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Serum microRNA expression in pregnancies with preeclampsia

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ABSTRACT. Preeclampsia continues to be a mortal disease of pregnant women throughout the world. Recently, geneticists, allied with obstetricians, have opened new frontiers. MicroRNAs (miRNAs) are members of a class of small, noncoding RNA molecules. They are critical posttranscriptional regulators of gene expression. We extracted circulating miRNA from maternal plasma and quantified mir-152 and mir-210. We found up-regulated miR-210 levels as well as down-regulated mir-152 levels in preeclampsia patients.We propose that detection of increased mir-210 levels in maternal serum could be used to improve prediction methods for noninvasive prenatal diagnosis of preeclampsia.

Key words: MicroRNA; Preeclampsia; Non-invasive prenatal diagnosis

INTRODUCTION

Preeclampsia is a potentially dangerous disorder specific to the second half of pregnancy, complicating 2.5-3% of pregnancies (Redman and Sargent, 2005). It is the most frequent pregnancy-associated disorder (Smets et al., 2006) and still constitutes a leading cause of maternal and perinatal mortality and morbidity (Mütze et al., 2008). Although the onsets of clinical symptoms are late, systemic and maternal, the origin of preeclampsia is early, localized and placental (Smets et al., 2006). Currently, testing for this disorder includes the use of protein markers in plasma or serum (Smets et al., 2006; Carty et al., 2008). However, they lack sufficient discrimination (Tjoa et al., 2003; Qiu et al., 2004). There are still weeks of delay in diagnosis after the initiation of placental pathology (22-24 weeks).

Identification of placental products rather than cells in the maternal circulation has taken a drastic turn for the better by the observation that cell-free DNA and RNA originating from the placenta circulates in a protected form in the maternal blood and can be easily obtained from the maternal plasma (Ng et al., 2003; Tsui et al., 2004). The essential change in feasibility and potential of using fetal DNA and RNA has triggered the pursuit of non-invasive prenatal diagnosis, including presymptomatic detection of pregnancy-associated diseases such as preeclampsia (Smets et al., 2006). Investigations have focused on which fetal RNA and DNA markers or their modifications can be screened in maternal plasma, for the purpose of presymptomatic detection of pregnant women at risk for preeclampsia.

miRNAs are short (19-25 nucleotides), single-stranded, and nonprotein-coding RNAs (Lee and Ambros, 2001; Lagos-Quintana et al., 2001) that regulate gene expression by binding to the 3' untranslated region of the target mRNAs (Lai, 2002). They have important roles in diverse biological processes, such as development (Krichevsky et al., 2003), differentiation (Esau et al., 2004), apoptosis (Baehrecke, 2003), and oncogenesis (Michael et al., 2003; Calin et al., 2005).

Recent studies on microRNAs (miRNAs) offer possibilities for developing a novel class of fetal nucleic acid markers in maternal plasma. An intriguing possibility is that these small molecules are taken up by cells exposed to the maternal circulation and may modulate gene expression of the maternal compartment (Chim et al., 2008).

By considering these results, we hypothesized that the concentrations of placental miRNA in maternal plasma may also show differences between preeclamptic and non-preeclamptic pregnant women, according to gene expression differences. Recent studies has indicated that it would be useful to investigate whether the aberrant concentrations of miRNAs in placentas involved in preeclampsia are reflected in maternal plasma (Pineles et al., 2007; Chim et al., 2008); however, miRNA expression differences have been investigated in preeclamptic placentas (Pineles et al., 2007). Here, we showed that miRNas are in a body fluid and represent new biomarkers.

MATERIAL AND METHODS

The ethics committee of Istanbul University, Cerrahpaşa Medical Faculty endorsed the study design (protocol number 2535-18/2009).

Sample collection

Among the patients in the maternity ward at Istanbul University Cerrahpaşa Medical Faculty and Istanbul Medical Faculty, Department of Obstetrics and Gynecology pregnant women who carried fetuses from 26 to 40 weeks of gestation were selected for study. Plasma samples were obtained from a total of 40 women in two groups; 20 being preeclamptic and 20 being healthy pregnancies, forming the control group. None of these subjects had undergone an invasive procedure. A volume of 5 mL maternal venous blood was drawn and collected in an ethylenediamine tetraacetic acid (EDTA) tube. The blood samples were centrifuged initially at 1200 *g* for 10 min and a second time at 10,000 *g* for 10 min, and 500 µL from the supernatant layer were used. The supernatant layer of the plasma was taken to Istanbul University, Department of Molecular Biology and Genetics, Molecular Diagnosis Laboratory for storage at -80°C until the time of study.

