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# Neutrophil Activation and Maternal Plasma Exosomal miR-301b-3p Decrease in Patients with Preeclampsia

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**Abstract:** Background: To evaluate the diagnostic value of circulating plasma exosomal miR-301b-3p and neutrophils ($NE^{\#}$) for preeclampsia (PE). Methods: The study cohort consisted of pregnant women with Hypertensive disorders of pregnancy (HDP) who were selected from Guangdong Women and Children Hospital between January 2017 and November 2017. A total of 80 cases with gestational hypertension (GH n=31) and preeclampsia (PE n=49) were chosen at random for each group. Furthermore, a group of healthy pregnant women was chosen as the control group (Control n=83). Quantitative reverse-transcription PCR (qRT-PCR) was used to detect the relative expression of miR-301b-3p in exosomes found in maternal plasma. Results: The antenatal Control group had higher miR-301b-3p expression levels than the GH and PE groups ($P < 0.05$). The ROC curve analysis indicates a predictive value for case groups ($P < 0.05$). The Pearson correlation analysis indicates an inverse correlation between miR-301b-3p levels and illness severity in pathological patients ($P < 0.05$). Conclusion: miR-301b-3p is closely associated with the severity of vascular inflammation in PE.

**Keywords:** Hypertensive Disorders of Pregnancy; Preeclampsia; Plasma Exosomes microRNA; qRT-PCR.

## 1. Introduction

HDP is a prominent cause of severe maternal morbidity and mortality globally, accounting for 7% of US maternal deaths [1]. PE is the most dangerous stage of GH, causing maternal death, severe maternal morbidity, maternal critical care hospitalization, caesarean section, and preterm birth [2,3]. Disordered inflammatory responses and imbalanced angiogenic patterns contribute to the pathophysiology [4,5]. The abnormal remodelling of the uteroplacental spiral arteries causes placental oxidative stress, ischemia, and increased production of inflammatory and anti-angiogenic factors such as sFlt1[6]. Placental growth factor and vascular endothelial growth factor binding are disrupted by sFlt1. MicroRNA, a 22-nucleotide short RNA, regulates cell proliferation, death, differentiation, metastasis, and invasion [7-9]. A growing body of research indicates that cardiovascular problems modify circulating miRNA expressions during pregnancy [10]. Some miRNAs may predict PE onset; however, the results are uncertain [11]. Most of this research uses animal models and species-specific placental miRNAs for PE. Differences in placenta miRNA expression and direction are discordant with patient variables (e.g., race, gestational week, presence or absence of delivery, preterm birth or term delivery) [12]. MiR-301b-3p targets TLR4 and is linked to inflammation [13]. TLR4 signalling upregulates pro-inflammatory factors and triggers inflammatory responses [14]. We statistically analyzed GH and PE women's clinical data to answer these questions.

## 2. Material and Methods

(1) Pregnant women and sample collection

From January to November 2017, Guangdong Women And Children Hospital's obstetrics department admitted 80 pregnant women with HDP, who were separated into two groups: (1) Gestational Hypertension (GH, n=31; SBP ≥140mmHg and/or DBP ≥ 90mmHg, BP normalized within 12W postpartum); (2) Preeclampsia (PE, n=49; SBP ≥140mmHg and/or DBP ≥90mmHg with proteinuria 0.3g/24h or thrombocytopenia ($<100\times10^9$/L), Kidney injury (blood creatinine level > 1.1 mg/dl). The control group was the regular pregnancy group (n=83) with the same physical evaluation. Clinically, all study participants met the International Society for the Study of Pregnancy-induced Hypertension (ISSHP) diagnostic criteria. Acute and chronic infections, autoimmune illnesses, haematological diseases, malignancies, and other obstetric problems are excluded. Pregnancy weeks, plasma haemoglobin (Hb), systolic blood pressure (SBP), diastolic blood pressure (DBP), 24-hour urine protein (24UPro), blood creatinine level (Scr), plasma platelet volume (PLT), glutamate transaminases (ALT, AST), body mass index (BMI), neonatal birth weight (Neonatal Weight), D-dimer (D-DIC), fibrinogen level (FIB), and serum Total $Ca^{2+}$, plasma WBC, and neutrophils ($NE^{\#}$) were recorded. The review committees of each agency approved the trial, and all patients gave informed permission.

(2) Plasma collection and RNA preparation

After admission, 10ml of fasting venous blood was obtained with an EDTA anticoagulant tube in the morning of the next day before delivery and stored at 15-25 ℃ or lower. Exosomes were extracted from 0.6 mL of maternal plasma samples using the mercury Exosome Isolation Kit-Serum and Plasma (Exiqon, Woburn, MA, USA, no: 300101) as per manufacturer's instructions.

