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QUANTIFICATION OF VARIED MIRNAS IN EARLY AND LATE-ONSET PREECLAMPSIA COMPLICATING PREGNANCIES IN SOUTH INDIAN WOMEN- A COHORT STUDY

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Keywords:

Early Onset Preeclampsia (EOPE), Late-Onset Preeclampsia (LOPE) microRNA (miR), Next-Generation Sequencing (NGS), Placenta, Real-time Polymerase Chain Reaction (rt-PCR)

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ABSTRACT: Introduction: Preeclampsia (PE) is a common pregnancy-related hypertensive disorder that may result from abnormal placental development and is a major threat to the mother's and foetus's life. **Methods:** Placental miRNAs (miRNAs) profile was investigated using next-generation sequencing for the identification of potential biomarkers playing a role in aberrant placental development and to understand the pathophysiology(space between 'the' and 'pathophysiology') of PE. Placental tissues were collected from three patient groups (Control [n = 30], Early Onset of Preeclampsia (EOPE) [n = 30], and Late Onset of Preeclampsia (LOPE) [n = 30]) and performed miRNA profilingby Illumina sequencing, downstream analysis for identification, quantification and expression profiling is done by the quantifier.pl, script, and miRDeep2.pl, script. **Results:** Sequence analysis showed novel miRNAs that were disease-specific and common among all studied groups, suggesting the underlying placental pathologies in EOPE and LOPE. The real-time PCR analysis of 3 miRNAs (miR4743-5p, miR149-5p, and miR331-5p) was done, and observed differential expression in all three patient groups. MiRNA4743-5p was found to be significantly expressed EOPE when compared to control and LOPE samples. Similarly, the expression of miR331-5p was significant in LOPE samples as compared to other groups, and the expression of miR149-5p was significantly observed in the control group. **Conclusion:** The integrative expression analysis of these 3 miRNAs has given hope for early diagnosis of PE and associated complications in pregnant women. The study explains for the first time the role of microRNA 4743-5p, 149-5p & 331-5p in early and late-onset preeclampsia.

INTRODUCTION: Preeclampsia (PE) isone of the manifestations of hypertensive disorder (140 mmHg systolic and 90 mmHg diastolic) with or without proteinuria (300 mg or more per 24-hour urine collection) [1-3]. Preeclampsia usually arises after the 20th week of pregnancy, and poses a major threat to the life of the mother as well as the child if left untreated.

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This disease of complex pathophysiology has a prevalence rate of 8-10 % nationally and 2-5% globally [4, 5]. The symptoms originate from defective placental formation, and shallow trophoblast infiltration, with no actual cure except delivery of the placenta. Other symptoms like

thrombocytopenia, impaired liver functions, and renal insufficiency (Serum creatinine concentrations greater than 1.1 mg/dL or a doubling of the serum creatinine concentration in the absence of another renal disease), or other neurological symptoms are also observed in PE patients [6, 7].

Turco *et al* (2018) [8] explained the concept of preeclampsia as a two-stage syndrome that involves impaired trophoblast migration, invasion, and impaired angiogenesis in the first stage and dysregulation of the maternal immune system in the second stage. It is now being proposed that, at the stage of remodeling the vessels supplying the intervillous space follows abnormal placentation. Anna C Staff [9] also supported the theory of two-stage disorder. Based on gestational age PE can be divided into Early-Onset Preeclampsia (EOPE; < 34 weeks) and Late-Onset Preeclampsia (LOPE; >34 weeks) [10]. Furthermore, EOPE is considered severe and with an increased risk of cardiovascular disease suggesting extensive involvement of the maternal constitution [11].

Since PE is a disease with complex pathogenesis, early detection can help the care provider with measures to alleviate the symptoms and initiate preventive prophylaxis such as Aspirin and achieve better control of hypertension and other development of associated complications that arise during disease progression[12, 13]. There is a need for the identification of clinically relevant biomarkers that can specifically predict the onset of the disease. Predictive molecular markers like miRNAs are detected in the plasma of PE women even before the 20th week of pregnancy.

MiRNAs are small (~22–25 nucleotides), endogenous, single-stranded, non-coding RNAs that regulate gene expression preferentially by binding to the untranslated region (3′UTR) of a target gene. They play an important role in the post-transcriptional regulation of gene expression by causing translational inhibition or mRNA cleavage thereby silencing gene expression. Development, cell differentiation, and migration are a few processes that are controlled by miRNAs [14]. Cells and tissues express miRNAs outside of the cell, such as serum, plasma, saliva, urine, and milk. These circulating miRNAs are extracellular miRNAs. miRNAs have up to 10 times more stability than messenger RNAs. Because they're non-intrusive, circulating in nature, stable, and simple in quantification, considering miRNAs as potential indicators for a number of pathologic disorders, such as cancer, cardiovascular conditions, and consequences from pregnancy. Recent data indicate that miRNAs are involved in regulating placentation, angiogenesis, blood pressure, and inflammatory response, underlining their participation in the disease's aetiology and a crucial function as potential biomarkers for early PE diagnosis [15, 16].

