This study included (40) asthma patients with a range of ages between 25-62 years. And an average (40,15 years). (20 males and 20 females), and (20) apparently individuals (controls) (10 males and 10 females), and their age range between 23-42 years. And an average (30,2 years).They were selected in Al-Kut-Jafriya, Iraq. Oral consent was taken from all participants. Age, gender, weight, body mass index, smoking, medications, and medical or family history of asthmatic were measured using standardized questionnaires. Three milliliters of blood were collected from all participants. Three ml of whole blood was withdrawn from a vein and delivered quickly to a plastic tube containing EDTA-K3. Plasma was collected by centrifugation of bloodat 2500 rpm for 10 min, divided into several aliquots, and stored in deep freeze until analysis.

DNA Extraction

Plasma cf- DNA was extracted using Quick-gDNA TM Blood Mini Prep Catalog Nos. D3072& D3073 following the protocol of the manufacturer.

Plasma cf-DNA Quantification by RT-PCR

Baseline plasma cf-mt DNA and cf-n DNA were quantified by real-time PCR using KAPA SYBR® FAST qPCR Master Mix(2X) kit. Primers sequences used in RT-qPCR β-globin gene forward 5'-GTGCACCTGACTCCTGAGGAGA-3', Rever 5'-CCTTGATACCAACCTGCCCAG-3', MT-ND1 forward 5'-AACATACCCATGGCCAACCT -3', Rever 5'- AGCGAAGGGTTGTAGTAGCCC-3'. The gene expression levels and fold changes were quantified by measuring the threshold cycle (Ct) employing the KAPA SYBR® FAST qPCR Master Mix (2X) Kit components. Every reaction was done in a duplicate and included a non-template control (NTC), non-amplification control (NAC), and non-primer control (NPC) as a negative control. The thermal profile for cf-DNA and

mt-DNA expression was as follows: denaturation at 95°C for 5 min, followed by 40 cycles of 20 sec at 95 °C, 20 sec at 60 °C and 20 sec at 72°C. DNA concentrations expressed as ng/µl.

Statistical analysis

Data were analyzed with the SPSS 21.0 software (SPSS Inc., chicago, IL, USA). The comparison of significant (P-value) in any test was: S = Significant difference (P<0.05), HS = Highly Significant difference (P<0.01), and NS = Non Significant difference. Analysis of variance (ANOVA) test was used to determine the differences between the studied groups.

Results and Discussion

Table 1 reveals the results of this study.

Table 1: Comparison between cells free nuclear DNA and mt-DNA in Ct (mean± SD), Δ Ct, ΔΔ Ct, and fold.

Study groups	Mean Ct of n DNA	Mean Ct of mt DNA	ΔCt (Ct of mt-n	ΔΔ Ct	Fold of gene expression
Control	24.0 ± 0.5	30.8±0.2	6.8	0.05	0.965
Patients	22.1 ± 0.2	33.6± 0.8	11.5	4.68	0.039
P-value	0.001				
Significance	Significant				

*Significant at p-value ≤ 0.05, significant at p-value ≥ 0.0.

The calculation of gene expression fold change was made using relative quantification. This depends on the normalization of Ct values calculating the ΔCt.

Results of ΔCt for the control group displayed a mean of 6.8, whereas for patients group 11.5 with a significant difference p=0.001 when compared patients to controls. Results of ΔΔ Ct showed a significant difference for the patient's group with a mean of 4.68. The fold of gene expression in the patient's group of cf-mt DNA was 0.039 lower than that of the control group. These results indicate that cf-mt DNA down regulated significantly in the patient's group.

Echem et al., (2019) observed that the activation of TLRs by DAMPs such as mt DNA is linked to arterial hypertension (AH), which is considered a chronic inflammatory condition. Also, they noticed that male patients with AH had a 4-fold greater amount of mt DNA in their blood than female patients. Su et al., (2016) used real-time PCR to reveal that the average number of mt DNA copies in PTC tumors was approximately four times greater than that in surrounding normal tissues. In breast cancer, a similar phenomenon has been reported, in which a rise in tissue mt DNA copies is associated with a reduction in cf-mt DNA copies (Shen et al., 2010). Since this number of mitochondrial genomes in a cell ranges from several hundred to more than 10,000 copies, and each mitochondrion contains between two and ten mt DNA molecules, Higuchi (2007) determined that the levels of cf-mt DNA in circulation are substantially greater than those of cf-n DNA. In endometriosis and lung cancer, a rise in plasma cf-mt DNA has been reported, which is generally described by a compensatory mechanism of the cells in response to a loss in respiratory function or redox imbalance (Bonner et al., 2009). According to Mehra et al., 2007 prostate cancer patients had a three-fold increase in the

number of mt DNA copies compared to healthy controls. Rosa et al., (2020) found that diabetic patients had 2.33 times more cf-mt DNA in their plasma and 2.08 times more in their serum than healthy controls. (corresponding to 230 and 208 percent of healthy controls' levels, respectively). An increase in mitochondrial copy number has been hypothesized as a possible compensatory effect for the overall loss in mitochondrial respiratory function caused by oxidative damage. The lack of histones and other DNA-protecting proteins, as well as less effective DNA repair mechanisms than those found in n DNA, allows for increased mt DNA degradation, which could lead to ROS generation and cell death (Kumar et al., 2017). Under diabetic conditions, mt DNA damage is significantly more prevalent and lasts much longer than nuclear DNA damage in cells experiencing oxidative stress (Cao et al., 2019). According to various studies, diabetics' peripheral blood mt DNA content is lowered, which may be linked to the development of T2DM (Singh et al., 2007; Xu et al., 2012). Several recent studies have found a link between mitochondrial dysfunction or anomalies in mitochondrial biogenesis and thedevelopment of insulin resistance or type 2 diabetes. Reduced respiratory enzyme complex activity, decreased expression of mitochondrial biogenesis genes, mutation or deletion of mitochondrial DNA, decreased bioenergetics capacity or problems in respiratory enzyme complex activityhave been found in the tissues of mice and humans with insulinsensitivity or type 2 diabetes, according to these studies (Wang et al., 2012).

The results reveal that both cf-n DNA and cf-mt DNA could be significant biomarkers for asthma.

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