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Classification and Regression Tree Model for the Differential Diagnosis of Preeclampsia Based on Clinicopathological Features and miR Signatures

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Abstract

Preeclampsia (PE) is a pregnancy complication characterized by the onset of high blood pressure after 20 weeks of gestation with proteinuria and abnormal liver enzymes. The early diagnosis and prophylactic use of aspirin can reduce the long-term complications of PE. In the current study, we utilized machine learning tools for the differential diagnosis of EOPE and LOPE based on demographic, clinical, and biochemical data. We employed SYBR green-based real-time PCR to study the differential expression of hsa-miR-4743-5p, miR-149-5p, miR-331-5p, and miR-483-5p in both forms of PE. A classification and regression tree (CART) model was developed to differentiate between EOPE and LOPE. This was achieved by determining thresholds of systolic blood pressure (SBP), Diastolic Blood Pressure (DBP), Mean Arterial Pressure (MAP), Body Mass Index (BMI), urine protein, and SGOT. The RT-PCR-based DEM profile identified an association of miR-4743-5p with both forms of PE; miR-149-5p with EOPE, and miR-331-5p and miR-483-5p with LOPE. MiRDip analysis revealed that genes targeted by these miRs influence TGF beta signaling in EOPE; cholesterol and lipid homeostasis and NOTCH2 signaling in LOPE. In conclusion, SBP, MAP, BMI, urine protein, DBP, and SGOT are key determinants of EOPE and LOPE. The DEM profile clearly distinguished EOPE and LOPE.

Keywords: *Classification and Regression Tree Model, Machine Learning, MIRS, Preeclampsia.*

Introduction

Preeclampsia is a pregnancy complication characterized by the onset of high blood pressure after 20 weeks of gestation with proteinuria, and abnormal liver enzymes. This condition increases the risk of morbidity and mortality in both the mother and the fetus. Long-term complications of preeclampsia can be reduced by early diagnosis followed by the use of aspirin as a prophylactic measure throughout pregnancy.

Yang et al. reported 2.9% and 2.3% prevalence of preeclampsia in the Swedish and Chinese populations, respectively [1]. Obesity and nulliparity were strongly associated with PE in both populations [1]. Tyrmi et al reported 19 genome-wide associations with PE which regulate blood pressure traits, placental development, uterine spiral artery remodeling,

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renal function, and proteostasis maintenance in pregnancy serum [2]. Muldoon et al. observed that prophylactic use of aspirin is not beneficial in reducing the recurrence among individuals with twin pregnancies, previous history of preeclampsia, or hypertension [3]. Hercus et al. have shown that new paternity and increasing birth and pregnancy intervals were associated with increased risk of PE in multiparous women [4]. Stitterich et al identified family history of PE, hypertension, high mid-upper arm circumflex, urinary tract infection/diarrhea during pregnancy, low socioeconomic status, inadequate fruit intake, and unhygienic conditions as risk factors for PE among Africans [5]. Dai et al demonstrated an association of in vitro fertilization and growth discordance with PE in dichorionic twins [6]. Fox et al. observed the association of egg donation and pre-pregnancy obesity with PE risk [7]. Jaatinen et al have shown an increased risk of PE in women with type 1 diabetes, chronic hypertension, dyslipidemia, early menarche, depression, and subfertility [8].

Weitzner et al. have shown that second-trimester maternal markers can distinguish early-onset PE (EOPE) from late-onset PE (LOPE), specifically alpha-fetoprotein (AFP) and unconjugated estriol (UE3) showing higher multiple of the medians in EOPE than LOPE [9].

Suksai et al. proposed a multivariate risk-scoring model for PE with age, BMI, number of fetuses, history of PE, adverse prenatal outcomes, interval between pregnancies, parity, presence or absence of renal disease, hypertension, autoimmune disease, diabetes, and mean arterial pressure (MAP) as predictors [10]. This model showed an AUC of 0.77 in predicting PE and the need for aspirin prophylaxis [10].

Choorakuttil et al used an integrated first-trimester ultrasound assessment of PE at 11 to 14 weeks of gestation with mean arterial blood pressure and mean uterine artery pulsatility index (UTPi), which showed 90.4% sensitivity, 98.1% negative predictive value, 16.7-fold odds ratio, and 6.64-folds likelihood ratio for preterm PE [11]. Chaemsaithong et al observed higher multiples of the median (MoM) for MAP, UTPi, and lower MoM for a mean placental growth factor (PlGF) in women with preterm preeclampsia [12].