(3) MicroRNA extraction

MicroRNA extraction underpins and verifies qRT-PCR. This experiment used a blood (serum, plasma) miRNA quick extraction kit (centrifugal column) type III (Bioteke Corporation) to extract the target gene with less blood and high-quality miRNA, following the instructions. To normalize qRT-PCR, 1.6x108 copies/μL of synthetic cel-miR-39-3p (Qiagen) was spiked before RNA isolation.

(4) RNA concentration determination

UV spectrophotometry was used to quantify miRNA levels in this investigation. RNA purity was initially assessed using the OD260/OD280 ratio. The ratio between 1.8 and 2.0 suggests good RNA quality; more than 2.0 indicates contamination; less than 1.8 shows degradation.

(5) First-strand cDNA synthesis (miRNA reverse transcription)

The first strand of cDNA was synthesized using Gene Copoeia All-in-One ™ miRNA qRT-PCR Detection Kit (cargo number: QP015) and operated in strict accordance with the instructions.

(6) The miRNAs were quantified by qRT-PCR

Using the GeneCopoeia All-in-One ™ miRNA qRT-PCR Detection Kit (cargo id: QP015) kit, the design and synthesis of microRNA and reference cel-miR-39-3p primers were provided by GeneCopoeia company (number HmiRQP0379) according to the instructions.

# 3. Statistical Analysis

Statistics were done using GraphPadPrism 9 (GraphPad Software, San Diego, CA, USA). Clinical samples were examined as triplicates, with normally distributed data expressed as mean ± standard deviation (Mean±SD). Skewed data were log-transformed. A median with an interquartile range was used to represent skewed data after transformation and assessed by the Mann-Whitney test. Statistical treatment included a Student's t-test between the two groups and linear regression analysis to establish factor correlation. Two groups and the above data were analyzed using covariance. Chi-square tests on contingency tables assessed categorical variables. P-values under 0.05 were significant. The curve was produced simultaneously during ROC curve analysis to assess clinical parameter distinction.

# 4. Results

(1) General clinical data

General clinical data in Table 1 show similar ages in the control and case groups and no significant difference in gestational weeks or Hb (P>0.05). Pregnant women's SBP, DBP, 24UPro, Scr, PLT, ALT, AST, BMI, Neonatal Weight, D-DIC, FIB, serum Total $Ca^{2+}$, WBC, and $NE^{\#}$ showed significant (P <0.05) results. The above statistical characteristics suggest that patients' pathological symptoms and analytical characteristics are typical of HDP and that BMI, D-DIC, Fib, and Neonatal Weight had high sensitivity but no apparent relationship with severity, whereas the 24UPro and PLT were significantly different in the PE group. However, SBP, DBP, WBC, $NE^{\#}$, and Total $Ca^{2+}$ had high sensitivity and specificity. WBC elevation is mainly induced by $NE^{\#}$.

(2) Plasma exosomal miR-301b-3p expression levels in each group

All quantitative polymerase chain reaction values for Fig. 1 were normalized to cel-miR-39-3p and expressed as fold change. Melting curve analysis revealed individual peaks in each sample. The postpartum groups are blood samples of pregnant women in the Antenatal groups on the 5th day after delivery. Four samples were lost in the control group, namely n=79, one sample in GH group was substandard, namely n=30, and two samples PE group were lost to follow-up, namely n=47.

Fig.1A & Table.2 Antenatal miR-301b-3p relative expression levels, into a normal distribution. There was a difference in the relative expression of plasma miR-301b-3p among the three groups (p<0.05), and there was also a difference between the two groups (p<0.05), with the PE group having the lowest expression—Postpartum miR-301b-3p relative expression levels into a normal distribution. The relative expression difference in plasma miR-301b-3p between the three groups was significant (P <0.05) and between pairs (P <0.05), with the PE group having the lowest expression. Fig. 1B &Table 2. The difference in the relative expression of miR-301b-3p in plasma exosomes in the same case group (P <0.05).

