Assessment of HCMV-encoded microRNAs in Plasma as Potential Biomarkers in Pregnant Women with Adverse Pregnancy Outcomes

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Research

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Abstract

Background: Human cytomegalovirus (HCMV) is the most frequent cause of congenital infections and can lead to adverse pregnancy outcomes (APO). HCMV encodes multiple microRNAs (miRNAs) that have been reported to be partially related to host immune responses, cell cycle regulation, viral replication and viral latency, and can be detected in human plasma. However, the relevance of HCMV-encoded miRNAs in maternal plasma as an indicator for APO has never been evaluated.

Methods: The expression profiles of 25 HCMV-encoded miRNAs were first measured in plasma samples from 20 pregnant women with APO and 28 normal controls by quantitative reverse-transcription polymerase chain reaction (RT-qPCR) technology. Next, markedly changed miRNAs were validated in another independent validation set consisting of 20 pregnant women with APO and 27 control subjects. HCMV DNA in peripheral blood leukocytes (PBLs) and anti-HCMV immunoglobulin M (IgM) and anti-HCMV immunoglobulin G (IgG) in plasma were also examined in both the training and validation sets. Diagnostic value and risk factors were compared between adverse pregnancy outcome cohorts and normal controls.

Results: The analysis of training and validation data sets revealed that plasma concentrations of hcmv-miR-UL148D, hcmv-miR-US25-1-5p and hcmv-miR-US5-1 were obviously increased in pregnant women with APO compared with normal controls. Hcmv-miR-US25-1-5p presented the largest area under the receiver-operating characteristic (ROC) curve (0.735; 95% CI, 0.635–0.836), with a sensitivity of 68% and specificity of 71%. Furthermore, the plasma levels of hcmv-miR-US25-1-5p and hcmv-miR-US5-1 were obviously positively correlated with APO (P = 0.029 and 0.035, respectively). Nevertheless, neither the concentration of HCMV DNA in PBLs nor the positivity rates of anti-HCMV IgM and IgG in plasma showed statistically significant correlation with APO.

Conclusion: We identified a unique signature of HCMV-encoded miRNAs in pregnant women with APO, which may be useful as a potential noninvasive biomarker for predicting and monitoring APO during HCMV infection.

Background

Fetal structural anomaly (FSA) and spontaneous abortion (SA) are two important types of APO. In the clinically recognized pregnancy, the incidence of FSA is about 3% [1, 2], and the cumulative risk of SA is about 11–20% [3, 4]. Although various types of genetic variations can cause FSA and SA, HCMV is one of the most important nonhereditary cause of APO [5–8]. By mother–child transmission, nearly 30%-40% of primary HCMV infected pregnant women had congenital infected fetus [9, 10]. In the United States, 4–5% of live births have congenital HCMV infection [11]. Among them, approximately 10%-15% have birth defects [12]. To date, there is no evidence that immune pregnant women had lower congenital HCMV infections risk[13]. Furthermore, despite HCMV DNA positive pregnant women showed 3-fold greater chance of congenital infection, there is no positive correlation between HCMV DNA positive and APO [14]. Therefore, identification of non-invasive predictors of APO for pregnant women with HCMV infection is an important gap in our knowledge.

MiRNAs are a type of ~ 22-nt noncoding RNA nucleotides that play a critical role in various biological processes [15]. Previous studies have revealed that a variety of miRNAs derived from the host itself and other species are highly stable in plasma and serum and may serve as noninvasive biomarkers for various diseases [16–21]. In addition, small noncoding RNAs can be transmitted through the mammalian placenta and directly regulate fetal gene expression [22].

HCMV encodes at least 26 mature miRNAs during its infectious life cycle [23]. The HCMV miRNAs can target both host and viral genes to regulate various aspects of cellular and viral biology, such as host immune responses [24–26], cell cycle regulation [27], viral replication [28], and viral latency [29–32]. HCMV-encoded miRNAs have been confirmed to exist in plasma, and they are associate with hypertensive, chronic hepatitis B and oral lichen planus [33–35]. However, the plasma signature of HCMV-encoded miRNAs in pregnant women with APO and its clinical relevance have not been studied.

We postulated that there is a unique plasma profile of HCMV-encoded miRNAs in pregnant women with APO, and used RT-qPCR assays to test this hypothesis. Furthermore, we evaluated the diagnostic potential of the significantly altered HCMV miRNAs in the plasma as a noninvasive predictor of APO for pregnant women with HCMV infection.

Methods

Patient characteristics and clinical features

A total of 95 pregnant women were enrolled from the First, Second and Third Affiliated Hospitals of Qiqihar Medical University and Jianhua Hospital (Qiqihar, China). Normal or APO were determined based on clinical examinations and laboratory assessments. As shown in Table 1, 40 pregnant women with APO and 55 pregnant women with normal pregnancy participated in our study. All blood samples were collected in EDTA tubes, and centrifuged at room temperature at 3000 × g for 10 min. The supernatant (plasma) and the blood cells were stored separately at − 80 °C until analysis.