In the current study, we have used machine learning tools for the differential diagnosis of EOPE and LOPE based on demographic, clinical, and biochemical data. In a few representative samples, we have studied the expression of four microRNAs in EOPE and LOPE to verify their utility in the differential diagnosis and to evaluate the role of miR-targeted genes in explaining the disease pathophysiology of both forms of PE.

Materials and Methods

Recruitment of Subjects

In a multicentric study, 99 women (33 healthy pregnant women, 33 EOPE women, and 33 LOPE women) in the Department of Obstetrics and Gynecology, Saveetha Institute of Medical and Technical Sciences, Chennai, Fernandez hospitals and and ESIC hospitals Hyderabad between January 2020 and March 2022. All participants consented to the study. The Institutional ethics committees of Saveetha, ESI (008/09/2019/IEC/SMCH: ESIC-ESICMC/SNR/IEC-S101/12-2020) and Fernandez hospital (Fernandez-EC Reference No. 32 2020) approved the study protocol. The study complies with the Declaration of Helsinki.

Documenting Demographic, Clinical, and Biochemical Data

We have collected demographic details such as age, height, weight, and gestational age. The blood pressure was monitored in both arms. Mean arterial blood pressure was calculated using the following formula:

$$\text{MAP} = 1/3\ (\text{Systolic blood pressure}) + 2/3\ (\text{Diastolic blood pressure}).$$

A battery of routine tests specifically SGOT, SGPT, urine protein, and urine creatinine was performed on all the subjects. Heart rate and respiratory rate were also monitored. Women with persistent hypertension, gestational diabetes, autoimmune disorders, and other inflammatory disorders were excluded.

Real-Time PCR for Differential Expression of miRs

We collected whole blood samples in special microRNA Pax tubes and stored them at -20°C until processing. We have used SYBR green-based real-time PCR to study the differential expression of hsa-miR-4743-5p, hsa-miR-149-5p, hsa-miR-483-5p, and hsa-331-5p miRs in EOPE and LOPE. miRDip module was used to identify genes targeted by these miRs. STRING database was used to explore protein-protein interactions.

Classification and Regression Tree Model for Predicting EOPE/LOPE

We have used machine learning tools to generate a classification and regression tree (CART) model. The input variables were age, height, weight (body mass index), systolic blood pressure (SBP), diastolic blood pressure (DBP), Mean arterial pressure (MAP), urine protein, urine creatinine, SGOT and SGPT.The output variables are controls, EOPE, and LOPE. The model identifies the most significant variable and determines its threshold that distinguishes one group from another at each step. The tree's apex was the most significant variable, while the branches are other variables at different levels of significance in establishing the differential diagnosis.

Statistical Analysis

We used the Student t-test to compare the distribution of continuous variables between two groups. ANOVA was used since there were more than two groups. Pearson correlation coefficient was used to establish the correlation between the two given variables. Logistic regression was used for multivariate analysis to assess the contribution of many variables towards EOPE and LOPE.

