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Association between serum microRNA-605 and microRNA-623 expression and essential hypertension in Egyptian patients

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ABSTRACT

Essential hypertension is widespread and has significant morbidity and mortality. However, the genetic basis of the disease is unknown. MicroRNAs are post-transcriptional regulators and have been implicated in development of the disease. The aim of this study was to assess the relationship between serum microRNA-605 and microRNA-623 expression and essential hypertension in Egyptian patients. MicroRNA expression in serum was determined in hypertensive patients and normotensive controls by quantitative real-time polymerase chain reaction. Hypertensive patients had significantly higher expression of both microRNA-605 (11 fold increase, $p < 0.001$) and microRNA-623 (22 fold increase, $p = 0.001$) than normotensive controls. Neither microRNA-605 nor microRNA-623 expression was significantly correlated with systolic or diastolic blood pressure. MicroRNA-605 expression was positively correlated with elevated serum total cholesterol, low density lipoprotein, and was negatively correlated with serum high density lipoprotein (HDL). MicroRNA-623 expression was negatively correlated with serum HDL. Results from the study indicate that the microRNAs studied are not directly associated with presence of essential hypertension, but are associated with dyslipidemia commonly associated with the disease.

1. Introduction

Causing complications in many vital organs, hypertension is a major public health concern. Hypertension is a predisposing factor for cardiovascular disease, chronic kidney disease, stroke and peripheral vascular disease (Romaine et al., 2016). Several mechanisms lie behind the pathogenesis of hypertension, including systemic inflammation, oxidative stress, impaired angiogenesis and endothelial dysfunction (Huang et al., 2017). Since it causes complications in many vital organs, hypertension is a major public health concern. However, information on biomarkers for hypertension is scarce. Therefore, it is important to study genetic loci associated with hypertension and assess if there are any biomarkers significantly associated with the disease.

MicroRNAs (miRs) are non-coding RNAs approximately 22 base pairs in length which act as post-transcriptional regulators of expression in eukaryotic cells (Romaine et al., 2016). MiRs typically exert their action through binding to complimentary sequences on the 3'untranslatted region of the target messenger RNA (mRNA), thereby inducing either mRNA degradation or repression of translation (Romaine et al., 2015). MiRs are involved in several processes as apoptosis, cell differentiation and proliferation, and cell cycle. MiRs are present in tissues and also exist in body fluids such as plasma, serum and urine in stable forms resistant to degradation by endogenous RNase (Sayed et al., 2014). The cardiovascular system as a whole is affected by circulating miRs, and aberrant levels have been associated with hypertension (Li et al., 2011), acute myocardial infarction (Wang et al., 2010), heart failure (Tijsen et al., 2010) and stroke (Tan et al., 2009). It follows that miRs have potential roles in prognosis of diseases.

Associations between circulating miRs and essential hypertension have been reported (Li et al., 2011; Karolina et al., 2012; Yang et al., 2014). A study on 13 hypertensive patients and 5 control subjects identified 27 miRs differentially expressed in patients with hypertension. The most notable of these include upregulation of Hcmv-miR-UL122, let-7e, miR-516b, miR-600, miR-605 and miR-623. Among these, miR-605 and mir-623 showed 3- and 1-fold upregulation in hypertensives, respectively (Li et al., 2011).

There is limited information on gene targets of miR-605 and miR-623. MiR-605 has been shown to be a part of the tumor suppressor p53 pathway, and has a marked effect on timing of apoptosis by upregulation of p53 (Zhou et al., 2014). MiR-605 is also a modifier gene of the Li-Fraumeni syndrome phenotype, where its overexpression causes a decrease in cell proliferation, clonogenicity and reduced migration in

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rhabdomyosarcoma cell lines with mutations in the TP53 gene (Said and Malkin, 2015). Mir-605 expression was also upregulated in blood of patients with stroke (Yuan et al., 2016). MiR-605 expression was downregulated in fetal single ventricle malformation. A predicted target gene of miR-605 is FOXP1, which plays a critical role in cardiac development (Yu et al., 2012). Another target gene of upregulated miR-605 is *SDC4*. *SDC4* encodes the protein syndecan-4, a heparan sulfate proteoglycan whose over-production is associated with elevated body mass index (BMI), coronary heart disease and blood pressure fluctuation (Rose et al., 2015). MiR-623 expression is positively correlated with smoking history, a predisposing factor to hypertension, as well as lymphatic metastasis, and it can function as a tumor suppressor, particularly in lung adenocarcinoma (Wei et al., 2016). MiR-623 expression is also upregulated during T cell intracellular antigen protein depletion in HeLa cells. This causes a decrease in nitric oxide (NO) production by endothelial NO synthase, which could have negative impacts on the cardiovascular system (Ibiza et al., 2006). Nitric oxide has many vital functions, including regulation of regional blood flow and vascular tone, modulation of thrombosis and suppression of smooth muscle cell proliferation in vascular tissue (Ibiza et al., 2006). MiR-623 is overexpressed in patients showing symptoms of Fabry disease. Transcriptomic analysis showed that miR-623 targets the NOG gene which encodes for noggin, a protein involved in repair and development of cardiac tissue (Pasqualim et al., 2017).

The aim of this study is to investigate serum miR-605 and miR-623 expression in patients with essential hypertension, as well as correlate plasma expression of both miRs with clinical markers of essential hypertension such as systolic and diastolic blood pressure, BMI and serum triglyceride and cholesterol levels.