Total RNA extraction

Total RNA was isolated from plasma using Trizol (Invitrogen, Carlsbad, CA) following the manufacturer protocol. Total RNA was analyzed using the commercially available Bioanalyzer Agilent RNA 6000 picoassay.

MicroRNA isolation from maternal plasma

MicroRNAs were obtained with the High-Specificity miRNA QRT-PCR detection kit (Stratagene-an Agilent Technologies Company, USA-Canada) according to manufacturer recommendations. Micro RNAs were subjected to a polyadenylation reaction as previously described. Next, using an oligo dT primer harboring a consensus sequence, reverse transcription was performed using an Affinity Script RT/RNase Block Enzyme mixture (Stratagene). miRNA was anayzed using the Bioanalyzer 2100- Agilent Small RNA assay.

QRT-PCR platform

The cDNA was amplified by real-time PCR. The real-time PCR analysis was performed on a Stratagene Mx3005P. This reaction contained a microRNA-specific forward primer, a TaqMan probe complementary to the 3' of the specific microRNA sequence, as well as part of the polyA adaptor sequence, and a universal reverse primer complementary to the consensus 3' sequence of the oligodT tail.

RESULTS

Total RNA was analyzed using the commercially available Bioanalyzer Agilent RNA 6000 picoassay. Forty subjects were enrolled in this study and grouped as target and control, each group having an equal number of individuals.

Total RNA extraction from maternal plasma was successful in both preeclamptic (Figure 1A) and control groups (Figure 1B) miRNA from maternal plasma samples from healthy pregnant women (Figure 2A) and preeclamptic maternalplasma samples (Figure 2b) was analyzed. The very low-abundant miRNA fraction was clearly detected and its concentration