(3) Comparison of the diagnostic efficacy of various clinical indicators on disease severity

In general clinical data, BMI, Neonatal Weight, D-DIC, Fib, Scr, Hb, ALT, and AST are not highly selective for disease severity, and the increase in WBC is mainly caused by higher $NE^{\#}$. HDP can vary. Thus, we compared miR-301b-3p's relative expression to 24hUPro, PLT, $NE^{\#}$, and Total $Ca^{2+}$.Fig. 2A. ROC curve analysis of 24hUPro, PLT, $NE^{\#}$, and Total Ca in GH cohort for GH diagnosis: AUC area of 24hUPro:0.5663 (95%CI 0.4462-0.6864), PLT:0.5649 (95%CI 0.4403-0.6915), $NE^{\#}$:0.6403 (95%CI 0.5169-0.7691), Total $Ca^{2+}$:0.7198 (95%CI 0.6227-0.8168), miRNA-301b-3p: 0.7606.HDP clinical indicators, including 24hUPro and PLT, have low diagnostic values congruent with clinical symptoms. Thus, SBP and DBP are primarily utilised to diagnose HDP clinically; however, their daily fluctuations are large. It depends on patients' moods, antihypertensive medicines, and the medical staff's technical level. Thus, a steady, non-invasive clinical indicator must be used for case group screening. $NE^{\#}$, Total $Ca^{2+}$, and miR-301b-3p expression demonstrated higher diagnostic value than other clinical signs. Figure 2B. ROC curve analysis of 24hUPro, PLT, $NE^{\#}$, and Total $Ca^{2+}$ in PE cohort for PE diagnosis: AUC area of 24hUPro:0.8865 (95%CI 0.8169–0.9562), PLT:0.6236 (95%CI 0.5175–0.7296), $NE^{\#}$:0.9189 (95%CI 0.5175–0.7296), Total $Ca^{2+}$:0.9208 (95%CI 0.8707–0.9670), miR-301b-3p: 0.9860.Fig.2 shows that $NE^{\#}$, Total $Ca^{2+}$, and miR-301b-3p may diagnose HDP and PE accurately and identify illness severity. Thus, $NE^{\#}$, Total $Ca^{2+}$, and miR-301b-3p are better diagnostics than 24hUPro and PLT.

(4) Analysis of the relationship between various indicators and SBP in the preeclampsia group

24hUPro, PLT, $NE^{\#}$, Total $Ca^{2+}$, and miR-301b-3p expression levels have good sensitivity and specificity for GH/PE diagnosis. What is the correlation between "gold standard" SBP? In pregnant women with PE, 24hUPro does not affect SBP: r= 0.03, P=0.81. Because of high proteinuria, 24hUPro cannot predict disease severity. PLT and SBP in pregnant women with PE: r= -0.59, P <0.01 (Fig.3B). Thrombocytopenia may indicate significant disease progression, which matches clinical features.$NE^{\#}$ is strongly linked with SBP in pregnant women in the PE group (Fig.3C, r=0.81, P <0.0001). It shows that $NE^{\#}$ is closely associated with SBP and can predict SBP increases. Figure 3D: Pregnant women in the PE group had a negative connection between total $Ca^{2+}$ and SBP (r=-0.67, p<0.0001). Total $Ca^{2+}$ and SBP are moderately associated, and a reduction in Total $Ca^{2+}$ predicts an increase in SBP. In Fig.3E, miR-301b-3p negatively correlates with SBP (r= -0.85, P <0.0001). This suggests that miR-301b-3p is correlated with SBP and that its decrease can anticipate SBP increases.

(5) Correlation Between PLT, Total $Ca^{2+}$, $NE^{\#}$ and miR-301b-3p Levels

Fig. 4A: PLT-Total $Ca^{2+}$ Correlation in PE Group: r=0.54,

p<0.0001. This fits clinical characteristics. PLT effects might trigger Total $Ca^{2+}$ reduction. Figure 4B shows a correlation (r=0.55, p<0.0001) between PLT and miR-301b-3p levels in the PE group. Thus, miR-301b-3p levels positively connect with PLT. Figure 4C shows a correlation (r=0.65, p<0.0001) between the PE group's Total $Ca^{2+}$ and miR-301b-3p levels.MiR-301b-3p levels are positively associated with Total $Ca^{2+}$. The correlation between inflammatory measure $NE^{\#}$ and miR-301b-3p levels in the PE group is -0.65, P <0.0001 (Fig. 4D). Thus, miR-301b-3p levels correlate well with $NE^{\#}$. Reduced miR-301b-3p levels are linked to inflammation decline.