2. Materials and methods

2.1. Study subjects

Subjects enrolled in the case-control study were from the North Sinai Area, Egypt. Fifty subjects were enrolled in the study, 25 with essential hypertension and 25 normotensive controls. A standard mercury sphygmomanometer was used for blood pressure (BP) measurement for all subjects. BP was measured when subjects were in a sitting position after at least 10 min rest. The mean value of at least 2 measurements was taken for each subject. Criteria for hypertension were diastolic BP greater than or equal to 90 mm Hg and/or systolic blood pressure greater than or equal to 140 mm Hg and/or if the subject had been taking anti-hypertensive medication. Any subjects displaying evidence of secondary hypertension were not included. Body mass index (BMI) was calculated as weight (kg)/height (m^2). Subjects with BMI $\geq 30\,kg/m^2$ were classified as obese. All study subjects provided written informed consent. The Research Ethics Committee of the Faculty of Pharmacy, Suez Canal University approved the study protocol. The study protocol conformed with the principles of the 2013 Helsinki Declaration.

2.2. Sample collection

Whole blood was collected from all study subjects. Serum was isolated by centrifugation for 10 min at 1200 $\times g$ at 4 °C. Supernatant was gathered into sterile tubes and re-centrifuged at 4000 $\times g$ for 10 min to remove remaining cells. Sera were partitioned into two aliquots and stored at −80 °C. One aliquot was used for RNA isolation. The other aliquot was used for lipid analysis. Total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C) and triglycerides (TG) were measured by an automated chemical analyzer A15 (Biosystem, USA). Low density lipoprotein-cholesterol (LDL-C) was calculated by the Friedwald's formula. Very low density lipoprotein-cholesterol (VLDL-C) was calculated as 0.2 × total TG concentration expressed in mg/dL (Friedwald et al., 1972).

2.3. RNA isolation

Total RNA was extracted from serum with the Qiagen miRNeasy Mini kit (Qiagen, Hilden, Germany), as per manufacturer instructions. RNA quality and concentration were spectrophotometrically determined with a NanoDrop 1000 spectrophotometer (Thermo scientific, USA).

2.4. Quantitative real time polymerase chain reaction (qRT-PCR)

MicroRNA (miR) expression was measured using qRT-PCR. Serum samples were diluted in RNase-free water before use to allow equal amounts of RNA in the reverse transcriptase (RT) reactions. RNA was reverse transcribed to complimentary DNA (cDNA) with the miScript RT Kit (Qiagen, Germany) in a 20 μL final volume using oligo-dT primers. The RT reaction consisted of 4 μL 5× miScript HiSpec buffer, 2 μL miScript reverse transcriptase mix, 2 μL 10× miScript nucleics mix, 5 μL (10–20 ng) RNA, and 10 μL RNase-free water. RT reactions were carried out at 37 °C for 60 min, then 95 °C for 5 min to inactivate reverse transcriptase. All reactions were cooled and stored at −20 °C till use.

Quantitative PCR was done in a PikoReal-Time PCR machine (Applied Biosystems, USA), using the miScript SYBR Green PCR Kit (Qiagen, Germany). All cDNA samples were diluted ten times with RNase-free water to provide 20 ng of cDNA in each reaction. qPCR reactions were performed in a 20 μL final volume and consisted of 10 μL QuantiTect SYBR Green PCR master mix, 1 μL cDNA, 2 μL 10× miScript primer assay, 2 μL 10× miScript Universal primer, and 5 μL RNase-free water. Cycling conditions were 95 °C for 15 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 30 s and 70 °C for 30 s. All reactions were run in duplicate. A no-template control was run for all samples. The internal control for normalization was U6. Primers used for amplification were: miR-605 5′-AATCCCATGGTGCC-3′, miR-623 5′-TCCCTTGCAGGGG-3′, and U6 5′-GCTTCGGCAGCACATATACTAAAAT-3′. Primer specificity was assessed by melt curve analysis.

Expression of miRs was calculated by the comparative cycle threshold (ΔCt) method. Mean Ct values were calculated for samples. Ct values of miR U6 were subtracted from Ct values of the target miR to obtain the ΔCt value. Relative expression of miRs was assessed by the standard $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

2.5. Statistical analysis

Patient and control data were expressed as mean ± standard deviation. Statistical comparison of quantitative variables between 2 groups was by independent *t*-test. Differences in relative expression of miRs were determined by the one way analysis of variance test (ANOVA). Bivariate correlation of relative expression of miRs with serum lipid parameters was assessed with Pearson's correlation coefficient. A p value < 0.05 was considered as statistically significant. Data was processed using the SPSS 17.0 software package (SPSS, Chicago, IL, USA).

3. Results and discussion

Essential hypertension is a growing public health risk and its incidence is increasing worldwide. MicroRNAs have been shown to influence development of hypertension either directly or indirectly. In this study, the association between expression of miR-605 and miR-623 and presence of essential hypertension was studied.

Fifty subjects, 25 with essential hypertension and 25 healthy controls, were recruited for the study. General characteristics of the study population are shown in Table 1. There was no difference in age, BMI, and smoking among the two groups. Hypertensive patients had significantly higher systolic and diastolic blood pressure. Hypertensive patients also had an aberrant lipid profile, with higher serum triglycerides (TG), total cholesterol (TC), VLDL-C, LDL-C, and lower HDL-C