Table 1
Demographic and clinical features of pregnant women with APO and controls in the present study

Variable	Training set			Validation set			HCMV titers set		
	APO	Controls	P value	APO	Controls	P value	APO	Controls	P value
Number	20	28		20	27		40	55	
Age, years[a]	27 ± 5.04	29 ± 6.24	0.28[b]	28 ± 3.87	29 ± 5.76	0.47[b]	27 ± 4.49	29 ± 5.95	0.20[b]
Type of Adverse pregnancy, n									
Fetal structural abnormalities	13(65%)			12(60%)			25(62.5%)		
spontaneous abortion	7(35%)			8(40%)			15(37.5%)		
Anti-HCMV IgM, n									
Positive	1(5%)	0(0%)	0.42[c]	0(0%)	0(0%)	> 0.99[c]	0(0%)	1(1.8%)	> 0.99[c]
Negative	19(95%)	28(100%)		20(100%)	27(100%)		40(100%)	54(98.2%)	
Anti-HCMV IgG, n									
Positive	19(95%)	27(96%)	0.63[c]	20(100%)	27(100%)	> 0.99[c]	38(95.0%)	54(98.2%)	0.57[c]
Negative	1(5%)	1(4%)		0(0%)	0(0%)		2(5.0%)	1(1.8%)	
HBV, n									
Positive	0(0%)	0(0%)	> 0.99[c]	0(0%)	0(0%)	> 0.99[c]	0(0%)	0(0%)	> 0.99[c]
Negative	20(100%)	28(100%)		20(100%)	27(100%)		40(100%)	55(100%)	
HIV, n									
Positive	0(0%)	0(0%)	> 0.99[c]	0(0%)	0(0%)	> 0.99[c]	0(0%)	0(0%)	> 0.99[c]
Negative	20(100%)	28(100%)		20(100%)	27(100%)		40(100%)	55(100%)	
Syphilis, n									
Positive	0(0%)	0(0%)	> 0.99[c]	0(0%)	0(0%)	> 0.99[c]	0(0%)	0(0%)	> 0.99[c]
Negative	20(100%)	28(100%)		20(100%)	27(100%)		40(100%)	55(100%)	
a Age data are presented as the mean ± SD									
b Student t test									
c Two-sided $\chi 2$ test									

RNA Isolation and RT-qPCR

Total RNA was extracted from the plasma using phenol/chloroform purification protocol. Briefly, 100 μL of plasma was diluted with 300 μL of ribonuclease-free water and 200 μL of acid phenol (Sigma), and then 20 μL of synthetic MIR2911 was added. After the mixture was vortex-mixed vigorously, 200 μL of chloroform was added. The sample was incubated at room temperature for 15 minutes and then centrifuged at 16,000 × g for 20 minutes. The aqueous supernatant was transferred to a fresh 1.5 mL microcentrifuge tube and mixed with 2 volumes of isopropyl alcohol and 1/10-volume of 3 M sodium acetate (pH 5.3). Next, the mixture was stored at - 20 °C for 2 h and then centrifuged at 16,000 × g for 20 minutes. Subsequently, the RNA pellet was collected, washed with 75% ethanol and dried for 10 minutes at room temperature. Finally, the pellet was dissolved in 30 μL of ribonuclease-free water and stored at - 80°C until further analysis.

We performed a TaqMan probe-based RT-qPCR assay (Additional file 1: Table S1) to investigate the differential expression of HCMV-encoded miRNAs between normal and APO groups as described previously [33, 34]. The RT-qPCR assays were performed in triplicate using an ABI 7300 instrument (Applied Biosystems, Foster City, CA). Expression levels of miRNAs were calculated using the CT values. We assessed the detection limits of the RT-qPCR assay by performing calibration curves developed with synthetic miR-16 oligonucleotides (Additional file 2: Fig. S1). All reactions were performed in triplicate.

Human Cytomegalovirus Titers

To investigate the copy numbers of HCMV in peripheral blood leukocytes (PBLs), RT-qPCR was performed on samples from 40 pregnant women with APO and 55 pregnant women with normal pregnancy. DNA was extracted from PBLs using a Blood Genomic DNA Midi Kit (CWBIO, China) according to the manufacturer's protocols. We amplified HCMV DNA by TaqMan real-time PCR with the following HCMV specific primers: HCMV DNA forward: 5′-CACGGTCCCGGTTTAGCA-3′, HCMV DNA reverse: 5′-CGTAACGTGGACCTGACGTTT-3′. A tenfold diluted recombinant plasmid containing the HCMV target sequence was used as a template for standard curve preparation (Additional file 2: Fig. S2). The procedure consisted of 45 cycles of denaturation at 95 °C for 15 seconds, annealing at 60 °C for 30 seconds and extension at 72 °C for 30 seconds. Plasmid DNA containing the HCMV target sequence was used in separate reactions serving as a positive control. Results were expressed as copies per 1 mL blood.