The present study is an attempt to find out the potential placental miRNAs which could be used as some sensitive and specific biomarkers for the early prediction of PE. Hence three miRNAs were selected with differential expression in PE conditions and normal pregnancy.

MATERIALS AND METHODS: This is a multicentric study in the Department of Obstetrics and Gynaecology conducted by collaboration between tertiary care hospitals such as Fernandez Hospital Hyderabad, SIC (Employees State Insurance Corporation), Apollo Institute of Medical Sciences, and Department of Molecular Genetics, Pathcare Labs, Red cliff labs. The study was conducted over the period of 2 years and 3 months from January 2020 to March 2022.

Ethical consideration: Ethical clearance was obtained for this study from the institutional ethical committee of the respective institutes (EC approval numbers: 008/09/2019/IEC/SMCH: ESIC-ESICMC/SNR/IEC-S101/12-2020: Fernandez -EC Reference No. 32_2020). Written informed consent was obtained from the participants included in the study. We ensured that the study complies with international ethical norms according to Helsinki Declaration – Ethical Principles for Medical Research Involving human subjects [17].

The inclusion and exclusion criteria followed in the recruitment of patients: Thirty normal pregnant women above 34 weeks of pregnancy were included in the study as the control group. Similarly, thirty early onsets and thirty late onsets of PE were included in the study, after the diagnosis of PE was based on blood pressure with

systolic ≥140mmHg and diastolic ≥ 90mmHg and proteinuria and Pregnant women who had co-morbidities or medical complications like chronic hypertension, autoimmune disease, gestational diabetes, or under any other medication with any other disease were excluded. Detailed clinical history and drug history were documented in a structural proforma.

Methodology: Placental tissues were collected from three patient groups (control [n = 30], early onset of preeclampsia (EOPE) [n = 30], and late onset of preeclampsia (LOPE) [n = 30]), and total RNA was purified. RNA sequencing was done by Illumina sequencing and generated files for downstream analysis with the miRDeep2. Identification, quantification, and expression profiling are done by the quantifier. pl, script, and miRDeep2.pl, script. The real-time expression of miRNA and their related genes were quantified by Mir-X™ miRNA qRT-PCR TB Green® Kit' in three study groups.

Quantitative PCR Reactions were set up using the following Reagents: Taq-man Universal Master Mix (Applied Biosystems. USA), dH2O, and relevant TaqMan probe (Applied Biosystems. USA). miRNA protocol was run on an ABI PRISM 7900HT PCR system at the following settings: 95^{O}C, 10 min; followed by 95 ^{O}C, 15 s; 60 ^{O}C, 1 min 40 cycles.

Statistical Analysis: The experimental data were expressed as mean ± standard deviation. Statistical analysis of all data was used SPSS 24.0 (SPSS Inc., Chicago, IL, USA) and Graph Pad Prism 7.04 software (Graph Pad, San Diego, CA, USA).

The statistical differences between groups were compared by Student's *t*-test and one-way ANOVA. Pearson's correlation coefficient was applied for the correlation analysis. $P< 0.05$ was considered a statistically significant criterion. Each experiment runs at least three times.

RESULTS: miRNA profiling by NGS study showed the expression of a total of 9miRNAs (4743-5p, 149-5p, 331-5p, 514b-3p, 517-5p, 373, 1287-5p, 21, 508-3p) in early-onset, late-onset, and control samples together and among them, only three miRNAs have shown significant expression in PE samples. miRNA149-5p (TCTGGCTCCGTGTCTTCACTCCC) was significantly expressed in control samples, whereas miRNA4743-5p (TGGCCGGATGGGACAGGAGGCAT) was found exclusively in EOPE and similarly miRNA331-5p(GCAGCTAGGTATGGTCCCA) was significantly expressed in LOPE samples. Target Scan (v7.2) bioinformatics software (Whitehead Institute for Biomedical Research in the Massachusetts Institute of Technology) (http://www.targetscan.org/vert_72/) was used to predict target genes of 4743-5p, 149-5p, and 331-5p.