# 5. Discussion

This study examined many detection indicators, including PLT, WBC, NE#, and Total Ca2+, which differed significantly among Control, GH, and PE groups (P < 0.05). Meanwhile, we measured miR-301b-3p expression in the same group before and after delivery. The PE group showed a considerable decrease in miR-301b-3p expression before delivery, with significant differences between the three groups (P < 0.05). The lowest relative expression levels of miR-301b-3p in PE within the three postpartum groups (P < 0.05). The case group showed significantly greater miR-301b-3p expression after delivery (P < 0.05), but the postpartum case group showed no inhibition. After evaluating the diagnostic efficacy of illness severity by clinical index, we observed that NE#, Total Ca2+, and miR-301b-3p were considerably better than 24UPro, PLT for GH or PE. Our analysis revealed a strong negative correlation between miR-301b-3p expression and SBP in PE patients with typical clinical alterations (r=-0.85, p<0.0001). The diagnostic effectiveness was much higher than 24UPro and PLT. Our research revealed a strong correlation between miR301b-3p and inflammatory index NE# in PE patients (r= -0.65, P <0.0001), which is linked to PE in numerous studies [15,16].PE patients' decreased Total Ca2+, and PLT may be linked to endothelial cell damage and blood hypercoagulability. This is supported by various studies[17,18]. MiR-301b-3p was linked with Total Ca2+ (r=0.65, p<0.0001) and PLT (r=0.55, p<0.0001). The low expression of miR-301b-3p in PE patients may be linked to endothelial damage and hypercoagulability, not a single inflammatory trigger. All data suggest that reduced miR-301b-3p expression is linked with worsening PE. Even commonly used diagnostic indicators like PAPP-A, PlGF, and sFlt1 do not prove PE. Because some have the best specificity and sensitivity when assessed at a set time [19,20]. Early pregnancy PAPP-A levels showed a strong correlation with PE[21]. Doppler ultrasound can predict PE. However, recent investigations of the cardiovascular index have not linked it to placental perfusion and functional markers, and cardiovascular indicators did not improve PE screening [22,23]. Thus, finding additional preeclampsia markers is sensible.

Recent evidence suggests that miR-301b-3p drives various types of human cancer, closely related to cancer cell proliferation, migration, and invasion[24,25]. Under inflammation and long-term hypoxia, trophoblast cells may inhibit miR-301b-3p expression, causing HDP pathological changes. Our research results' rationale is clarified. In low-calcium diets, oral calcium reduces preeclampsia[26]. Low-dose aspirin prevents preeclampsia by reducing inflammation and platelet aggregation[27,28]. Our research has been drug-verified, making miR-301b-3p drug research easier—another benefit. The sample size needs to be raised in this study. Other deficiencies include the necessity for animal and prospective cohort studies to verify miR-301b-3p-targeting medicines. In conclusion, miR-301b-3p can diagnose PE and differentiate clinical kinds to delay or stop its progression.

# References


[1] Sharma, G., Hays, A. G. & Blumenthal, R. S. Can We Reduce Premature Mortality Associated With Hypertensive Disorders of Pregnancy?: A Window of Opportunity. *J Am Coll Cardiol* 77,1313-1316, doi: 10.1016/j.jacc.2021.01.021 (2021).

[2] Rana, S., Lemoine, E., Granger, J. P. & Karumanchi, S. A. Preeclampsia: Pathophysiology, Challenges, and Perspectives. *Circ Res* 124, 1094-1112, doi:10.1161/ CIRCRESAHA. 118. 313276 (2019).

[3] Ford, N. D. *et al.* Hypertensive Disorders in Pregnancy and Mortality at Delivery Hospitalization - United States, 2017-2019. *MMWR Morb Mortal Wkly Rep* 71, 585-591, doi:10. 15585/ mmwr.mm7117a1 (2022).

[4] Aplin, J. D., Myers, J. E., Timms, K. & Westwood, M. Tracking placental development in health and disease. *Nat Rev Endocrinol* 16, 479-494, doi:10.1038/s41574-020-0372-6 (2020).

[5] Khosla, K. *et al.* Long-Term Cardiovascular Disease Risk in Women After Hypertensive Disorders of Pregnancy: Recent Advances in Hypertension. *Hypertension* 78, 927-935, doi:10. 1161/HYPERTENSIONAHA.121.16506 (2021).

[6] Qu, H. & Khalil, R. A. Vascular mechanisms and molecular targets in hypertensive pregnancy and preeclampsia. *Am J Physiol Heart Circ Physiol* 319, H661-H681, doi:10.1152/ ajpheart. 00202.2020 (2020).

[7] Bacon, S. *et al.* Can placental growth factors explain birthweight variation in offspring of women with type 1 diabetes? *Diabetologia* 64, 1527-1537, doi:10.1007/s00125-021-05438-y (2021).

[8] Wang, G. *et al.* Contribution of placental 11beta-HSD2 to the pathogenesis of preeclampsia. *FASEB J* 34, 15379-15399, doi:10.1096/fj.202001003RR (2020).

[9] Ghafouri-Fard, S. *et al.* Exploring the role of non-coding RNAs in autophagy. *Autophagy*, 1-22,doi:10. 1080/ 15548627. 2021. 1883881 (2021).