Enzyme-Linked Immunosorbent Assay

Anti-HCMV IgG and IgM antibodies were detected with a commercially available ELISA kit (MEDSON, NJ, USA) according to the manufacturer's instructions. For the IgG-ELISA, a value of < 0.5 IU/ml was considered a negative result, and a value of > 0.5 IU/ml was considered a positive result, indicating prior exposure to HCMV. For the IgM-ELISA, the test results were calculated using the optical density (OD) value at 450 nm, and the cut-off value for positivity was OD > 1.2.

Statistical Analysis

Clinical characteristics between the two study cohorts were statistically analyzed using a Student's t-test and χ^2 test (Table 1). Data from the RT-qPCR assays were also statistically analyzed using a Student's t-test. The results are presented as the mean ± SEM for miRNAs and the mean ± SD for other variables. Univariate logistic regression analyses were performed to analyze the associations between plasma HCMV-encoded miRNAs and APO. A *P* value of < 0.05 was considered statistically significant. All statistical analyses were performed using GraphPad Prism 7.0 software (GraphPad Software, USA).

Results

Expression profiles of HCMV-encoded miRNAs in plasma from pregnant women

The experimental design of this study is shown in Fig. 1. We enrolled 95 pregnant women to participate in our study, and the samples were randomly divided into a training set and a validation set. In the training set, we detected 25 HCMV-encoded miRNAs in plasma from 20 pregnant women with APO and 28 normal controls by TaqMan probe-based RT-qPCR. Our data showed that the plasma of all pregnant women contained a specific level of the examined HCMV-encoded miRNAs. As shown in Table 2, 5 out of 25 HCMV-encoded miRNAs, including hcmv-miR-US25-1-5p, hcmv-miR-UL148D, hcmv-miR-US33-3p, hcmv-miR-US5-1 and hcmv-miR-UL59, were expressed at higher levels in plasma from pregnant women with APO than in that from normal controls ($P < 0.05$). These results indicate that the altered levels of plasma HCMV-encoded miRNAs may be specific to pregnant women with APO.

Table 2
Expression profile of HCMV-encoded miRNAs in pregnant women with APO and controls in the training set

HCMV encoded miRNAs	APO (n = 20) Mean ± SEM	Controls (n = 28) Mean ± SEM	Fold change	*P* value[a]
hcmv-miR-UL148D	6.12 ± 0.89	3.54 ± 0.31	1.73	0.003
hcmv-miR-US25-1-5p	0.17 ± 0.02	0.10 ± 0.01	1.75	0.003
hcmv-miR-US33-3p	0.11 ± 0.02	0.06 ± 0.01	1.76	0.018
hcmv-miR-US5-1	3.70 ± 0.55	2.32 ± 0.27	1.59	0.019
hcmv-miR-UL59	1.26 ± 0.14	0.95 ± 0.08	1.33	0.048
hcmv-miR-UL112-3p	5.82 ± 1.01	4.03 ± 0.43	1.44	0.078
hcmv-miR-US4-3p	1.30 ± 0.31	0.80 ± 0.13	1.63	0.105
hcmv-miR-US29-5p	20.58 ± 2.42	16.77 ± 1.63	1.23	0.181
hcmv-miR-UL36-3p	0.44 ± 0.12	0.28 ± 0.06	1.59	0.183
hcmv-miR-US29-3p	0.21 ± 0.06	0.13 ± 0.03	1.54	0.197
hcmv-miR-US25-1-3p	0.83 ± 0.31	0.47 ± 0.12	1.74	0.24
hcmv-miR-UL22A-5p	1.03 ± 0.22	0.76 ± 0.13	1.36	0.259
hcmv-miR-US25-2-5p	3.73 ± 1.14	2.53 ± 0.54	1.47	0.304
hcmv-miR-UL69	1.52 ± 0.59	0.88 ± 0.32	1.73	0.304
hcmv-miR-US33-5p	17.33 ± 6.67	11.97 ± 2.35	1.45	0.397
hcmv-miR-UL112-5p	1.17 ± 0.50	0.78 ± 0.16	1.5	0.401
hcmv-miR-US22-3p	0.97 ± 0.22	0.80 ± 0.11	1.22	0.439
hcmv-miR-US5-2-3p	0.10 ± 0.02	0.08 ± 0.01	1.24	0.446
hcmv-miR-US22-5p	39.84 ± 8.33	33.44 ± 6.52	1.19	0.543
hcmv-miR-UL70-5p	0.86 ± 0.28	1.09 ± 0.26	0.79	0.56
hcmv-miR-UL22A-3p	0.19 ± 0.07	0.16 ± 0.04	1.21	0.651
hcmv-miR-US4-5p	0.46 ± 0.11	0.40 ± 0.09	1.14	0.692
hcmv-miR-UL36-5p	16.21 ± 1.54	17.00 ± 1.44	0.95	0.713
hcmv-miR-US5-2-5p	5.92 ± 1.71	5.23 ± 1.03	1.13	0.72
hcmv-miR-US25-2-3p	8.21 ± 1.90	7.99 ± 0.88	1.03	0.908