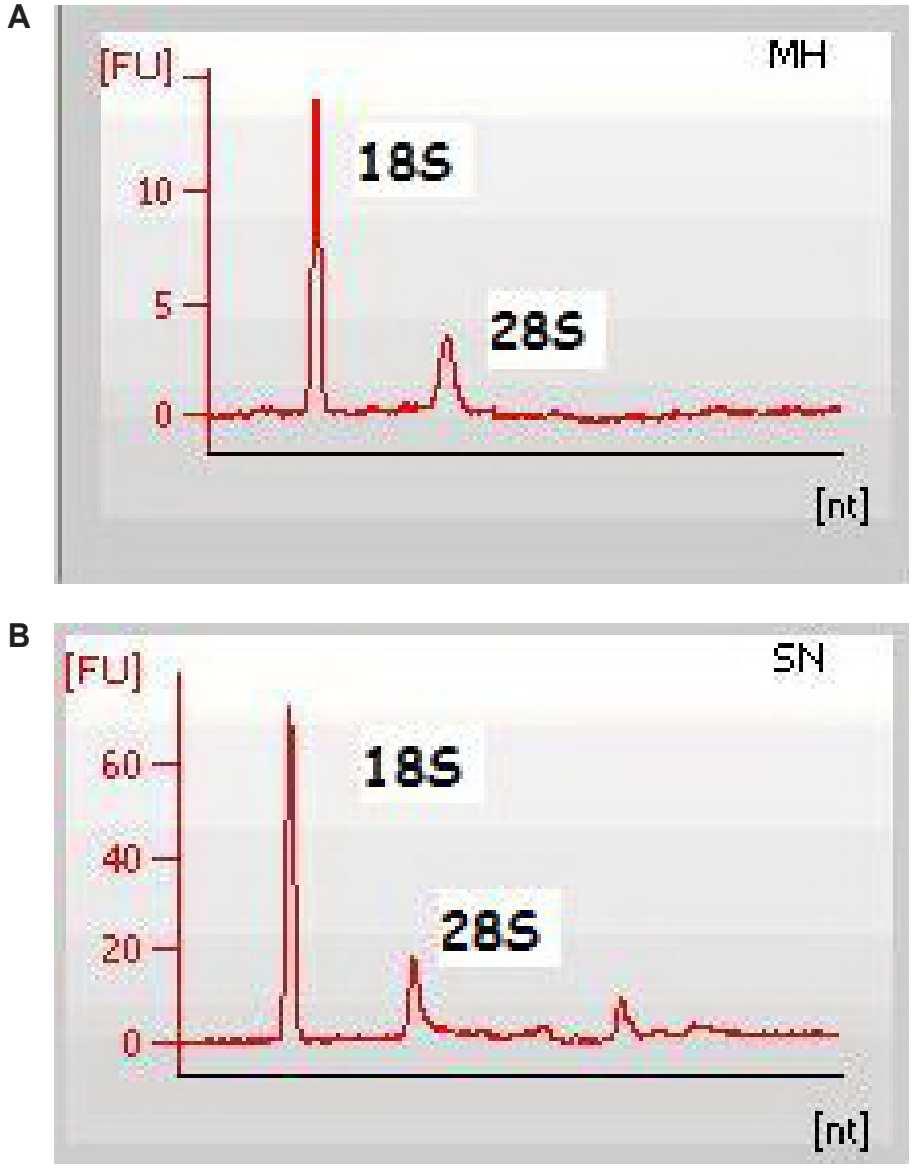

Figure 1. A. Bioanalyzer results for microRNAs obtained from maternalplasma of healthy pregnant women. **B.** Bioanalyzer results for microRNAs obtained from maternalplasma of preeclamptic pregnant women.

determined by the sofware.

In the preeclamptic group, mir-RNAs showed increased expression, where they represented 25% of total RNA concentration (Figure 2B).

We also examined the levels of two microRNAs in plasma samples. We found miR-210 levels to be more than 2-fold higher than miR-153 levels in preeclamptic pregnant women.