Results

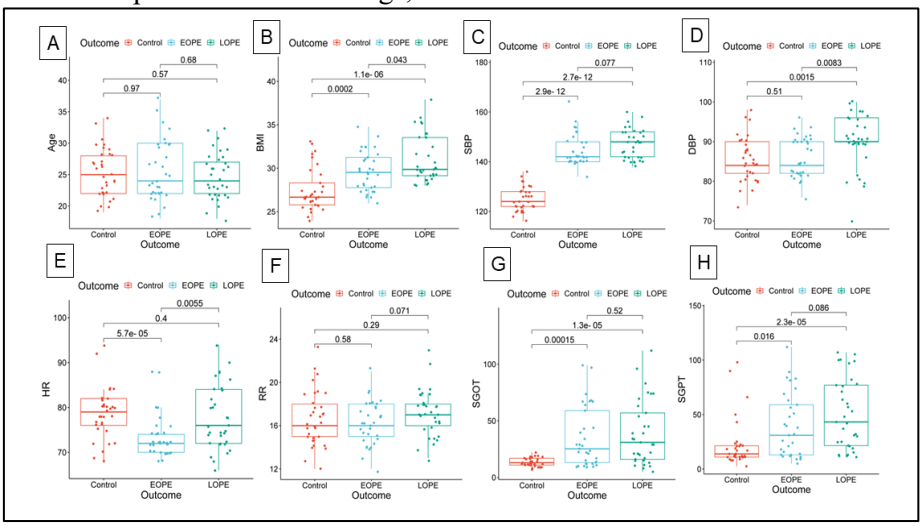

Figure 1. Distribution of demographic, clinical, and biochemical variables in EOPE and LOPE. (A) Age showed no significant association with EOPE and LOPE. (B) Body mass index (BMI) is higher in LOPE followed by EOPE than the controls. (C) Systolic blood pressure (SBP) is higher in LOPE and EOPE than in the controls. (D) Diastolic blood pressure (DBP) is higher in LOPE than controls. No such association with EOPE. (E) Heart rate (HR) in EOPE is lower than that of controls. (F) Respiratory rate (RR) has no significant association with EOPE and LOPE. (G)SGOT levels are higher in LOPE followed by EOPE. (H) SGPT levels are higher in LOPE followed by EOPE.

As shown in Figure 1, age showed no statistically significant association with either EOPE or LOPE. LOPE cases exhibited higher body mass index (31.03 ± 2.71 kg/m^2,

p=0.000001) followed by EOPE (29.62±2.28 Kg/m^2, p=0.0002) than the controls (27.47 ±2.45 Kg/m^2).BP is elevated in LOPE (146.97 ± 6.00 mmHg, p =2.7 x 10^{-12}) and EOPE (144.27 ±6.73 mmHg, p=2.9 x 10^{-12}) when compared to controls (124.69 ±4.86 mmHg). DBP is elevated only in LOPE (90.18 ± 6.84 mmHg, p=0.0015), but not EOPE (86.30 ±5.12 mmHg) in comparison to controls (85.52 ±5.66 mmHg). MAP levels are higher in LOPE (109.17 ±5.35 mmHg, p= 5.8 x 10^{-9}) followed by EOPE (105.63 ±3.29 mmHg, p=4.5 x 10^{-8}) than the controls (98.63 ±4.61 mmHg).

Heart rate z(HR) is indistinguishable between LOPE and controls (77.70 ±7.10 vs. 78.70 ±5.65), however, heart rate is lowest in EOPE (74.06 ±4.75, p= 5.7 x 10^{-5}). Respiratory rate (RR) showed no significant association with EOPE/LOPE.

SGOT levels are higher in both LOPE (38.98 ± 28.19 U/L, p=1.3 x 10^{-5}) and EOPE (35.14 ± 26.57 U/L, p=0.00015) than the controls (13.52 ± 3.81 U/L). SGPT levels are also higher in both LOPE (50.81 ± 32.37 U/L, p=2.3 x 10^{-5}) and EOPE (41.38 ± 29.44 U/L, p=0.016) than the controls (22.58 ± 22.40 U/L).

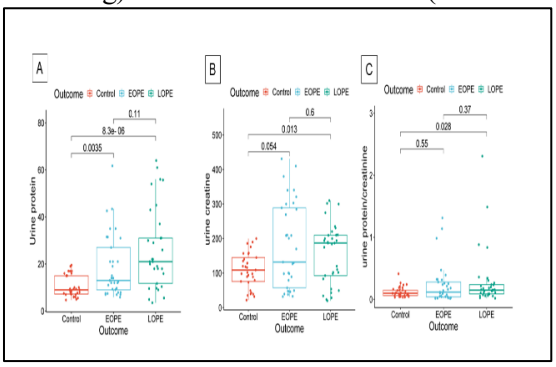

Figure 2. Association of urinary biomarkers with EOPE and LOPE. (A) Urine protein levels are elevated in LOPE followed by EOPE. (B) Urine creatine levels are higher in LOPE than controls. (C) The urine protein/creatinine ratio is higher in LOPE than in controls.

As shown in Figure 2, Higher urine protein levels were observed in LOPE cases (25.98 ± 17.34 mg/dl, p=8.3 x 10^{-6}) followed by EOPE (18.84 ± 13.43 mg/dl, p=0.0035) than the controls (10.20 ± 4.41 mg/dl). Urine creatine is elevated in LOPE (156.82 ± 86.45 mg/dl, p=0.013) and EOPE (174.77 ± 125.62 mg/dl, p=0.054) than the controls (108.54 ± 49.86 mg/dl). The urine protein/creatinine ratio is elevated in LOPE (0.30 ± 0.47, p=0.028) than in controls (0.12 ± 0.08), but not in EOPE (0.24 ± 0.32, p=0.55).