Data are presented as the mean ± SEM

[a] Student t test

Further validation of HCMV-encoded miRNAs in the plasma from pregnant women by RT-qPCR

Subsequently, we confirmed the 5 altered HCMV miRNAs in an additional independent sample set (validation set) consisting of 20 pregnant women with APO and 27 normal subjects (Fig. 2). As shown in Fig. 2a-c and Additional file 1: Table S2, pregnant women with APO had higher plasma levels of hcmv-miR-US25-1-5p, hcmv-miR-US5-1 and hcmv-miR-UL148D than normal controls in the validation set. However, the plasma levels of hcmv-miR-US33-3p and hcmv-miR-UL59 showed no significant difference between pregnant women with APO and normal controls (Fig. 2d-e and Additional file 1: Table S2). The observed differences in the 3 identified miRNAs between 40 pregnant women with APO and 55 control individuals are illustrated in Fig. 3a-c and Additional file 1: Table S3.

Plasmas expression levels of the 3 identified HCMV-encoded miRNAs in different types of APO

FSA and SA are common categories of APO. These APO are devastating for couples, and most FSA and SA are unexplained. We analyzed the altered expression of the above 3 HCMV miRNAs in pregnant women with different types of APO (FSA (n = 25) and SA (n = 15)) who were enrolled in this study. The results showed that hcmv-miR-UL148D and hcmv-miR-US25-1-5p were only elevated in pregnant women with FSA, whereas hcmv-miR-US5-1 was significantly upregulated in pregnant women with both FSA and SA. Additionally, the plasma level of hcmv-miR-US25-1-5p was much higher in pregnant women with FSA than in pregnant women with SA (Fig. 3d-f and Additional file 1: Table S4).

Diagnostic value of the 3 altered HCMV-encoded miRNAs as a predictor of APO for pregnant women with HCMV infection

To assess the diagnostic accuracy of the 3 plasma HCMV miRNAs for APO, we performed ROC curve analysis. The area under the ROC curve (AUC) of the 3 altered miRNAs (hcmv-miR-UL148D, hcmv-miR-US25-1-5p and hcmv-miR-US5-1) was 0.689, 0.735 and 0.688, respectively (Additional file 1: Table S5). Among them, hcmv-miR-US25-1-5p presented the largest AUC (0.735; 95% CI, 0.635–0.836), with a sensitivity of 68% and specificity of 71%. Although we also investigated the diagnostic value of the combinations of the 3 selected miRNAs, the AUC of all the combinations was lower than that of hcmv-miR-US25-1-5p alone (Additional file 1: Table S5). Our data indicate that plasma hcmv-miR-US25-1-5p may be a potential predictor of APO for pregnant women with HCMV infection.

Increased plasma levels of HCMV-encoded miRNAs in pregnant women correlate with APO

To further evaluate the risk prediction power of the three increased HCMV miRNAs in plasma from pregnant women, we used APO as the dependent variable and the risk score as the covariate to conduct a univariate logistic regression analysis. Hcmv-miR-US25-1-5p and hcmv-miR-US5-1 were independently correlated with APO (Additional file 1: Table S6). The odds ratios (ORs) of these 2 HCMV miRNAs for pregnant women with APO were as follows: hcmv-miR-US25-1-5p (OR = 26500.657, 95% CI 2.848–246567602.202, $P = 0.029$) and hcmv-miR-US5-1 (OR = 1.406, 95% CI 1.024–1.931, $P = 0.035$). The results suggest that the elevated plasma levels of hcmv-miR-US25-1-5p and hcmv-miR-US5-1 are potential risk factors for APO.

Detection of human cytomegalovirus in pregnant women

Because the expression of the 3 HCMV miRNAs were obviously increased in the plasma from pregnant women with APO, we investigated the discrepancy in HCMV load between pregnant women with APO and normal controls. A previous study reported that HCMV DNA in PBLs was more sensitive for diagnosing HCMV infection than that in plasma [36]. Therefore, we used RT-qPCR to detect HCMV DNA in PBLs from both the training and validation sets. Nevertheless, we found no significant differences in HCMV DNA levels between pregnant women with APO and normal controls, with HCMV virus titers of (33.46 – 2307) ± 678.9 copies/mL in APO and (9.08 – 6370) ± 1088.0 copies/mL in normal controls ($P = 0.60$) (Additional file 2: Fig. S3a). We further examined the concentrations of anti-HCMV IgM and anti-HCMV IgG in plasma from all 40 pregnant women with APO and 55 control individuals by ELISA. Our results indicated that there was no significant difference in the positivity rates of anti-HCMV IgM and anti-HCMV IgG between the two study cohorts (0% versus 1.8%, $P >$

0.99; 95.0% versus 98.2%, *P* = 0.57, respectively) (Table 1) or in the plasma concentrations of anti-HCMV IgG (Additional file 2: Fig. S3b).