TABLE 1: EXPRESSION LEVEL (FOLD CHANGE) OF MIR4743-5pin EOPE, LOPE, AND CONTROL

S. no.	hsa-miR 4743-5P		
	Control	**EOPE**	**LOPE**
1	0.8955	6.825	3.15
2	1.155	5.996	2.55
3	0.8415	6.8952	1.955
4	0.6885	7.256	2.007
5	0.5894	6.512	3.255
6	0.8547	6.211	1.2525
7	1.12	5.8889	2.145
8	1.3853	6.0025	1.0447
9	0.6506	8.6982	2.0147
10	0.9159	6.125	2.104
11	1.1812	7.996	1.857
12	0.4465	5.8952	1.478
13	0.7118	7.896	1.895
14	0.9771	8.512	3.5855
15	0.7424	5.211	3.2587
16	0.5077	5.5889	3.1475
17	0.773	6.0025	2.155
18	0.7383	6.6982	2.1785
19	0.7036	8.225	1.9885
20	0.5689	6.596	2.1785
21	0.8342	7.7852	2.5895
22	1.0995	9.256	2.9885
23	0.7648	5.8512	3.447
24	0.6301	7.0525	2.5895
25	0.8954	6.049	3.2585
26	0.5607	6.144	3.5685
27	0.426	6.5963	2.9455
28	0.8555	9.6725	3.1885
29	1.0255	7.07715	2.2558
30	0.7885	7.4818	2.8588
Mean	0.811	6.933	2.496
STDEV	0.229	1.132	0.696
p Value	0.3705	0.0001	0.0016

Table 1 shows the expression of miRNA -4743-5p in control, EOPE & LOP. The expression level the expression of miRNA 4743-5p is significantly increased in EOPE as compared to LOPE and control.

The expression level is 4 fold higher in EOPE as compared with LOPE. The expression level in EOPE is 6-fold higher as compared with the control. The value of fold change, <1 means that it is down regulated and a fold change value >1 shows that it is upregulated. The expression pattern is higher in Early-onset PE as compared with Late-onset PE and control samples fold change>1 shows upregulation and fold change<1 shows down regulation.

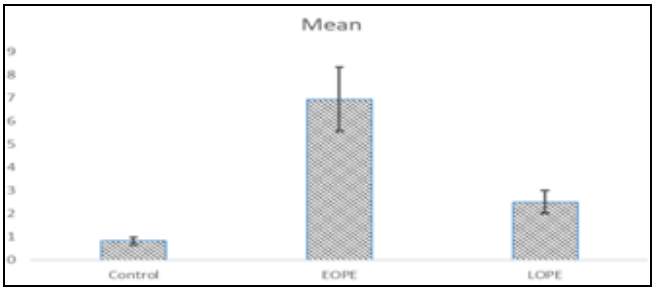

FIG. 1: MICRORNA-4743-5P EXPRESSION IN CONTROL, EOPE & LOPE

MiR4743-5p shows an increased expression level in Early Onset Preeclampsia as compared to late-onset preeclampsia. The individual fold change was observed in control, EOPE & LOPE patients. The miR 4743-5p was highly expressed in EOPE as compared to LOPE & control. The fold change in EOPE vs control is >5. Fold change in EOPE vs LOPE is >2.4. Yaxis shows the fold change in each group.

TABLE 2: DIFFERENTIAL EXPRESSION LEVEL OF MIR-149-5P IN EOPE, LOPE, AND CONTROL

S. no.	miR -149-5P		
	Control	EOPE	LOPE
1	7.825	0.7955	3.1475
2	6.996	1.0155	3.5544
3	5.892	1.0145	2.9955
4	7.256	1.0674	2.8587
5	5.512	1.9335	3.255
6	8.211	1.0193	3.4525
7	6.1889	1.4525	3.7145
8	5.0025	1.0712	4.0447
9	6.2982	1.0971	4.0147
10	6.8925	1.2231	4.2104
11	7.1476	1.4105	3.8857
12	5.7252	1.1175	3.6478
13	6.5496	1.0095	3.7895
14	7.0512	1.269	3.5855
15	6.4811	1.0525	3.5517
16	6.0089	0.8788	3.0475
17	7.0152	1.0475	4.1522
18	5.7782	1.3207	4.1785
19	5.1525	0.9665	3.4785
20	6.1256	0.9826	4.1785
21	7.2582	1.0855	4.5895
22	9.0046	1.1345	3.9885
23	5.88857	0.8045	3.5447
24	6.8914	0.8764	3.7795
25	6.6949	1.1235	3.9585
26	6.5544	1.3583	3.3185
27	5.6983	1.0645	4.2045
28	7.6725	0.902	3.1885
29	8.0775	1.1615	4.2558
30	7.4818	1.421	3.4228
Mean	6.678	1.123	3.700
STD.EV	0.940	0.232	0.436
p Value	0.0001	0.4263	0.0016