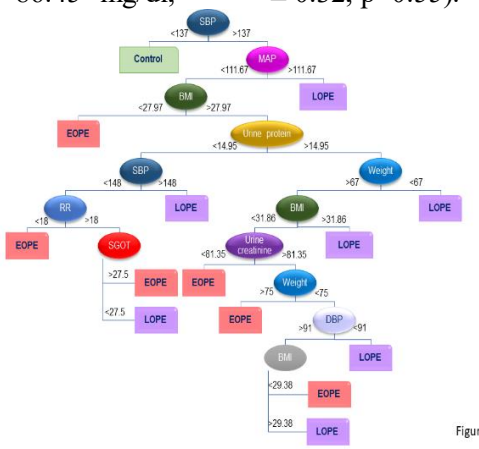

Figure 3. Classification and regression tree (CART) model for the differential diagnosis of preeclampsia. Systolic blood pressure (SBP), mean arterial pressure (MAP), body mass index (BMI), urine protein, SGOT and diastolic blood pressure (DBP) are the key determinants whose thresholds were established for the differential diagnosis of preeclampsia.

As shown in Figure 3, SBP emerged as the most significant determinant of preeclampsia. All the control women had SBP<137 mmHg while all women with PE had SBP >137 mmHg.

MAP>111.67 mmHg is associated with LOPE. Women with MAP<111.67 mmHg and BMI <27.97 Kg/m2 had EOPE. Women with MAP<111.67 mmHg, urine protein >14.95 mg/dl, and BMI >31.86 Kg/m2 had LOPE. In women with BMI<31.86 Kg/m2, urine creatinine >81.35 mg/m2 and DBP <91 mmHg is associated with LOPE. DBP>91 mmHg contributes to EOPE in women with BMI<29.38 while BMI>29.38 leads to LOPE. In women with urine protein <14.95mg/dl, SBP>148 mmHg was associated with LOPE. If SBP<148 mmHg, RR>18 and SGOT>27.5 contribute to EOPE. This model showed 100% accuracy in distinguishing controls, EOPE and LOPE.

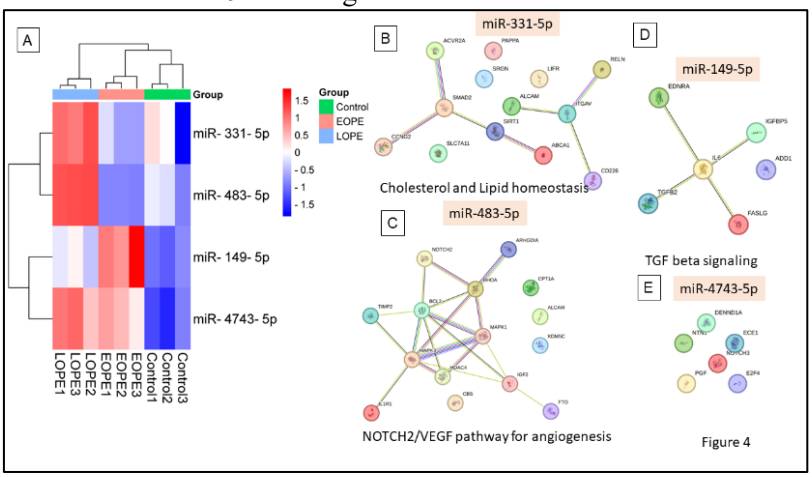

Figure 4. Differential expression of miRs and its target genes in EOPE and LOPE. (A) Heat map analysis of bidirectional clustering analysis of differential expression of miRs indicates the association of miR-331-5p, miR-483-5p with LOPE; miR-149-5p with EOPE; and miR-4743-5p with EOPE and LOPE. (B) STRING analysis reveals that miR-331-5p affects cholesterol and lipid homeostasis. (C) STRING analysis reveals that miR-483-5p affects NOTCH2/VEGF pathway for angiogenesis (D)STRING analysis reveals that miR-149-5p affects TGF beta signaling. (E) STRING analysis reveals that miR-4743-5p targets PGF, which one of the key biomarker in both EOPE and LOPE.

As shown in Figure 4, Clustering-based heat map analysis of RT-PCR data revealed upregulation of miR-149-5p in EOPE. Two miRs i.e. miR-331-5p and miR-483-5p showed upregulation in LOPE. miR-4743-5p was upregulated in both EOPE and LOPE. miRdip analysis revealed that miR-149-5p targets EDNRA, TFGB2, IL6, IGFBP5, FASLG and ADD1 genes and hence likely to influence TGF beta signaling. miR-331-5p targets ABCA1, SIRT1, SMAD2, ACVR2A, CCNG2, SLC7A11, SRGN, PAPPA, ALCAM, ITGAV, RELN, CD226, SRGN, and LIFR and hence likely to affect cholesterol and lipid homeostasis. miR-483-5p targets ALCAM, ARHGDIA, BCL2, CBS, CPT1A, FTO, HDAC4, IGF2, IL1R1, KDM5C, MAPK1, MAPK3, NOTCH2, RHOA, and TIMP2 and hence likely to affect NOTCH2/VEGF pathway for angiogenesis. miR-4743-5p targets DENND1A, ECE1, NOTCH3, E2F4, NTN1, and PGF.