[10] Aryan, L., Medzikovic, L., Umar, S. & Eghbali, M. Pregnancy-associated cardiac dysfunction and the regulatory role of microRNAs. *Biol Sex Differ* 11, 14, doi:10.1186/ s13293-020-00292-w (2020).

[11] Murugesan, S. *et al.* Role of exosomal microRNA signatures: An emerging factor in preeclampsia-mediated cardiovascular disease. *Placenta* 103, 226-231, doi:10.1016/ j.placenta. 2020. 10.033 (2021).

[12] Frazier, S., McBride, M. W., Mulvana, H. & Graham, D. From animal models to patients: the role of placental microRNAs, miR-210, miR-126, and miR-148a/152 in preeclampsia. *Clin Sci (Lond)* 134, 1001-1025, doi:10.1042/ CS20200023 (2020).

[13] Li, K. *et al.* Acute Exposure of Atmospheric Ultrafine Particles Induced Inflammation Response and Dysregulated TGFbeta/ Smads Signaling Pathway in ApoE(-/-) Mice. *Cardiovasc Toxicol* 21, 410-421, doi:10.1007/s12012-021-09633-6 (2021).

[14] Li, W. *et al.* Ferroptotic cell death and TLR4/Trif signaling initiate neutrophil recruitment after heart transplantation. *J Clin Invest* 129, 2293-2304, doi:10.1172/JCI126428 (2019).

[15] Liu, D. *et al.* Placenta-derived IL-32beta activates neutrophils to promote preeclampsia development. *Cell Mol Immunol* 18, 979-991, doi:10.1038/s41423-021-00636-5 (2021).

[16] Miller, D. *et al.* Cellular immune responses in the pathophysiology of preeclampsia. *J Leukoc Biol* 111, 237-260, doi:10.1002/JLB.5RU1120-787RR (2022).

[17] Barnes, J. N. *et al.* Cerebrovascular Reactivity and Vascular Activation in Postmenopausal Women With Histories of Preeclampsia. *Hypertension* 71, 110-117, doi:10.1161/ HYPERTENSIONAHA. 117.10248 (2018).

[18] Ozdemirci, S. *et al.* Predictivity of mean platelet volume in severe preeclamptic women. *Hypertens Pregnancy* 35, 474-482, doi:10.1080/10641955.2016.1185113 (2016).

[19] Chiu, C. P. H. *et al.* Prediction of spontaneous preterm birth and preterm prelabor rupture of membranes from maternal factors, obstetric history and biomarkers of placental function at 11-13 weeks. *Ultrasound Obstet Gynecol*, doi:10.1002/ uog. 24917 (2022).

[20] Licini, C. *et al.* Pre-eclampsia predictive ability of maternal miR-125b: a clinical and experimental study. *Transl Res* 228, 13-27, doi: 10.1016/j.trsl.2020.07.011 (2021).

[21] Rolnik, D. L. *et al.* ASPRE trial: performance of screening for preterm pre-eclampsia. *Ultrasound Obstet Gynecol* 50, 492-495, doi:10.1002/uog.18816 (2017).

[22] Cheng, Y. *et al.* First trimester screening for pre-eclampsia in Chinese pregnancies: case-control study. *BJOG* 125, 442-449, doi:10.1111/1471-0528.14970 (2018).

[23] Zhu, J., Zhang, J., Syaza Razali, N., Chern, B. & Tan, K. H. Mean arterial pressure for predicting preeclampsia in Asian women: a longitudinal cohort study. *BMJ Open* 11, e046161, doi:10.1136/bmjopen-2020-046161 (2021).

[24] Zhou, S. L. *et al.* A Positive Feedback Loop Between Cancer Stem-Like Cells and Tumor-Associated Neutrophils Controls Hepatocellular Carcinoma Progression. *Hepatology* 70, 1214-1230, doi:10.1002/hep.30630 (2019).

[25] Li, P. *et al.* microRNA-301b-3p downregulation underlies a novel inhibitory role of long non-coding RNA MBNL1-AS1 in non-small cell lung cancer. *Stem Cell Res Ther* 10, 144, doi:10.1186/s13287-019-1235-8 (2019).

[26] Chappell, L. C., Cluver, C. A., Kingdom, J. & Tong, S. Pre-eclampsia. *The Lancet* 398, 341-354, doi:10.1016/s0140-6736(20)32335-7 (2021).