Discussion

Although various types of genetic variations can cause FSA and SA, congenital HCMV infection is one of the most important nongenetic causes for APO. However, there are no reliable qualitative or quantitative methods for early prediction of APO due to HCMV infection in pregnant women. This study for the first time examined the relationship between HCMV-encoded miRNAs in maternal plasma and APO. Furthermore, our results indicated that plasma hcmv-miR-US25-1-5p may be a noninvasive biomarker of APO for pregnant women with HCMV infection.

Our study identified that 3 out of the 25 HCMV-encoded miRNAs, including hcmv-miR-US25-1-5p, hcmv-miR-US5-1 and hcmv-miR-UL148D, were markedly increased in pregnant women with APO. More importantly, we observed a significant difference in the concentrations of detected miRNAs among pregnant women in different types of APO. Compared with the normal controls, the 3 identified HCMV miRNAs in plasma were all obviously increased in pregnant women with FSA, whereas only hcmv-miR-US5-1 was significantly upregulated in pregnant women with SA. Most notably, pregnant women with FSA had higher hcmv-miR-US25-1-5p levels than those with SA. These findings suggest that the increased expression of these three HCMV miRNAs may have a greater impact on FSA. Among the 3 miRNAs, hcmv-miR-US25-1-5p and hcmv-miR-US5-1 were significantly related to APO, suggesting that these miRNAs are potential risk factors for APO. Hcmv-miR-US25-1-5p presented the largest AUC (0.735; 95% CI, 0.635–0.836), indicate that plasma hcmv-miR-US25-1-5p may be a potential predictor of APO for pregnant women with HCMV infection.

In the present study, we tested HCMV DNA in PBLs from our studied pregnant women, there was no significant difference in HCMV DNA between pregnant women with APO and normal controls. HCMV DNA in maternal blood and/or serological investigation have become key indicators for the diagnosis of primary infection [36]. However, the correlation between HCMV DNA and congenital infection remains controversial [14, 36–38]. Additionally, HCMV DNA is not correlated with APO [14]. It is consistent with our finding. Our results showed that more than 90% of the pregnant women in both study cohorts were anti-HCMV IgG positive, and no significant difference in the positive rates of anti-HCMV IgG or IgM. There is also no strong evidence that antiviral antibody responses provide protection against maternal infection, intrauterine transmission, and APO [39]. Currently, predictors of APO in pregnant women with HCMV infection are not well defined, and solving this problem has important clinical value. Our results showed that HCMV-encoded miRNAs could distinguish pregnant women with APO from normal controls.

The prognosis only can be determined until late in pregnancy if fetus has no ultrasound features or severe ultrasound abnormalities [40, 41]. Sometimes invasive operations are required to obtain fetal blood for some prognostic parameters' measurement [42–44]. Therefore, the use of plasma HCMV miRNAs as a noninvasive predictor of APO for pregnant women with HCMV infection have great clinical significance.

Our study identified the three altered HCMV-encoded miRNAs in pregnant women. Based on the fact that exogenous/endogenous small noncoding RNA in the maternal system can be transferred through the mammalian placenta and influence fetal development and health by directly regulating fetal gene expression [22], whether the three HCMV-encoded miRNAs can influence fetal development are promising field for future study.

The validated downstream target genes for hcmv-miR-UL148D include IER5, ACVR1B, RANTES, and IEX-1 [23, 29, 45, 46], for hcmv-miR-US25-1-5p include CD147, cyclin E2, BRCC3, YWHAE, UBB, NPM1, and HSP90AA [27, 47–49], and for hcmv-miR-US5-1 include GMNN, IKKα, IKKβ, and US7 [50–53]. These validated target genes can be divided into four categories: viral latency (IER5, ACVR1B, and RANTES), viral replication (YWHAE, UBB, NPM1, HSP90AA, GMNN, and US7), immune evasion (ACVR1B, RANTES, IKKα, and IKKβ), and cell processes (IEX-1, CD147, cyclin E2, BRCC3). The downregulation of these genes by HCMV-encoded miRNAs may interfere with hosts' immune responses and cell cycle, as well as leading to

abnormalities in embryonic development. The above results indicate that these identified HCMV-encoded miRNAs in our study may contribute to the pathogenesis of HCMV infection-related diseases as well as in APO, but further research is needed.

The strengths of our study are that the HCMV-encoded miRNAs in plasma are safe, stable and specific biomarker candidates for APO monitoring and prognosis. Moreover, the required testing costs are relatively low. The factors leading to APO of pregnant women are complex, thus it is necessary to further exclude the known confounding factors that affect the results of the maternal cohort study. This study only compared the two major types of APO (FSA and SA) with normal pregnant, it may require more detailed classification and larger sample size for prospective studies. Although we have clarified the relationship between plasma HCMV-encoded miRNAs and APO in pregnant women, the function of these identified HCMV miRNAs on intrauterine transmission and the pathogenic mechanism have not yet been clarified. Therefore, further careful design and implementation of prospective studies will be required.