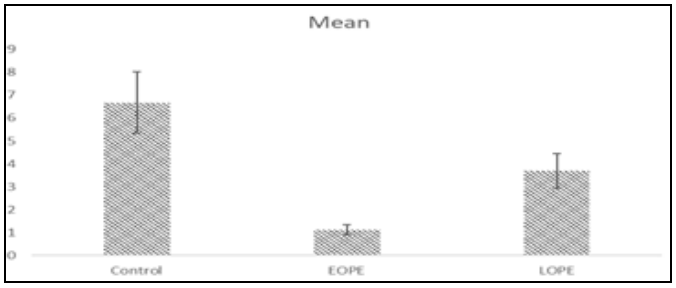

FIG. 2: MIRNA149-5P EXPRESSION IN CONTROL, EOPE, AND LOPE

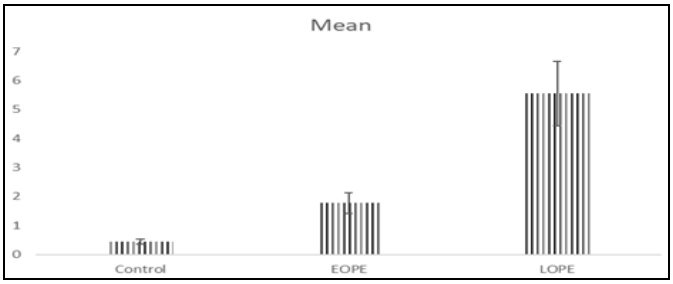

FIG. 3: MI RNA 331-5P EXPRESSION IN EOPE, LOPE, AND CONTROL SAMPLES

miR149-5p expression was higher in control samples in comparison to Late-onset Preeclampsia samples and significantly higher than Early onset Preeclampsia **Table 2** and **Fig. 2**. The individual fold change was observed in control, EOPE & LOPE patients. The miR149-5p was highly expressed in controls compared to EOPE & LOPE. The fold change in control vs EOPE is >5. Fold change in control vs LOPE is >2.

TABLE 3: EXPRESSION LEVEL OF MIR-331-5P IN EOPE, LOPE AND CONTROL

	miR-331-5p		
S. no.	**Control**	**EOPE****	**LOPE*****
1	0.284	2.435	2.115
2	0.606	1.403	6.028
3	1.022	0.305	1.295
4	0.503	6.115	2.451
5	0.214	1.225	2.343
6	0.122	0.234	4.994
7	0.535	2.108	8.333
8	0.619	1.204	5.558
9	0.581	0.129	9.121
10	0.305	0.351	8.119
11	0.308	1.007	9.352
12	0.415	2.007	5.695
13	0.099	1.836	7.239
14	0.282	1.233	1.944
15	0.885	4.219	4.339
16	0.327	2.351	8.331
17	0.575	1.238	6.236
18	1.008	2.463	5.613
19	0.673	3.106	7.339
20	0.042	0.911	2.031
21	0.348	1.003	4.104
22	0.315	1.942	8.337
23	0.339	1.009	5.341
24	0.297	2.354	9.333
25	0.358	1.106	8.124
26	0.997	3.006	5.225
27	0.486	4.006	6.239
28	0.223	1.576	6.332
29	0.342	0.217	3.881
30	0.214	1.292	1.382
Mean	0.439	1.775	5.553
STDEV	0.265	1.328	2.530
p Value	0.589	0.0016	0.0001

miR331-5p is highly expressed in LOPE samples as compared with Control samples. The expression is even reduced in LOPE even though it was significant **Table 3** and **Fig. 3**. The individual fold change was observed in control, EOPE & LOPE patients. The miR 331-5p was highly expressed in LOPE as compared to EOPE & control. The fold change in LOPE vs control is >5. Fold change in LOPE vs EOPE is >2.

The Novelty of the Research: The current study identified the differential expression of microRNAs 4743-5p, 149-5p, and 331-5p in placental tissues for the first time in the Indian population.

DISCUSSION: Preeclampsia is a multifactorial and multisystem disorder, with unknown pathophysiology of the disease, but factors such as defective placentation due to defective trophoblast invasion and narrowing of spiral arteries may be contributing to the onset of PE by leading to a hypoxic, inflammatory environment, intrauterine growth restriction, the altered ratio of angiogenic and anti-angiogenic factors in maternal blood causing to oxidative stress, both in the mother and foetus that affects maternal end organs and also the growth of the foetus. During normal implantation, trophoblasts invade the decidualized endometrium, leading to spiral artery remodelling and obliteration of the tunica media of myometrial spiral arteries, allowing increased blood flow to the placenta, all independent of maternal vasomotor changes. In preeclampsia, trophoblasts fail to adopt an endothelial phenotype, which leads to impaired trophoblast invasion and incomplete spiral artery remodelling [18]. Early in pregnancy, inappropriate maternal immune responses to trophoblast may cause aberrant placentation and pave the way for clinical preeclampsia later on. Endothelial dysfunction and systemic inflammatory response to placental oxidative stress are features of established

preeclampsia [19]. However, genetic factors, immune factors, and systemic inflammation are also related to the pathophysiology of this syndrome. Early prediction of PE may improve the surveillance and prognosis of hypertension in pregnant women in preventing associated complications. Therefore, it is essential to find markers for the detection of developing PE early in the pre-symptomatic stage. Several studies have shown differential expression of miRNA in pregnant patients with PE.