[27] Ives, C. W., Sinkey, R., Rajapreyar, I., Tita, A. T. N. & Oparil, S. Preeclampsia-Pathophysiology and Clinical Presentations: JACC State-of-the-Art Review. *J Am Coll Cardiol* 76, 1690-1702, doi: 10.1016/j.jacc.2020.08.014 (2020).

[28] Force, U. S. P. S. T. *et al.* Aspirin Use to Prevent Preeclampsia and Related Morbidity and Mortality: US Preventive Services Task Force Recommendation Statement. *JAMA* 326, 1186-1191, doi:10.1001/jama.2021.14781 (2021).

# Supplemental Information

**Table 1.** The clinical characteristics of the participants (Mean±SD)

| | Control n=83 | GH n=31 | PE n=49 | P1 | P2 | P3 | P4 |
|---|---|---|---|---|---|---|---|
| Age (years) | 27.31±5.21 | 29.20±4.14 | 32.81±5.37 | 0.45 | 0.32 | 0.06 | 0.07 |
| Gestational weeks | 36.74±1.36 | 37.37±1.79 | 37.24±1.53 | 0.57 | 0.19 | 0.07 | 0.06 |
| Hb(g/L) | 119.63±17.72 | 113.63±15.7 | 113.4±17.3 | 0.13 | 0.27 | 0.81 | 0.13 |
| BMI ($kg/m^2$) | 23.19±4.04 | 24.37±3.18 | 27.01±4.13 | 0.27 | <0.05 | 0.19 | <0.05 |
| D-DIC (mg/L) | 0.54±0.17 | 1.73±0.52 | 1.97±0.81 | <0.05 | <0.05 | 0.11 | <0.05 |
| FIB(g/L) | 4.42±1.01 | 5.17±1.31 | 5.37±1.55 | <0.05 | <0.05 | 0.85 | <0.05 |
| Neonatal Weight(kg) | 3.22±0.69 | 3.13±0.52 | 2.91±0.55 | 0.17 | <0.05 | 0.65 | <0.05 |
| 24UPro (mg/24h) | 137.93±68.71 | 182.51±71.6 | 771.93±647.6 | 0.95 | <0.05 | <0.05 | <0.05 |
| Scr (umol/L) | 55.42±17.81 | 51.28±10.57 | 59.55±18.63 | 0.87 | <0.05 | 0.08 | <0.05 |
| PLT (10^9/L) | 188.83±48.03 | 179.6±59.65 | 158.23±71.55 | 0.79 | <0.05 | 0.29 | <0.05 |
| ALT(U/L) | 18.13±6.24 | 47.58±10.23 | 55.71±14.05 | <0.05 | <0.05 | 0.35 | <0.05 |
| AST(U/L) | 21.27±6.92 | 37.25±7.9 | 40.7±12.3 | <0.05 | <0.05 | 0.38 | <0.05 |
| SBP (mmHg) | 115.42±11.93 | 145.74±5.74 | 158.52±12.14 | <0.05 | <0.05 | <0.05 | <0.05 |
| DBP (mmHg) | 68.25±8.31 | 72.64±10.73 | 87.81±12.37 | <0.05 | <0.05 | <0.05 | <0.05 |
| WBC(*10^9/L) | 8.57±2.55 | 11.94±2.47 | 12.39±2.43 | <0.05 | <0.05 | <0.05 | <0.05 |
| NE#(*10^9/L) | 5.19±1.66 | 6.73±2.73 | 9.18±1.93 | <0.05 | <0.05 | <0.05 | <0.05 |
| Total $Ca^{2+}$ (mmol/L) | 2.29±0.21 | 2.10±0.14 | 1.94±0.09 | <0.05 | <0.05 | <0.05 | <0.05 |
| $P_1$: Control vs GH, $P_2$ : Control vs PE, $P_3$:GH vs PE , $P_4$: Comparison of three groups | | | | | | | |

**Table 2.** Relative expressions of plasma exosomal miR-301b-3p (Mean±SD)

| | Control n=83 | GH n=31 | PE n=49 | P1 | P2 | P3 | P4 |
|---|---|---|---|---|---|---|---|
| Antenatal | 1.07±0.33 | 0.65±0.37 | 0.33±0.15 | <0.05 | <0.05 | <0.05 | <0.05 |
| | n=79 | n=30 | n=47 | | | | |
| Postpartum | 1.03±0.22 | 0.87±0.07 | 0.79±0.08 | <0.05 | <0.05 | <0.05 | <0.05 |
| P5 | 0.83 | <0.05 | <0.05 | | | | |
| P1: Control vs GH, P2: Control vs PE, P3: GH vs PE P4: Comparison of three groups P5: Antenatal vs Postpartum | | | | | | | |