Conclusions

In summary, we first report a distinctive plasma signature of HCMV-encoded miRNAs in pregnant women with APO and that plasma levels of hcmv-miR-US25-1-5p and hcmv-miR-US5-1 in pregnant women are associated with APO. Furthermore, hcmv-miR-US25-1 may be one of the noninvasive biomarkers for predicting APO in pregnant women during HCMV infection. These findings indicate that HCMV-encoded miRNAs may provide pathophysiological clues for the development of APO and warrant further study.

Abbreviations

HCMV: Human-cytomegalovirus; MiRNAs: MicroRNAs; RT-qPCR: Quantitative reverse-transcription polymerase chain reaction; PBLs: Peripheral blood leukocytes; IgM: Immunoglobulin M; IgG: Immunoglobulin G; FSA: Fetal structural anomaly; SA: Spontaneous abortion; APO: Adverse pregnancy outcome; ROC: Receiver-operating characteristic; AUC: area under the ROC curve; ORs: Odds ratios.

Declarations

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Not applicable.

Authors' contributions

Study design: XC; CW; CZ and XZ. Acquisition of data: ZG. Analysis and interpretation of data: ZG; LZ; JB and MD. Collection of patient samples: ZG; DL; SZ; YL; XL; XW; MJ and HS. Drafting the article: ZG and LZ. Revising manuscript content: CW and XC. Study supervision: XC; CW; CZ and XZ. All authors read and approved the final manuscript.

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Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon request.

Ethics approval and consent to participate

This study was approved by the Qiqihar Medical University Ethics Committee (project code 19). All participants signed a consent form prior to providing a blood sample.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