Discussion

This study reported an association of BMI, SBP, DBP, HR, SGOT, SGPT, urine protein, and urine protein/creatinine ratio with PE. Among these variables, BMI, DBP, and HR are distinct between EOPE and LOPE. A CART model was developed for the differential diagnosis of PE, which identified SBP, MAP, BMI, urine protein, DBP, and SGOT as the key variables with decreased order of significance. This model is clinically actionable with high accuracy. RT-PCR-based DEM profile identified an association of miR-149-5p with EOPE; miR-331-5p and miR-483-5p with LOPE; and miR-4743-5p with both EOPE and LOPE. miRDip analysis revealed that genes targeted by these miRs influence TGF beta signaling in EOPE; cholesterol and lipid

homeostasis and NOTCH2/VEGF pathway for angiogenesis in LOPE. PAPPA and PLGF, the most important biochemical markers used for preeclampsia screening are targeted by miR-331-5p and miR-4743-5p, respectively.

A machine learning model by Maric et al for early prediction of preeclampsia also demonstrated preexisting hypertension, history of preeclampsia, MAP, and obesity as the key contributors of preeclampsia similar to our model [13]. Another machine learning model by Melinte-Popescu et al showed higher BMI, personal history of PE/hypertension, higher MAP, higher UTPi, lower levels of PAPP-A and PP-13, and higher levels of PLGF as the key determinants of PE [14]. PLGF and PP-13 levels were reported to be higher in EOPE compared to LOPE [14].

There are limited studies on the association of miR-149-5p with preeclampsia. Zhao et al demonstrated lower levels of miR-149-5p in LOPE [15]. Wang et al have shown that miR-149-5p mitigates the endothelial cell injury caused by oxidized low-density lipoprotein by inhibiting PAPP-A [16]. Our results corroborate with Mayor-Lynn et al in demonstrating the upregulation of miR-483-5p with LOPE [17]. To the best of our knowledge, there are no direct studies on the association of miR-331-5p with preeclampsia. However, it is reported to influence pathological remodeling of arteries through PPARγ-mediated inhibition of TGFB1-induced mitochondrial activation and vascular smooth muscle proliferation [18].

The genes targeted by miR-149-5p are related to TGF-β- signaling that was reported to play a pivotal role in human placental development by governing the differentiation of extravillous trophoblasts [19]. The association of two miRs in LOPE with cholesterol and lipid homeostasis by Antonic et al study showed an altered lipid profile in LOPE [20]. NOTCH2 and NOTCH3 mediate invasion and migration of trophoblasts thus playing an important role in the pathogenesis of PE [21].

The major strengths of our study were: i) evaluation of demographic, clinical, and biochemical characteristics of EOPE and LOPE and establishing differential diagnostic thresholds using machine learning tools; ii) exploring the distinct DEM profiles in EOPE and LOPE and correlating with aberrant pathways. Future studies are warranted by including first-trimester maternal screening markers specifically PAPP-A, PLGF, and UTPi along with a detailed follow-up throughout pregnancy to establish the interrelationships of these markers with clinicopathological variables and miRs.

To conclude, our study demonstrated the risk factors associated with EOPE and LOPE using well-documented cases and established differential diagnostic thresholds for these factors using a machine learning approach. miR-149-5p upregulated in EOPE whereas miR-483-5p and miR-331-5p were upregulated in LOPE. miR-4743-5p was upregulated both in EOPE and LOPE.

Statements and Declarations

Funding

No specific funding was obtained for this study.

Compliance with Ethical Standards

Disclosure of Potential Conflicts of Interest

All authors declare no conflicts of interest.

Research Involving Human Participants

The Institutional ethics committees of SIMATS, (008/09/2019/IEC/SMCH): ESI (ESIC-ESICMC/SNR/IEC-S101/12-2020) and Fernandez hospital (Fernandez-EC Reference No. 32 2020) approved the study protocol. The study complies with the Declaration of Helsinki.

Consent to Participate

Informed consent was obtained from all the study participants.

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