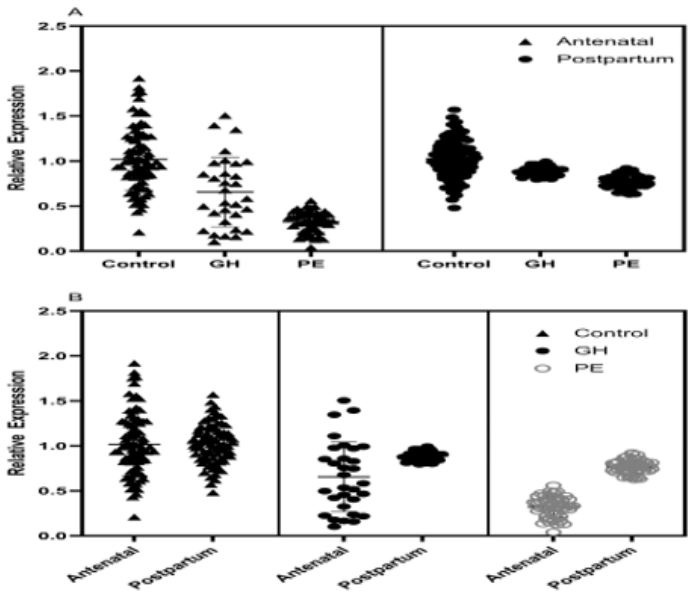


**Fig 1.** Relative expression of miR-301b-3p was measured by qRT-PCR

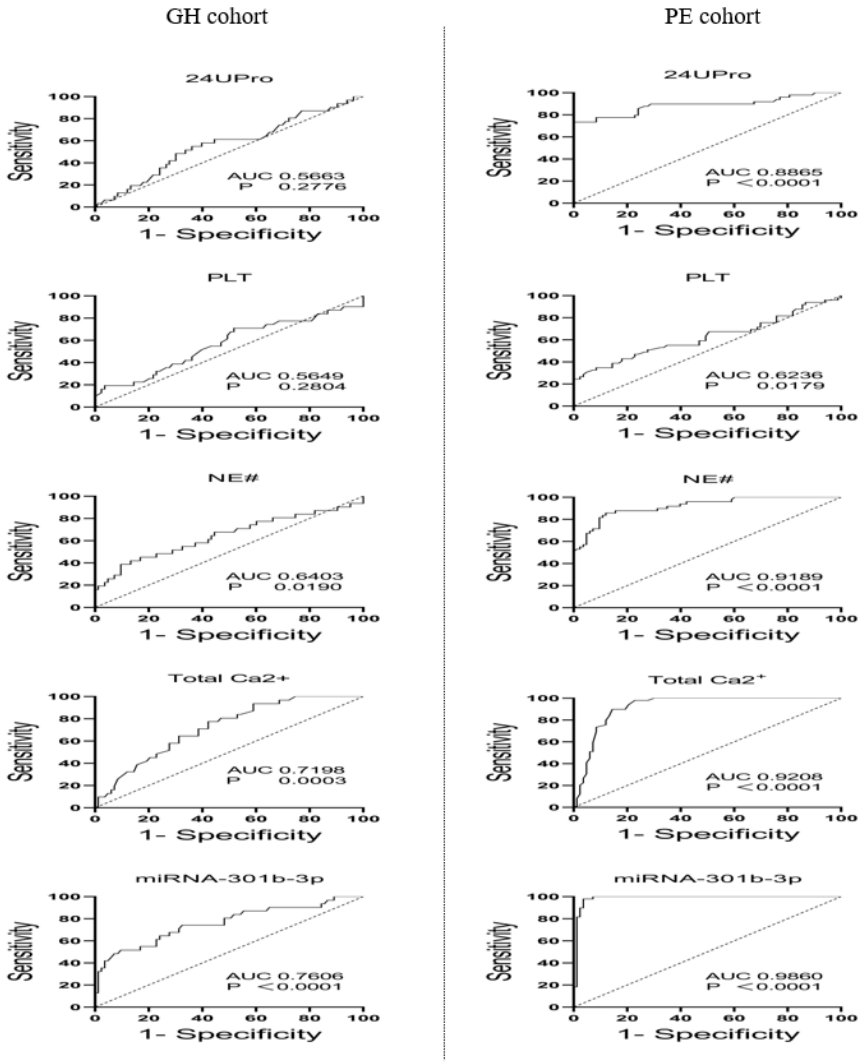


**Fig 2.** ROC curve for the predictiveness of GH, PE. The ROC curve was estimated by entering in the model 24hUPro, PLT,NE$^{\#}$, Total $Ca^{2+}$, miR -301b -3p before delivery. AUC: Area Under Curve.