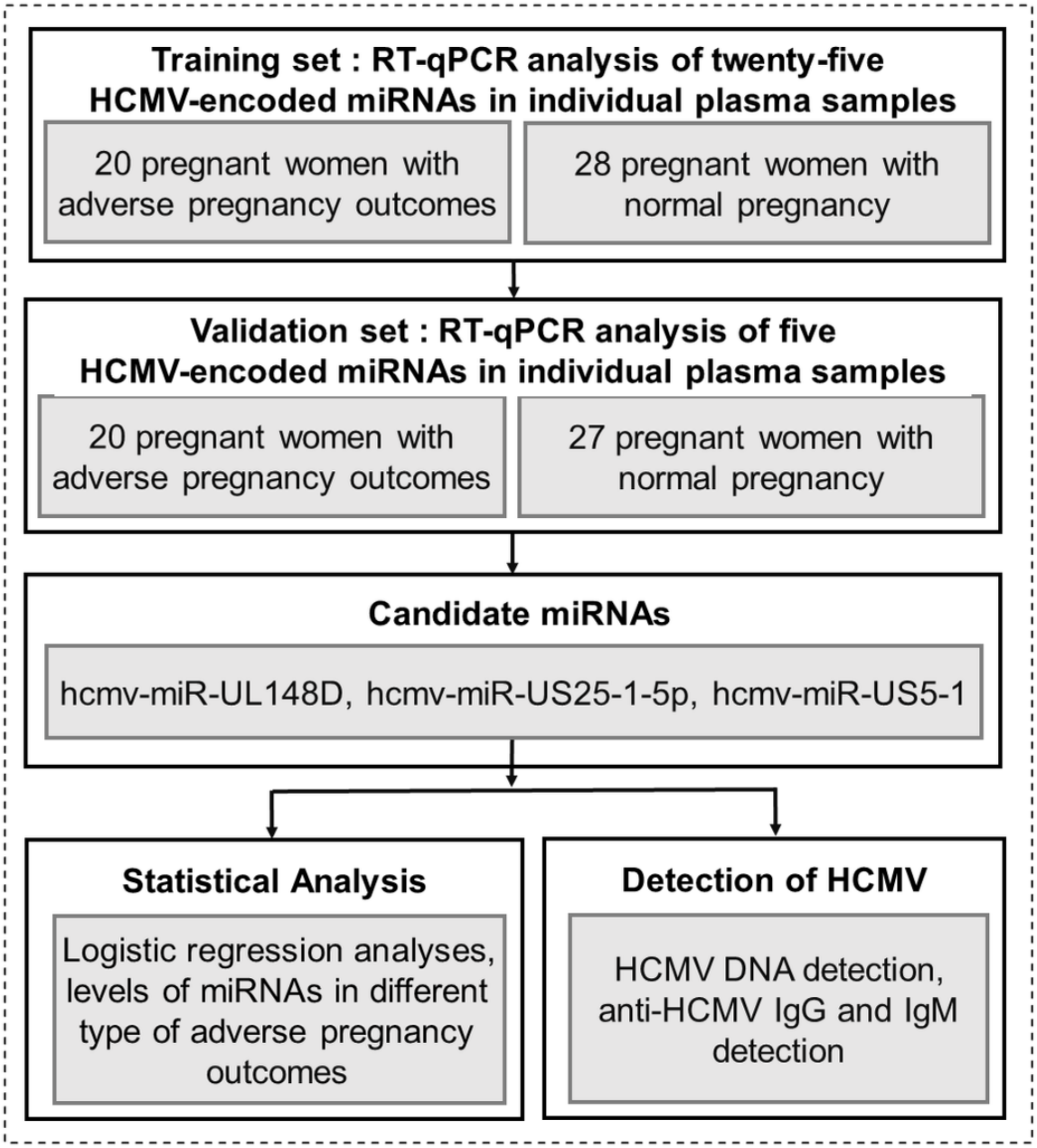

Figure 1

Overview of the experimental design.

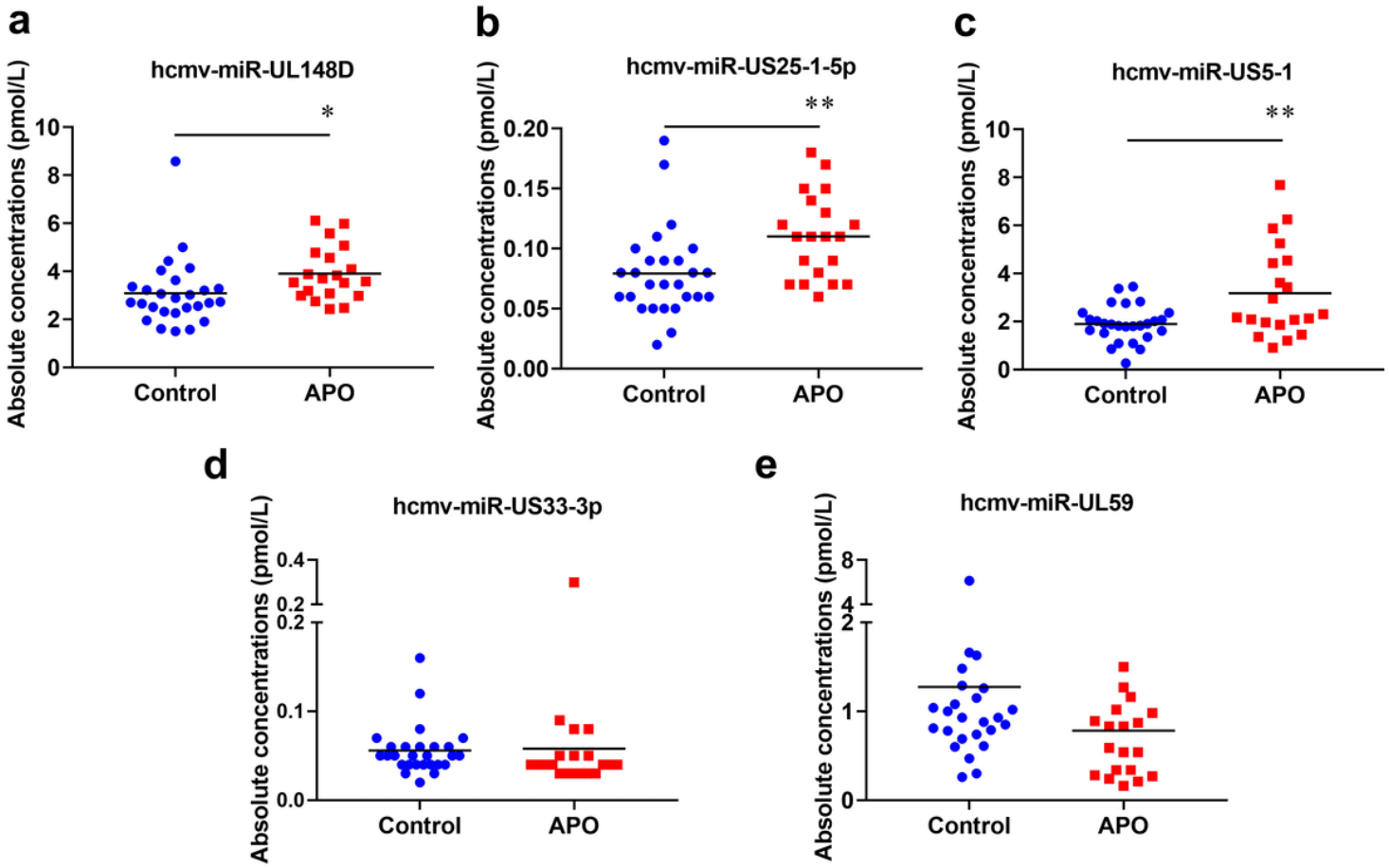

Figure 2

Elevated plasma levels of HCMV-encoded miRNAs in pregnant women with APO in the validation set. a–e The concentrations of 5 HCMV-encoded miRNAs were measured in 20 pregnant women with APO and 27 normal controls using a RT-qPCR assay. Cq values were converted to absolute values based on the standard curve. Each P value was derived from a 2-sided Student's t test. *P < 0.05; **P < 0.01.