Specific patterns of miRNA have been detected in the placenta, circulation, decidual-derived mesenchymal stem cells (MSCs), umbilical cord blood, and human umbilical vein endothelial cells. MiR-141, miR-23a, miR-136, and some novel miRNAs are highly enriched in the placenta [20]. miRNA clusters that are differentially regulated are functionally linked to various physiologic and pathologic conditions during pregnancy. These functions include regulation of cell growth (miR-3200), control of cell transition factors in the female reproductive tract by IGF receptor 1 and TGF-b receptor 2 functions (miR-3200-5p), anomalous placentation (miR-320a), epithelial-mesenchymal transition involved in tissue remodeling (miR-25-3p), gestational hypertension, preeclampsia, and intrauterine growth restriction (miR-143-3p), cell proliferation and migration (miR129-1 cluster), oocyte aging and embryogenesis (miR-203a-3p), cell proliferation and migration (miR-324-5p), MAPK function and induction of cell cycle arrest as seen in senescent uterine cells (miR129-1 cluster), and immune responses and inflammatory reactions (miR-6769b-5p) [21, 22].

Ilona Hromadnikova *et al* studied the usefulness of miRNAs as a predictive biomarker for early diagnosis of PE [23]. Literature review in the area of PE-related miRNAs has found many miRNAs that could be used as biomarkers in the early detection of PE *i.e.*, miRNA 210, miRNA155, miRNA26, and miRNAs in the 14th chromosome cluster, and these miRNAs were also observed in the present study. Even though there are several studies that proved the role of miRNAs in hypertensive complications of pregnancy like preeclampsia, the results are inconsistent due to many factors. The sampling techniques, sample size, source of sampling, and molecular techniques adopted by the authors in their study add to the discrepancy in the results. The present study focused on novel targets other than the previously discovered miRNAs which led to the following findings.

The gene targets for miR 4743-5p that were predicted using a computational method are Estrogen Receptor-1 (ESR-1), Notch receptor -1 and 3 (NOTCH-1 and NOTCH-3), and TNF Receptor super family member 8 (TNFRSF8). Estrogen exerts its biological effects in large part through intracellular activation of its principal receptor, Estrogen receptor-1 [24]. Inhibition of Estrogen Receptor-1 by miR 4743-5p leads to reduced expression of Estrogen and results in vasoconstriction of uterine arteries.

Another target of miRNA 4743-5p is Notch Receptor which controls trophectoderm formation, decidualization, placental branching morpho-genesis, and endovascular trophoblast invasion. In humans, the particular signalling cascade promotes the formation of the extravillous trophoblast lineage and regulates trophoblast proliferation, survival, and differentiation. Expression patterns as well as functional analyses indicate distinct roles of Notch receptors in different trophoblast subtypes. Inhibition of Notch-1 suppresses the NF-κBsignalling pathway by inhibiting the phosphorylation of nuclear factor kappa B (NF-κB p65) inhibitor (IκBα) and the subsequent nuclear translocation of NF-κB subunit p65 [25]. Deregulation of Notch signalling leads to aberrant placentation.

There were no articles found indicating the role of miRNA4743-5p in preeclampsia. The present study shows for the first time the differential expression of miRNA 4743-5p in preeclampsia compared to normal pregnancy

A previous literature search showed one study regarding the role of miRNA149-5p on normal pregnancy. MiR-149–5p could improve the invasion ability of trophoblast cells by targeting endoglin (ENG). Endoglin is a protein that is abundantly seen in the plasma of preeclamptic patients. The protein negatively affects the invasive ability of trophoblasts [26]. The expression of miRNA149-5p negates the influence of the endoglin protein. The high expression level of miR-

149-5P shows the importance of miR 149-5p in the normal development of the placenta. In a study done by Ronghui, Xiaoluand Qian [27] (2020), miRNA 149-5p showed lower expression in PE placental tissue.

Endothelin-1 (ET-1), one of the targets of miR-149-5p is a potent endogenous vasoconstrictor, mainly secreted by endothelial cells. It acts through two types of receptors: ETA and ETB. Apart from a vasoconstrictive action, ET-1 causes fibrosis of the vascular cells and stimulates the production of reactive oxygen species. miRNA149-5p was found to down-regulate endothelin receptor-1. This prevents vasoconstriction and the production of reactive oxygen species [28]. This proves the protective effect of miR-149-5p on normal pregnancy. It has to be understood that one miRNA can affect many genes and their functions by down-regulating them and one gene can also be a target for many miRNAs.