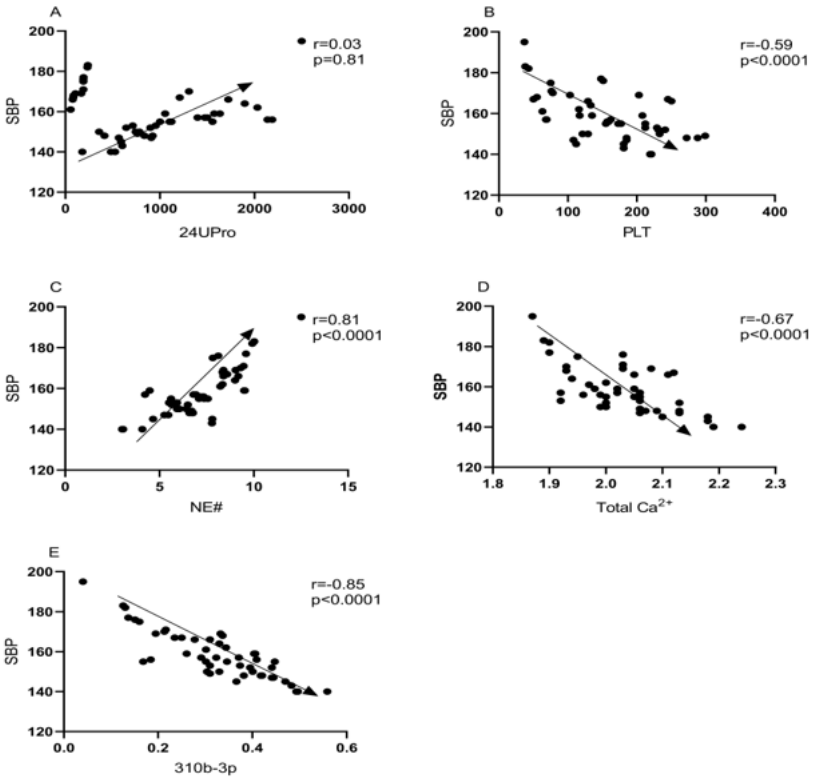


**Fig 3.** Correlation analysis between 24hUPro, PLT, NE#, Total $Ca^{2+}$, miR-301b-3p and SBP (mmHg) in PE(n=49).

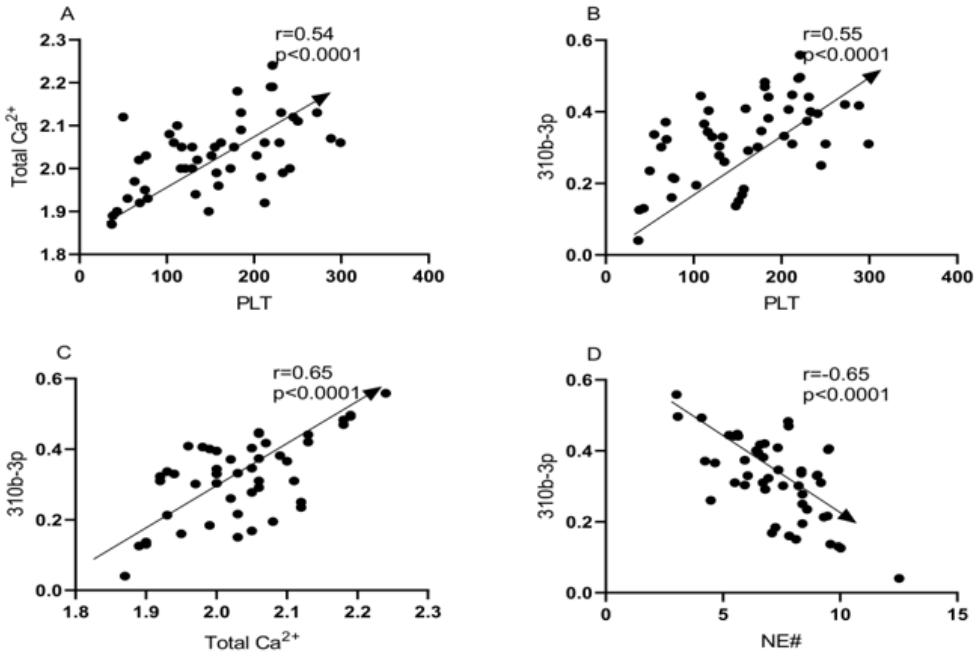


**Fig 4.** Correlation analysis between PLT, NE#, Total Ca2+ and miR-301b-3p levels in PE(n=49).