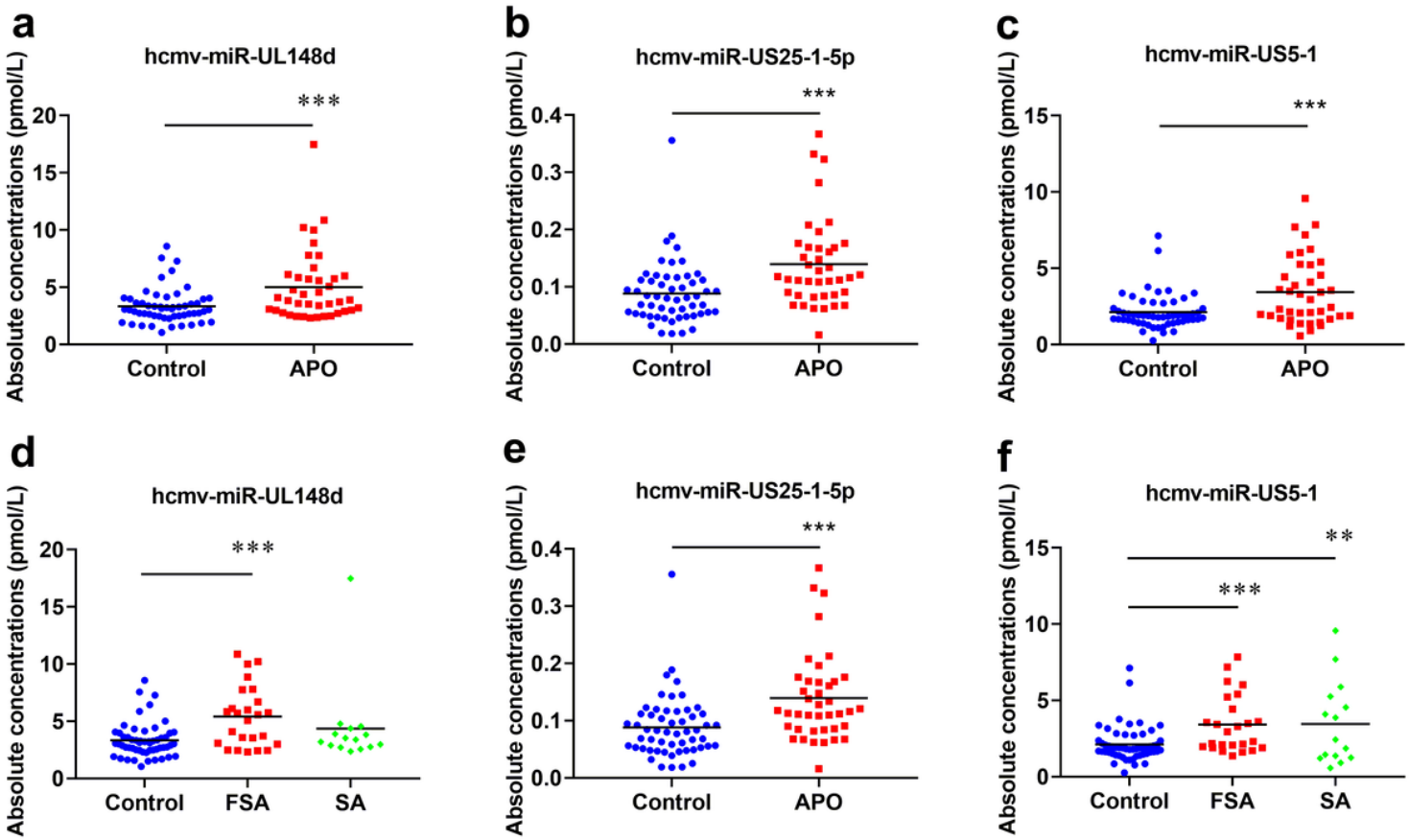

Figure 3

Plasma levels of 3 HCMV-encoded miRNAs upregulated in pregnant women with different types of APO. a–c In the training and validation sets, the concentrations of 3 HCMV-encoded miRNAs were measured in 40 pregnant women with APO and 55 normal controls using RT-qPCR assays. Each P value was derived from a 2-sided Student's t test. d–e The concentrations of 3 upregulated HCMV-encoded miRNAs were measured in 25 women with fetal structural anomalies, 15 women with spontaneous abortions and 55 normal controls using RT-qPCR. Each P value was derived from ANOVA. Cq values were converted to absolute values based on the standard curve. **P < 0.01; ***P < 0.001.

Supplementary Files

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