According to a study by Imperio *et al,* (2018), [29] miRNA 331-5p expression was elevated in the preterm placenta. Mirna331-5p negatively regulates P-gp expression. P-gp is a glycoprotein that is found in humans, encoded by the ABCB1 gene. P-gp is a well-characterized ABC-transporter. Placental ABC transporters exert a critical role in regulating steroid transport, immunological responses, and drug disposition. The ABC transporters act as "gatekeepers" protecting the fetus against the accumulation of potentially harmful factors that may be present in the maternal circulation. Given that ABC transporters extrude a range of specific substrates, alterations in their expression will likely lead to the modified transport of clinically relevant compounds as well as physiological factors.

Mirna 331-5p is also said to be involved in the downregulation of the PAPPA gene which encodes the protein Pappalysin 1 (Pregnancy Associated plasma protein A). There is a drastic difference in PAPP-A concentration in women with common anatomic uterine abnormalities (AUAs) compared to their age-matched control women with normal uteri [30]. Lower PAPP-A levels could be associated with smaller placental size rather than poor placentation and in future research, the calculation of the uterine cavity functional dimension may lead to a more accurate clinical assessment. There are no references to the study about the quantification of microRNAs in preeclampsia in Indian literature. Even though other international journals have demonstrated the expression of microRNA in preeclampsia, most of the articles did not estimate the differential expression.

CONCLUSION: The present preliminary study shows that epigenetic changes can also lead to pregnancy complications and miRNAs have a pivotal role in the progression of the disease. The study showed the expression of 9 differentially expressed miRNAs by the placental tissue. The selected miRNAs for the present study affect the genes which are involved in the normal development of the placenta. The altered level of miRNAs in the plasma of the mother in the early stages of pregnancy can be an indication of future pathogenesis. There has never been any research on microRNA in preeclampsia in the South Indian population.

The study also made use of next-generation sequencing, a molecular method that a few labs in the nation use. Overall, the study of differential miRNA expression seems a promising approach for the early prediction of PE, and the selected miRNAs 4743-5p,331-5p, and 149-5p can be used as a biomarker for the early detection of PE.

Although, additional research and well-designed prospective studies in a large sample pool are needed for validating these as reliable predictive tests for early diagnosis of PE. Since, the present study shows the role of miRNA 4743-5p, 149-5p, and 331-5pin normal pregnancy and preeclampsia, the study also explains the use of the above-mentioned miRNAs as early biomarkers of the disease and throws light on the exact pathophysiology of the disease.

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REFERENCES:

1. Scott G, Gillon TE, Pels A, von Dadelszen P and Magee LA: Guidelines similarities and dissimilarities: a systematic review of international clinical practice guidelines for pregnancy hypertension. Am J Obstet Gynecol [Internet] 2022; 226(2): 1222–36. Available from: http://dx.doi.org/10.1016/j.ajog.2020.08.018
2. Garovic VD, Dechend R, Easterling T, Karumanchi SA, McMurtry Baird S, Magee LA, Rana S, Vermunt JV and August: Hypertension in pregnancy: Diagnosis, blood pressure goals, and pharmacotherapy: A scientific statement from the American heart association. Hypertension [Internet]. 2022; 79(2): 21–41. Available from: http://dx.doi.org/10.1161/HYP.0000000000000208
3. Beech A and Mangos G: Management of hypertension in pregnancy. AustPrescr [Internet] 2021; 44(5): 148–52. Available from: http://dx.doi.org/10.18773/austprescr.2021.03
4. Macedo TCC, Montagna E, Trevisan CM, Zaia V, de Oliveira R, Barbosa CP, Laganà AS and Bianco B: Prevalence of preeclampsia and eclampsia in adolescent pregnancy: A systematic review and meta-analysis of 291, 247 adolescents worldwide since 1969. Eur J Obstet Gynecol Reprod Biol [Internet]. 2020; 248: 177–86. Available from: http://dx.doi.org/10.1016/j.ejogrb.2020.03.04
5. Wang W, Xie X, Yuan T, Wang Y, Zhao F, Zhou Z and Zhang H: Epidemiological trends of maternal hypertensive disorders of pregnancy at the global, regional, and national levels: a population-based study. BMC Pregnancy Childbirth [Internet] 2021; 21(1). Available from: http://dx.doi.org/10.1186/s12884-021-03809-
6. Sotiriou S, Satra M, Samara A, Vamvakopoulou D, Simou A, Tzelepis KSkentou, H, Vamvakopoulos, N and Garas, A:Maternal serum pregnancy-associated plasma protein-A concentration at 11–14 weeks of gestation and preeclampsia risk of women with common congenital anatomic uterine abnormalities. J Obstet Gynaecol [Internet]. 2022; 42(6): 1711–4. Available from: http://dx.doi.org/10.1080/01443615.2022.2031930
7. Tan KH, Tan SS, Sze SK, Lee WKR, Ng MJ and Lim SK: Plasma biomarker discovery in preeclampsia using a novel differential isolation technology for circulating extracellular vesicles. American Journal of Obstetrics and Gynecology 2014; 211: 380-381.
8. Turco MY, Gardner L, Kay RG, Hamilton RS, Prater M, Hollinshead MS, McWhinnie A, Esposito L, Fernando R, Skelton H, Reimann F, Gribble F. M, Sharkey A, Marsh S. G. E, O'Rahilly S, Hemberger M, Burton GJ and Moffett A: Trophoblast organoids as a model for maternal–fetal interactions during human placentation. Nature [Internet]. 2018; 564(7735): 263–7. Available from: http://dx.doi.org/10.1038/s41586-018-0753-3
9. Anne Cathrine Staff: The two-stage placental model of preeclampsia: An update. J Reprod Immunol [Internet]. 2019; 134–135: 1–10. Available from: http://dx.doi.org/10.1016/j.jri.2019.07.004
10. Wójtowicz A, Zembala-Szczerba M, Babczyk D, Kołodziejczyk-Pietruszka M, Lewaczyńska O and Huras H: Early- and late-onset preeclampsia: A comprehensive cohort study of laboratory and clinical findings according to the new ISHHP criteria. Int J Hypertens [Internet]. 2019; 4108271. Available from: http://dx.doi.org/10.1155/2019/4108271
11. Muijsers HEC, Roeleveld N, van der Heijden OWH and Maas AHEM: Consider preeclampsia as a first cardiovascular event. Curr Cardiovasc Risk Rep [Internet]. 2019; 13(7). Available from: http://dx.doi.org/10.1007/s12170-019-0614-0
12. Poon LC, Shennan A, Hyett JA, Kapur A, Hadar E, Divakar H, McAuliffe F, da Silva Costa F, von Dadelszen P, McIntyre H. D, Kihara A. B, Di Renzo G. C, Romero R, D'Alton M, BerghellaV, Nicolaides, K. H and Hod M: The International Federation of Gynecology and Obstetrics (FIGO) initiative on pre-eclampsia: A pragmatic guide for first-trimester screening and prevention. Int J GynaecolObstet [Internet] 2019; 145 1(1): 1–33. Available from: http://dx.doi.org/10.1002/ijgo.12802
13. Aneman I, Pienaar D, Suvakov S, Simic TP, Garovic VD and McClements L: Mechanisms of key innate immune cells in early- and late-onset preeclampsia. Front Immunol [Internet] 2020; 11. Available from: http://dx.doi.org/10.3389/fimmu.2020.01864
14. Chou CH, Shrestha S, Yang C-D, Chang NW, Lin YL, Liao KW, Huang WC, Sun TH, Tu SJ, Lee WH, Chiew MY, Tai CS, Wei TY, Tsai TR, Huang HT, Wang CY, Wu HY, Ho SY, Chen PR, Chuang CH, Hsieh PJ, Wu YS, Chen WL, Li MJ, Wu YC, Huang XY, Ng FL, Buddhakosai W, Huang PC, Lan KC, Huang CY, Weng SL, Cheng YN, Liang C, Hsu WL and Huang HD: miRTar Base update 2018: a resource for experimentally validated microRNA-target interactions. Nucleic Acids Res [Internet] 2018; 46(1): 296–302. Available from: http://dx.doi.org/10.1093/nar/gkx1067
15. Skalis G, Katsi V, Miliou A, Georgiopoulos G, Papazachou O, Vamvakou G, Nihoyannopoulos P, Tousoulis D and Makris T: MicroRNAs in preeclampsia. MicroRNA [Internet] 2019; 8(1): 28–35. Available from: http://dx.doi.org/10.2174/2211536607666180813123303
16. Danai M, Lykoudi A, George L, Papaioannou G and Kalantaridou S: Deep Sequencing Identified Dysregulated Circulating MicroRNAs in Late Onset Preeclampsia (2020). *In-vivo* [Internet] 34(5):2317–24. Available from: http://dx.doi.org/10.21873/*in-vivo*.12044
17. Shrestha B and Dunn L: The Declaration of Helsinki on medical research involving human subjects: A review of seventh revision. J Nepal Health Res Counc [Internet] 2020; 17(4): 548–52. Available from: http://dx.doi.org/10.33314/jnhrc.v17i4.1042
18. Ives C, Sinkey W, Tita T and Oparil Suzanne: Preeclampsia-Pathophysiology and Clinical Presentations JACC State-of-the-Art Review. Journal of the American College of Cardiology [Internet]. 2020; 76(14): 1690–702. Available from: http://dx.doi.org/10.1016/j.jacc.2020.08.014
19. Lokki AI, Heikkinen-Eloranta JK and Laivuori H: The immunogenetic conundrum of preeclampsia. Front Immunol [Internet]. 2018; 9: 2630. Available from: http://dx.doi.org/10.3389/fimmu.2018.02630