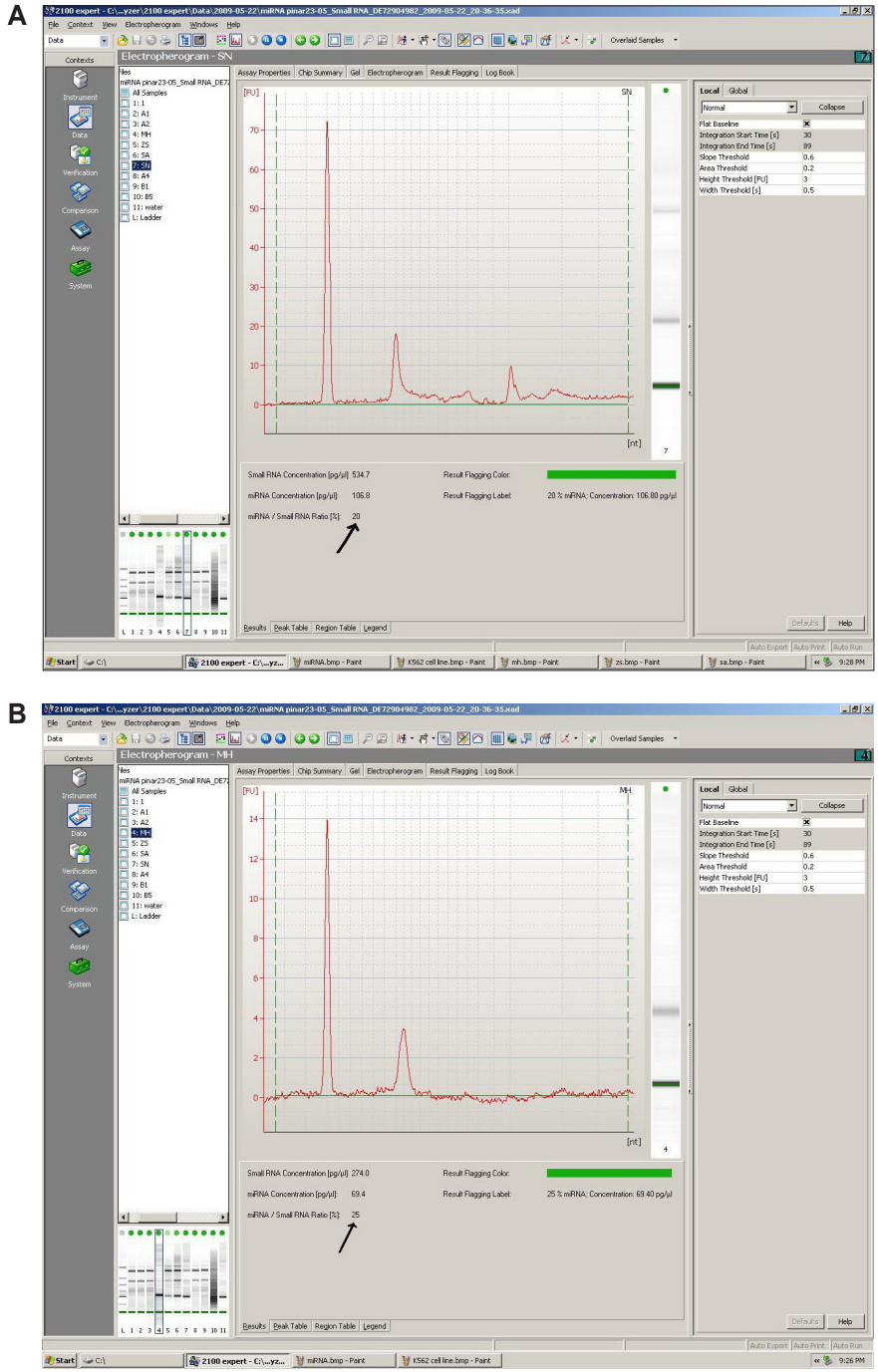

Figure 2. A. Bioanalyzer results for microRNAs obtained from maternal plasma of healthy pregnant women. **B.** Bioanalyzer results for microRNAs obtained from maternal plasma of preeclamptic patients.

DISCUSSION

Preeclampsia, defined as high blood pressure, proteinuria and edema in pregnancy, is one of the leading causes of maternal mortality, preceding hemorrhage and abortion (WHO, 2005).

The main characteristics of the disease prompt important clues on its pathogenesis. Most studies focus on the placenta due to sudden resolution of symptoms after placental birth. Impaired trophoblastic invasion, placental hypoxic degeneration and apoptosis have been shown by many studies, defining preeclampsia as a placental pathology (Hahn et al., 2006). Over the past decades, scientists have intensified studies on the prediction of the disease in order to diminish the mortality and morbidity of preeclampsia. Numerous methods have been tried in order to predict the disease; however, the most currently used method is the notching of uterine arteries (Chien et al., 2000).

Non-invasive and efficient prediction of preeclampsia gained acceleration by the detection of cell-free nucleic acids in maternal blood. The discovery of increased cell-free fetal DNA in maternal blood by Lo et al. (1997) proved that preeclampsia can be predicted in advance, long before the disease is clinically apparent. However, the main limitation of this technique was the restriction to preeclamptic patients carrying male fetuses.

With the discovery of microRNAs, genetic mechanisms of placental pathologies could be thoroughly understood. MicroRNAs are non-coding RNAs that regulate gene expression and play crucial roles in pathological processes. They are regulated and transcribed like protein coding genes (Gilad et al, 2008).

In 2007, Pineless et al. published a study on differential expression of miRNAsin placentas of preeclamptic patients. They screened 157 miRNAs, of which 153 were detected in the placental tissue. The expression of mir-210 and mir-182 was increased in placentas of preeclamptic patients. The elevation was more apparent for mir-210 compared to mir-182. The expression of mir-210 was localized to the syncytiotrophoblast, and the target gene was found to be responsible for increased immune response, apoptosis and lipid metabolism.

In the light of this information, we set forth to show an increase of mir-210 levels in maternal plasma in order to improve a prediction method for noninvasive prenatal diagnosis of preeclampsia. We hypothesized that since the expression of mir-210 is increased in preeclamptic placenta, its maternal shedding should increase proportionally. In addition to mir-210, we studied another microRNA with known functions in cytolysis and immune response, but it has not yet been studied in preeclampsia. We preferred mir-152, which represses HLA-G expression and induces NK cell-mediated cytolysis, causing increased immune response (Prieto and Markert, 2011). Our study on the expression differences of miRNAs from maternal plasma using quantitative real-time PCR revealed that mir-210 expression is elevated in preeclamptic patients and can readily be detected prenatally without the need for invasive procedures. However, in our study, mir-152 was not overexpressed in preeclamptic subjects, although it was readily extracted from control patients. This finding was interpreted as the exaggerated immune response seen in preeclampsia being of maternal origin, rather than fetal. We believe our study is encouraging in that it is a noninvasive and efficient method for prediction of preeclampsia, which also elucidates the underlying pathogenesis and origin of preeclampsia. In the search for novel biomarkers for preeclampsia, microRNAs are promising with the development of genetics.

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