20. Lv Y, Lu C, Ji X, Miao Z, Long W, Ding H and LvM: Roles of microRNAs in preeclampsia. J Cell Physiol [Internet]. 2019; 234(2): 1052–61. Available from: http://dx.doi.org/10.1002/jcp.27291
21. Laganà AS, Vitale SG, Sapia F, Valenti G, Corrado F, Padula F, Rapisarda AMC and D'Anna R: miRNA expression for early diagnosis of preeclampsia onset: hope or hype. J Matern Fetal Neonatal Med [Internet]. 2018; 31(6): 817–21. Available from: http://dx.doi.org/10.1080/14767058.2017.1296426
22. Menon R, Debnath C, Lai A, Guanzon D, Bhatnagar S, Kshetrapal PK, Sheller-Miller S, Salomon C and Garbhini Study Team: Circulating exosomal miRNA profile during term and preterm birth pregnancies: A longitudinal study. Endocrinology [Internet]. 2019; 160(2): 249–75. Available from: http://dx.doi.org/10.1210/en.2018-00836
23. Hromadnikova I, Kotlabova K, Dvorakova L and Krofta L: Maternal cardiovascular risk assessment 3-to-11 years postpartum in relation to previous occurrence of pregnancy-related complications. J Clin Med [Internet]. 2019; 8(4): 544. Available from: http://dx.doi.org/10.3390/jcm8040544
24. Richa Sharma, Basu Dev Banerjee, Manjeet Singh Bhatia, Amita Suneja, Kiran Guleria and Neetu Chaudhary: Association of Placental Tissue Estrogen Receptor Alpha Gene Expression with Postpartum Depression in Women at Risk: A Pilot Study. Journal of South Asian Federation of Obstetrics and Gynaecology. Volume 12 | Issue 6 | Year 2020https://doi.org/10.5005/jp-journals-10006-1842.
25. Orzechowska M, Anusewicz D and Bednarek AK: Functional gene expression differentiation of the Notch signaling pathway in female reproductive tract tissues-A comprehensive review with analysis. Front Cell Dev Biol [Internet] 2020; 8: 592616. Available from: http://dx.doi.org/10.3389/fcell.2020.592616
26. Xiaobo Z, Qizhi H, Zhiping W and Tao D: Down-regulated miR-149-5p contributes to preeclampsia *via* modulating endoglin expression. Pregnancy Hypertens [Internet] 2019; 15: 201–8. Available from: http://dx.doi.org/10.1016/j.preghy.2019.01.002
27. Liu R, Wang X and Yan Q: The regulatory network of lncRNA DLX6-AS1/miR-149-5p/ERP44 is possibly related to the progression of preeclampsia. Placenta [Internet] 2020; 93: 34–42. Available from: http://dx.doi.org/10.1016/j.placenta.2020.02.001
28. Granger JP, Spradley FT and Bakrania BA: The endothelin system: A critical player in the pathophysiology of preeclampsia. CurrHypertens Rep [Internet] 2018; 20(4). Available from: http://dx.doi.org/10.1007/s11906-018-0828-4
29. do Imperio GE, Bloise E, Javam M, Lye P, Constantino A, Dunk C, Dos Reis FM, Lye SJ, Gibb W, Ortiga-Carvalho TM and Matthews SG: Chorioamnionitis induces a specific signature of placental ABC transporters associated with an increase of miR-331-5p in the human preterm placenta. Cell PhysiolBiochem [Internet] 2018; 45(2): 591–604. Available from: http://dx.doi.org/10.1159/000487100
30. Sotiriou S, Satra M, Samara A, Vamvakopoulou D, Simou A, Tzelepis K, Skentou H, Vamvakopoulos N and Garas, A: Maternal serum pregnancy-associated plasma protein-A concentration at 11-14 weeks of gestation and preeclampsia risk of women with common congenital anatomic uterine abnormalities. J Obstet Gynaecol [Internet] 2022; 42(6): 1711–4. Available from: http://dx.doi.org/10.1080/01443615.2022.2031930.

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